

**PAT 201. APPLIED PLANT PATHOLOGY (1+1)**  
**(2003 Syllabus)**

repared by

**Dr. G. Arjunan, Professor and Head**  
**Dr. G. Karthikeyan, Assistant Professor**  
**Dr. D. Dinakaran, Associate Professor**

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**THEORY**

Principles of Crop disease management — Epidemiology of crop diseases— Disease surveillance, assessment of disease intensity - forecasting. Survival and mode of spread of plant pathogens – Types of resistance – Cross protection – mechanism of resistance,. Methods and management of plant diseases - Fungicides – Characteristics of an ideal fungicide - Classification – groups of fungicides – antibiotics. Formulations – compatibility. Phytotoxicity – precautions and safety measures in handling. Management of diseases – seed, soil foliar and post harvest diseases – seed health testing methods – Simple diagnostic techniques for identification of diseases. Biological control and their scope – biocontrol agents – Fungi, bacteria, vesicular arbuscular mycorrhizae – Plant products and antiviral principles. Biotechnological approaches in plant disease management.

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# 1

## Introduction

Agriculture is very important in countries like India. Higher food production depends on many factors. Among important factors knowledge on pests and diseases is very essential. Plant diseases caused by fungi, bacteria, viruses, phytoplasmas etc. cause heavy loss to the crop. Detection and identification of the cause for the diseases attacking seed or seed materials and plants is important. Importance of seed health testing has been recognized and is being used in several countries to check the spread of seed - borne diseases. Newer techniques like ELISA, ELFA, PCR, etc., have been employed to detect plant pathogens present in seed and planting materials.

Disease survey is an important component in the management of crop diseases. Regular surveillance of plant diseases has to be undertaken to obtain details on the intensity and incidence of the diseases and weather factors like temperature, relative humidity etc., and to forecast the plant diseases.

Plant pathogens spread from place to place or from one field to another by different means including seed, soil, wind, vectors(insects, mites, fungi, nematodes), human beings, implements etc., The severity of crop diseases is governed by pathogen, host and environmental factors. Epidemics occurred in food and other crops and factors responsible for epidemics have been worked out. Forecasting systems for many important crop diseases have been worked out and are being adopted for effective crop disease management.

Management methods for the control of plant diseases are many. Several fungicidal chemicals of systemic and non-systemic nature (including antibiotics) have been used to control the diseases attacking the crop. Fungicides are applied by different methods to seed soil or to the foliage with the help of equipments operated manually or with power. At present, the fungicide use has been reduced because of higher cost, development of resistance to several pathogens, health hazards to living beings and environmental pollution. Alternate methods

like use of cultural and biological means in plant disease management are used. Researches to find out newer biocontrol agents have been strengthened. Many biocontrol agents have been found out and they are mass cultured and distributed in suitable formulations to the farmers. Plant products have also been used for the control of crop diseases. Antiviral principles and cross protection are employed in the control of virus diseases. Biotechnological tools like genetic engineering and tissue culture are utilized in the control of fungal, bacterial and viral diseases. Meristem tip culture is used to obtain virus free plants in cassava, banana, potato and many ornamental plants. Plant diseases can be managed effectively by integrating the available methods without causing environmental pollution and ill-effects to human beings and other organisms.

# 2

## Survival of Plant Pathogens

Any pathogen can cause disease under favourable conditions. The only requisite factor is that the pathogen must come in contact with the host for the development of the disease. Pathogen itself or its parts that are capable of causing disease when brought near a host is called **inoculum**. Fungal pathogens are diversified where the vegetative body (hyphae), dormant mycelium, (embedded in the embryo of seeds or other plant parts), special reproductive structures (rhizomorphs, sclerotia, chlamydospores), various types of asexual spores (sporangia, sporangiospores, zoospores, conidia) and sexual spores (oospores, zygospores, resting spores, ascospores, basidiospores etc.), serve as inocula. In the case of viruses and plant pathogenic bacteria, the individuals are acting as inocula, since they do not produce any special type of infective units like resting spores or endospores etc. But in the case of *Streptomyces* sp. (Actinomycetes), fragments of filaments and spore-like cells serve as inocula. In phanerogamic parasites, seeds are the potential inocula. Seeds in the soil survive for longer period. *Orobanche* seeds survive for about 13 years. Seeds are abundantly produced for their multiplication which could attack the host plants. But dodder is an exception because broken bits of shoot can attack host plant. In any locality a time lag exists between harvest of a crop and subsequent sowing. Year after year, diseases appear in the newly sown crops. There should be some link between the previous crop and the subsequent new crop to revive or continue the life cycle. The existence of the pathogen between the two crop seasons is the vulnerable period in its life cycle. Hence knowledge of the survival of the pathogen in the off-season is useful for the plant pathologists to device effective control measures.

The establishment of a plant pathogen in a geographical location presupposes its ability to survive, not only during its parasitic relations with its hosts, but also during off-seasons in which the hosts are not growing. In temperate zones, plant pathogens must be adapted for survival overwinters or oversummers, like the powdery mildew pathogen that attacks fall-seeded wheat. In the Torrid zone, plant pathogens must be able to survive the dry seasons, during which susceptible plants are not growing.

These sources of survival of pathogens or the sources for renewal of infection chain can be grouped as follows:

1. Survival by means of specialized resting structures
2. Survival as saprophytes
3. Survival in vital association with living plants
4. Survival in association with insects

### 1. Survival by means of specialized resting structures

Enduring structures of plant pathogens may be as simple as conidia or as complex as perithecia. Apparently, ascospores or conidia derived from them, serve to carry the pathogen causing peach-leaf curl (*Taphrina deformans*) over the winter. Conidia of *Alternaria solani*, the pathogen of early blight of potato and tomato, survive for eighteen months in dried diseased leaves. Specialized thick-walled chlamydospores of *Fusarium* and other Imperfect fungi, spores of many smut fungi and the amphiospores, uredospores and teliospores of certain rust fungi also are important enduring structures. The resting spores of *Plasmodiophora brassicae* may survive for ten years in soils infested upon the disintegration of clubbed roots. The oospores of downy-mildew fungi survive in the soil between growing seasons. In fact, oospores of the fungus that causes onion mildew do not germinate until several years after their formation.

Some fungi survive unfavourable seasons in the form of sclerotia. Those produced by the omnivorous cottony-rot fungus, *Sclerotinia sclerotiorum*, can survive for years in a dry atmosphere. They decay rapidly, however, in warm moist soil. Cold induced dormancy probably accounts for their ability to survive winters in temperate zones. Some powdery mildew fungi and other ascomycetes survive with plant refuse. Parasitic phanerogams survive in the form of seeds and as eggs, cysts and larvae of parasitic nematodes serve as overseasoning structures.

### 2. Survival as saprophytes

The ability to live saprophytically enables many plant pathogens to survive in the absence of growing susceptible plants. Saprophytic survival usually occurs in or on the soil. Waksman (1971) distinguished between soil inhabitants and soil invaders; the former comprise the basic fungal flora of the soil, whereas the later are short lived exotics. As applied to the root infecting fungi **soil inhabitants** are unspecialized parasites with a wide host range that are able to survive indefinitely in the soil as saprophytes; **soil invaders** (root inhabiting fungi) are more specialized parasites that survive in soils in close association with their hosts. Most plant pathogenic fungi and bacteria are soil invaders, but some pathogens, notably *Rhizoctonia solani* and *Pythium debaryanum* which cause seedling blights and root rots, live saprophytically in the soils.

The microbiological balance in the soil markedly affects the survival of saprophytic plant pathogens there. Apparently, Sanford (1926) was the first to suggest that control of potato scab by green manuring with grass might be due to the antagonistic action of saprophytic organisms flourishing on the green manure. Not only do soil saprophytes antagonize other microorganisms by toxic action, but some such as *Trichoderma lignorum* actually parasitize *Rhizoctonia solani* and other soil-borne pathogens. Despite antagonism and parasitism by other organisms, many plant pathogens survive in the soil as inhabitants or invaders. The special conditions that favour biological control of plant pathogens in sterilized soil or in culture are nonexistent in field soil, in which there is a complex microflora and a low concentration of nutrients.

Certain plant pathogens survive between growing seasons as saprophytes in the dead tissues of susceptible plants. Such organisms are only incidentally associated with the soil, and live only as long as tissues of susceptible plants are available to supply nutrients. Most plant-pathogenic bacteria and many specialized parasitic fungi survive in this manner. The apple scab pathogen (*Venturia inaequalis*) lives parasitically in leaves and fruits during the growing season, but becomes saprophytic in fallen leaves. Perithecia form in these leaves during the winter, but ascospores do not form until spring. Ascospores of certain other ascomycetes mature during the winter, but are protected from adverse conditions by perithecial walls. Soil inhabitants include obligate saprophytes and facultative parasites and they are exo-pathogens. Whereas soil invaders (root inhabitants) include facultative saprophytes and obligate parasites and they are endopathogens (root infecting fungi).

Plant pathogenic bacteria can saprophytically survive or actively multiply in the rhizosphere or rhizoplane of healthy host and non-host plants. *Erwinia carotovora* subsp. *carotovora* has been considered to survive in soil. However, some recent studies have shown that soft rot *Erwinia* cannot persist for a long time in fallow soil. *E. carotovora* subsp. *carotovora* multiplies in the rhizosphere of many cruciferous plant species, where the population can readily increase from  $10^2$  cells /g in fallow soil to  $10^4$  to  $10^6$  cells /g in soil subjected to the rhizosphere effect of chinese cabbage. *Pseudomonas glumae*, the causal agent of bacterial grain rot of rice, remains on rhizosphere and/or rhizoplane of the rice plant from germination to tillering stage. *Burkholderia solanacearum* and species of *Agrobacterium* are best known with a prolonged soil phase which can be regarded as the true soil-borne pathogens.

### 3. Survival in vital association with living plants

Survival of the plant pathogens in vital association with living plants is grouped into

**a. Seed:** The pathogen of loose smut of wheat, *Ustilago nuda tritici*, enters the stigma and style and infects the young seed, in which it survives as mycelium. The seed-infecting pathogens that cause loose smut of wheat and loose smut of barley are strikingly different from other smut fungi that attack cereal crops. Most of the others survives from season to season either in non-pathogenic association with seed or as spores in the soil. *Colletotrichum lindemuthianum*, the causative organism of bean anthracnose, can also infect the seed; unless the seed is killed, new infections are initiated by the fungus in newly sprouted bean seedlings. The bacteria that cause bean blights and bacterial blight of cotton survive the winter in infected seed. In Mexico, the fungus of late blight of potatoes (*Phytophthora infestans*) produces oospores but in colder regions of the world, the fungus overwinters as mycelium in diseased tubers.

Lenticels of potato tubers may carry soft rot *Erwinia* at the maximum level of 100 cells/lenticel, although the infested tubers do not necessarily develop soft rot in the field. Examples of the plant pathogenic bacteria that survive in seed/planting materials are given in the following table.

**Table.** Plant pathogenic bacteria surviving in seeds and planting materials.

Sl.No	Disease	Bacteria
	<b>a. Seed</b>	
1.	Bacterial canker of tomato	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>
2.	Goss's bacterial wilt and blight of maize	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>
3.	Bacterial wilt of bean	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i>
4.	Bacterial brown stripe of rice	<i>Pseudomonas avenae</i>
5.	Bacterial grain rot of rice	<i>Pseudomonas glumae</i>
6.	Bacterial blight of soybean	<i>Pseudomonas syringae</i> pv. <i>glycinea</i>
7.	Angular leaf spot of cucurbits	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i>
8.	Halo blight of bean	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>
9.	Bacterial blight of pea	<i>Pseudomonas syringae</i> pv. <i>pisi</i>
10.	Black rot of crucifers	<i>Xanthomonas campestris</i> pv. <i>campestris</i>
11.	Bacterial blight of cotton	<i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>
12.	Common blight of bean	<i>Xanthomonas campestris</i> pv. <i>phaseoli</i>
	<b>b. Planting material</b>	
1.	Ring rot of potato	<i>Clavibacter michiganensis</i> subsp. <i>Sepedonicus</i>
2.	Silvering of tulip	<i>Curtobacterium flaccumfaciens</i> pv. <i>portii</i>
3.	Bacterial wilt of carnation	<i>Pseudomonas caryophylli</i>
4.	Gladiolus scab	<i>Pseudomonas gladioli</i> pv. <i>gladioli</i>
5.	Bacterial wilt of potato and ginger	<i>Burkholderia solanacearum</i>
6.	Bacterial leaf spot of <i>Photinia glabra</i>	<i>Pseudomonas syringae</i> pv. <i>photiniae</i>
7.	Leaf scald and white streak of sugarcane	<i>Xanthomonas albilineans</i>
8.	Bacterial leaf spot of Begonia	<i>Xanthomonas campestris</i> pv. <i>begoniae</i>
9.	Yellows of hyacinth	<i>Xanthomonas campestris</i> pv. <i>hyacinthi</i>
10.	Bacterial blight of iris	<i>Xanthomonas campestris</i> pv. <i>tardicrescens</i>

**b. Collateral hosts:** Collateral hosts are those which are susceptible to the plant pathogens of crop plants and provide adequate facilities for their growth and reproduction of these pathogens. Weeds which survive and live during non-cropping season provide for the continuous growth and multiplication of the pathogen. For example, the fungal pathogen for blast disease of rice, *Pyricularia oryzae* can infect the grass weeds like *Brachyarrhiza mutica*, *Dinebra retroflexa*, *Leersia hexandra*, *Panicum repens*, etc., and survive during off-season of rice-crop. As soon as a fresh rice crop is raised, the conidia (inoculum) liberated from the weed host disseminated by wind infects the fresh rice crop. Thus the weed hosts help to bridge the gap between two rice crops. Hence the pathogen can be able to line continuously in the vicinity on these hosts inspite of the non-cropping period intervening between two cropping periods. Intensive cultivation of particular crop repeatedly and constantly also provides perpetual inoculum. Powdery mildew and viral diseases of cucurbits are also best examples, where, number of cultivated crops serve as collateral hosts. The survival of the plant pathogens on collateral hosts/ alternative hosts which include the weed hosts also. The collateral / weed hosts which are present in the field and in the bunds harbour the plant pathogens during cropping season. But the collateral / weed hosts present in the bunds harbour the plant pathogens during off-season. The pathogens can survive in active sporulating stage on wild collateral hosts and from there the primary inoculum may be disseminated by wind or insect to the main cultivated crops. Plant pathogenic bacteria may be able to survive in the parasitic form on annual and perennial weeds. For example, the long term survival of *Pseudomonas avenae* in Florida is attributed to the association with a perennial grass, vaseygrass (*Paspalum urvillei*), through repeated infection of its vegetative growth as well as seed transmission. Bacterial leaf blight of maize may have its origin in the infected vaseygrass distributed in the field. This list of collateral weed hosts for the plant pathogens is given in the table.

Table. Collateral hosts (alternative hosts) of plant pathogens

Pathogen	Disease	Principal host	Collateral hosts / Alternative hosts
<b>1. Fungal Diseases</b>			
<i>Sclerospora graminicola</i>	Downy mildew	Pearlmillet Fox-tail Millet	<i>Echinochloa</i> sp., <i>Euchlaena</i> sp., <i>Panicum</i> sp.
<i>Peronosclerospora heterogoni</i>	Downy mildew	Corn	<i>Euchlaena</i> sp., <i>Heteropogon</i> sp.
<i>P.maydis</i>	Downy mildew	Corn	<i>Euchlaena</i> sp., <i>Tripsacum</i> sp.
<i>P.miscanthi</i>	Leaf splitting downy mildew	Corn	<i>Miscanthus</i> sp., <i>Plumosum</i> sp.
<i>P.philippinensis</i>	Philippine downy mildew	Corn	<i>Avena</i> sp., <i>Euchlaena</i> sp., <i>Miscanthus</i> sp., <i>Saccharum</i> sp., <i>Tripsacum</i> sp.
<i>P.sacchari</i>	Sugarcane	Corn	<i>Andropogon</i> spp.,



	downy mildew		<i>Bothriochloa</i> sp., <i>Euchlaena</i> sp., <i>Miscanthus</i> sp., <i>Schizachyrium</i> sp., <i>Tripsacum</i> sp.
<i>P.sorghii</i>	Sorghum downy mildew	Corn, Sorghum	<i>Andropogon</i> spp., <i>Euchlaena</i> sp., <i>Heteropogon contortus</i> , <i>Panicum typheron</i>
<i>P.spontanea</i>	Spontaneum Downy mildew	Corn	<i>Euchlaena</i> sp., <i>Iseilema</i> sp., <i>Miscanthus</i> sp.
<i>Sclerophthora macrospora</i>	Crazy top of corn / yellow wilt of rice	Corn, Finger millet, rice, wheat	140 grass hosts, <i>Echinochloa</i> sp., <i>Eragrostis</i> sp., <i>Iseilema</i> sp., <i>Miscanthus</i> sp., <i>Paspalum</i> sp., <i>Saccharum</i> sp,
<i>S.macrospora</i>	Downy mildew / Green ear	Finger millet	<i>Digitaria marginata</i> , <i>Eleusine indica</i> <i>Eragrostis cilianensis</i> , <i>E.pectinacea</i>
<i>S.rayssiae</i> var <i>zeae</i>	Brown stripe downy mildew	Corn	<i>Digitaria sanguinalis</i>
<i>Uromyces setariaeitalicae</i>	Rust	Fox-tail millet	<i>Setaria glauca</i> <i>S.verticillata</i> , <i>S.viridis</i>
<i>Sphaerotheca fuligena</i>	Curcubit powdery mildew	Curcubits	Many curcubitaceous weeds
<i>Erysiphe cichoracearum</i>	Curcubit powdery mildew	Curcubits	Many curcubitaceous weeds
<i>Rhizoctonia solani</i>	Web blight	Cowpea	<i>Amaranthus spinosus</i> , <i>Aspilia Africana</i> , <i>Fleurya estruans</i> , <i>Newbouldia laevis</i>
<i>Rhizoctonia solani</i>	Sheath blight	Rice	<i>Cynodon dactylon</i> <i>Echinochloa</i> sp.
<i>Gaeumannomyces</i>	Take-all disease	Wheat	<i>Agropyron repens</i> , <i>Holcus lanatus</i>
<i>Pyricularia oryzae</i>	Blast	Rice	<i>Arundo donax</i> , <i>Brachiaria mutica</i> , <i>Dinebra retroflexa</i> , <i>Leersia hexandra</i> , <i>Panicum repens</i> , <i>Digitaria marginata</i> , <i>Setaria intermedia</i>

<i>Helminthosporium oryzae</i>	Brown leaf Spot	Rice	<i>Echinochloa colona</i> <i>Leersia tenuifolia</i>
<i>Balansia oryzae</i>	Udbatta disease	Rice	<i>Eragrostis tenuifolia</i> , <i>Isachne elegans</i>
<i>Claviceps microcephala</i>	Ergot	Pearlmillet	<i>Cenchrus ciliaris</i> , <i>C.setigerus</i> , <i>Pennisetum alopecuroides</i> , <i>P.hohenackeria</i> , <i>P.purpureum</i> , <i>P.ruppelii</i> .
<i>Puccinia substriata</i> var. <i>penicillariae</i>	Rust	Pearlmillet	<i>Pennisetum leonis</i> , <i>P.spicatum</i>
<i>Pyricularia setariae</i>	Blast	Finger millet	<i>Dactyloctenium aegyptium</i> sp. <i>Digitaria Marginata</i>
<i>Helminthosporium nodulosum</i>	Leaf blight	Finger millet	<i>Echinochloa frumentacea</i> , <i>Eleusine indica</i> <i>Dactyloctenium aegyptium</i>
<i>Ustilago scitaminea</i>	Smut	Sugarcane	<i>Cyperus rotundus</i> <i>Erianthus saccharoides</i> , <i>Imperata arundinacea</i> , <i>Narenga</i> sp. <i>Saccharum barberi</i> , <i>S.robustum</i> , <i>S.sinensis</i> , <i>S.spontaneum</i> <i>Sclerostachya fusca</i>
<i>Puccinia melanocephala</i>	Rust	Sugarcane	<i>Erianthus fulvus</i> <i>Narenga porphyrocoma</i> <i>Saccharum spontaneum</i>
<i>P.kuehnii</i>	Rust		<i>Erianthus arundinaceum</i> , <i>Narenga</i> sp., <i>Saccharum spontaneum</i> , <i>Sclerostachya fuscum</i>
<i>Cercospora koepkei</i>	Yellow spot	Sugarcane	<i>Saccharum spontaneum</i>
<i>Phakopsora gossypii</i>	Rust	Cotton	<i>Azanza garkensa</i> , <i>Thespesia populnea</i>
<i>Colletotrichum capsici</i>	Anthracnose	Cotton	<i>Aristolochia bracteata</i> , <i>Hibiscus diversifolius</i>
<i>Alternaria brassicae</i>	Leaf blight	Rapeseed Mustard,	<i>Anagallis arvensis</i> , <i>Convolvulus arvensis</i>

		<i>Brassica</i> spp.	
<i>Puccinia helianthi</i>	Rust	Sunflower	<i>Helianthus cucumerifolius</i> , <i>H.tuberosus</i>
<i>Sclerotinia sclerotiorum</i>	Wilt	Safflower	<i>Argemone</i> sp. <i>Chenopodium album</i>
<i>Puccinia carthami</i>	Rust	Safflower	<i>Cathranthus oxycantha</i>
<i>Erysiphe cichoracearum</i>	Powdery mildew	Tobacco	<i>Nicotiana alata</i> , <i>N.megalosiphon</i>
<b>2. Bacterial diseases</b>			
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Bacterial leaf blight	Rice	<i>Cyperus</i> spp. <i>Leersia hexandra</i>
<i>Pseudomonas rubrilineans</i>	Red stripe and top rot	Sugarcane	<i>Sorghum halepense</i> <i>S.sudanense</i>
<i>X.campestris</i> pv. <i>vasculorum</i>	Gummosis	Sugarcane	<i>Brachiaria mutica</i> , <i>Dictyosperma album</i> , <i>Panicum maximum</i> , <i>Roystonea regia</i> , <i>Thysanolaena maxima</i>
<i>X.axonopodis</i> pv. <i>Malvacearum</i>	Bacterial blight	Cotton	<i>Eriodendron anfructuosum</i> , <i>Jatropha curcas</i> , <i>Lochnera pusilla</i> , <i>Thurbaria thespesoides</i>
<b>3. Viruses and phytoplasmas</b>			
Cucumber mosaic virus(Strain)	Mosaic	Safflower	<i>Amaranthus blitum</i> , <i>A.gangeticus</i> , <i>Commelina</i> sp., <i>Datura metel</i> , <i>Physalis minima</i> , <i>Solanum nigrum</i>
Cucumber mosaic virus	Cucumber mosaic	Cucurbits	Many Cucurbitaceous Weeds
Cowpea mosaic virus	Mosaic	Cowpea	<i>Phaseolus lathyroides</i>
Cowpea aphid borne mosaic virus Hoja blanca	Cowpea aphid borne mosaic Hoja blanca	Cowpea  Rice	<i>Chenopodium amaranticolor</i> <i>Echinochloa</i> sp. <i>Panicum</i> sp.
Rice tungro virus	Rice tungro	Rice	<i>Oryza</i> spp., <i>Echinochloa colona</i> , <i>E.crusgalli</i> , <i>Leersia hexandra</i>
Bhendi yellow vein mosaic virus	Bhendi yellow vein mosaic	Bhendi	<i>Hibiscus tetraphyllus</i>
RLO	Ratoon stunting	Sugarcane	<i>Chloris gayana</i> ,

			<i>Cynodon dactylon</i> , <i>Sorghum halepense</i> , <i>S.sudanense</i>
Groundnut rosette virus	Rosette	Groundnut	<i>Nicotiana clevelandii</i> , <i>Stylosanthes guyanensis</i> , <i>S.mucronata</i> , <i>S.sundaica</i> .
Sugarcane mosaic virus	Mosaic	Sugarcane	<i>Echinochloa crusgalli</i> <i>Eleusine indica</i> , <i>Saccharum spontaneum</i>
Cotton leaf curl virus	Leaf curl	Cotton	<i>Althaea rosea</i> , <i>Gossypium peruvianum</i>
Sunhemp mola virus	Mosaic	Sunhemp	<i>Crotalaria laburnifolia</i> , <i>C.lanceolata</i> , <i>C.mucronata</i> , <i>C.retusa</i> , <i>C.spectabilis</i> , <i>Nicandra physoides</i> , <i>Solanum nigrum</i>
Tobacco mosaic virus (Strain)	Southern Sunhemp mosaic	Sunhemp	<i>Cassia tora</i> , <i>Datura innoxia</i> , <i>Gomphrena globosa</i> , <i>Petunia hybrida</i>
Tomato spotted wilt virus	Ring mosaic	Groundnut	<i>Bidens pilosa</i> , <i>Tagetes sp.</i>
Cowpea mild mottle virus	Cowpea mild Mottle	Cowpea	<i>Cassia occidentalis</i> , <i>Nicotiana clevelandii</i>
Peanut chump virus	Peanut clump	Groundnut	<i>Nicotiana benthamiana</i> , <i>N.clevelandii</i>
Phytoplasma	Phyllody	Sesame	<i>Sesamum album</i> , <i>S. occidentale</i> , <i>S.orientale</i> , <i>S. prostratum</i> , <i>S.radiatum</i>
Phytoplasma	Little leaf	Brinjal	<i>Datura sp.</i> , <i>Catharanthus sp.</i>

Table: Some pathogens with wide host range which are aided by weeds

Sl. No.	Pathogen	Disease	Number of host species
1.	<i>Sphaerotheca fuliginea</i>	Powdery mildew	570

2.	<i>Leveillula taurica</i>	Powdery mildew	710
3.	<i>Sclerotinia sclerotiorum</i>	Blight, cottony rot, Stalk rot	361
4.	<i>Botrytis cinerea</i>	Grey mold	several thousands
5.	<i>Phymatotrichum omnivorum</i>	Root rot	1,300
6.	<i>Sclerotium rolfsii</i>	collar rot, stalk rot, white rot	500
7.	<i>Macrophomina phaseolina</i>	Charcoal rot	300
8.	<i>Verticillium albo-atrum</i> and <i>Verticillium dahliae</i>	Wilt	300
9.	<i>Cucumber mosaic virus</i>	Mosaic	775

**c. Alternate hosts:** The role of alternate hosts is not as important as of collateral hosts. However, when a pathogen has very wide host-range and is tolerant to wide range of weather conditions the alternate hosts become very important source of survival of the pathogen. These alternate hosts are very important for the completion of the life cycle of heteroecious rust pathogens. e.g. in temperate regions the alternate host *Berberis vulgaris* of *Puccinia graminis tritici* (black/stem rust pathogen on wheat), the barberry bush, grows side by side with the cultivated host, wheat. In such areas this wild host belonging to a different family is important for survival of the fungus. It helps in completion of heterogeneous infection chain of the rust fungus. The list of alternate hosts for important plant pathogenic survival is given in the table.

Table: Alternate hosts of plant pathogens

Fungal pathogen	Disease	Primary host	Alternate host
<i>Puccinia graminis tritici</i>	Stem rust / Black rust	Wheat	<i>Berberis vulgaris</i>
<i>Puccinia striiformis</i>	Stripe rust / Yellow rust	Wheat	?
<i>Puccinia recondita</i>	Leaf rust / Brown rust / orange rust	Wheat	<i>Thalictrum</i> sp.
<i>Puccinia coronata</i>	Crown rust	Oat	<i>Rhamnus</i>
<i>Puccinia anomala</i>	Brown rust	Barley	Lily
<i>Puccinia dispersa</i>	Brown rust	Rye	<i>Anchusa</i> sp.
<i>Puccinia purpurea</i>	Rust	Sorghum	<i>Oxalis corniculata</i>
<i>Puccinia substriata</i> var <i>penicillariae</i>	Rust	Pearl-millet	Brinjal
<i>Puccinia sorghi</i>	Leaf rust	Maize	<i>Oxalis</i>
<i>Cronartium ribicola</i>	Blister rust	Currant	Pine
<i>Gymnosporangium juniperi-</i>	Cedar rust	Cedar	Apple

*virginiana*

**d. Self sown crops:** Self sown plants, voluntary crops and early sown crops are reservoirs of many plant pathogens e.g., groundnut rust pathogen, *Puccinia arachidis* and ring mosaic of groundnut caused by tomato spotted wilt virus. Self sown rice plants harbour the pathogen as well as vector. e.g., rice tungro virus and its vector, *Nephotettix virescens*.

Perennial crops also play a major role in the survival of the plant pathogens. Pathogens which infect perennial plants can survive in them during low winter temperature and/or during the hot, dry weather of the summer. They survive in the lesions on perennial host plants which may be actively growing or are dormant. Disease like bunchy top of banana survives continuously in the suckers produced by the mother plants. In citrus canker the bacterium, *Xanthomonas axonopodis* pv. *citri* survive in the cankers formed on the leaves and twigs of the standing tree. They mostly survive in mild or vigorous active form on hosts, *Citrus* sp. Other examples, are *Erwinia amylovora* and *Xanthomonas campestris* pv. *pruni*.

**e. Ratoon Crops:** Sometimes ratoon crop also harbour the plant pathogens e.g., sugarcane mosaic.

**f. Survival by latent infection:** Latent infection refers to the conditions in which the plant pathogens may survive for a long time in plant tissue without development of visible symptoms. Eg. *Pseudomonas syringae* pv. *syringae* and *X. axonopodis* pv. *citri* can survive in apparently healthy bark tissues of their tree hosts. *Xylella fastidiosa*, the causal agent of Pierce's disease of grapevine and leaf scorch disease of various fruits and leaf ornamental trees infect diverse kinds of weeds without developing visible symptoms. Because these weeds are usually favourable habitats for the vector insects, latently infected weeds become an important source of the carrier insects.

**g. Survival as residents:** Plant pathogenic bacteria have the capacity to grow on the surface of host and non-host plants utilizing the small amount of nutrients that are secreted on the plant surface. Survival as residents in the phyllosphere by bacteria is given in the table.

**Table.** Plant pathogenic bacteria that survive in epiphytic form

Sl. No	Disease	Bacteria
1.	Fire blight of apple and pear	<i>Erwinia amylovora</i>
2.	Soft rot of chinese cabbage	<i>Erwinia carotovora</i> subsp. <i>Carotovora</i>
3.	Bacterial grain rot of rice	<i>Pseudomonas glumae</i>
4.	Bacterial blight of soybean	<i>Pseudomonas syringae</i> pv. <i>glycinea</i>
5.	Angular leaf spot of	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i>

- cucurbits
6. Bacterial canker of stone fruits *Pseudomonas syringae* pv. *Morsprunorum*
  7. Bacterial brown spot of bean *Pseudomonas syringae* pv. *syringae*
  8. Bacterial speck of tomato *Pseudomonas syringae* pv. *tomato*
  9. Bacterial blight of cotton *Xanthomonas axonopodis* pv. *malvacearum*
  10. Bacterial blight of cassava *Xanthomonas axonopodis* pv. *manihotis*
  11. Common blight of bean *Xanthomonas campestris* pv. *phaseoli*

#### 4. Survival in association with nematode and fungi

Plant viruses like wheat mosaic, wheat spindle-streak virus, lettuce big vein, tobacco necrosis, tobacco rattle and tobacco ringspot viruses survive with nematodes or fungi found in the soil between crop seasons. Tobacco ringspot virus is associated with the nematode, *Xiphinema americana*. The fungus, *Polymyxa graminis* (Barley yellow mosaic, oat yellow mosaic, wheat soil-borne mosaic, wheat spindle-streak mosaic) and *Spongospora subterranea* (potato mop top) carry the viruses internally and transmit them through their resting spore. Viruses are retained by nematode vectors for long times (stable). *Xiphinema* sp. retained viruses for a considerable length of time, while *Longidorus* spp. and *Trichodorus* spp. retained them for a much less period of one to two months only.

**Table.** Retention periods and acquisition feeding periods of some viruses on nematodes

Sl. No	Virus	Vector	Retention period
1.	Tobacco ringspot	<i>Xiphinema americanum</i>	12 months
2.	Arabis mosaic	<i>Xiphinema diversicaudatum</i>	8 months
3.	Grapevine fan leaf	<i>Xiphinema index</i>	8 months
4.	Tobacco rattle (Dutch isolate)	<i>Trichodorus pachydermis</i>	36 days
5.	Tobacco rattle (California isolate)	<i>Trichodorus allivus</i>	28 days
6.	Raspberry ringspot	<i>Longidorus elongatus</i>	1-2 months
7.	Tomato blackring	<i>Longidorus elongatus</i>	1-2 months

#### 5. Survival in association with insects

Many insects are carriers of inocula during the growing season and several important plant pathogens survive between growing seasons within insects. Some bacterial plant pathogens may survive within the insect body and over winter therein. The corn flea beetle, *Chaetocnema pulicaria* Melsh carries inside its body, the corn wilt pathogen, *Xanthomonas stewartii* and thus helps in its overwintering. The cucumber beetles, *Diabrotica vitata* Fabr. and *D. duodecimpunctata* Oliv., which chew the plant parts affected by *Erwinia*

*tracheiphila* carry the pathogen inside their body, where it overwinters. In the following seasons the insect passes on the bacterial pathogen to the host plant. The bacterial pathogen causing wilt of cucurbits is effectively transmitted by these insect vectors. It is reported that the bacterial pathogen and the insect vectors live in a symbiotic relationship, the insects helping the bacterium with protection from adverse weather conditions and the bacterium helping the insects with a supply of some digestive enzymes while it is inside the insect's body.

Plant viruses and phytoplasmas multiply within the vectors and can overwinter in those insects. Semi-persistent viruses are retained in the vectors for periods ranging from hours to days. Example, citrus tristeza virus is retained in the aphid, *Toxoptera citricida*. Persistent viruses retain the viruses from days to week. Most of the hopper-borne viruses multiply in their vectors. Viruses are retained through the moult and the vectors frequently remain viruliferous for life important vectors which retain the viruses are given below.

	Vector	Virus
Leafhopper	<i>Circulifer tenellus</i>	Beet curly top virus
Plant hopper	<i>Cicadulina mbila</i>	Maize streak virus
Green leaf hopper	<i>Nephotettix cincticeps</i>	Rice dwarf virus
Brown plant hopper	<i>Nilaparvata lugens</i>	Rice grass stunt virus
Hopper	<i>Agallia constricta</i>	Wound tumour virus

Transovarial transmission of the virus to the eggs of the vectors occurs, and the virus can multiply within a viruliferous hopper even if the insect is feeding on an immune host plant. Eggs carrying viruses may overwinter, and provide a source of virus to infect spring crops, even in the absence of diseased plants. Phytoplasmas attacking plants also multiply in the insects and remain infective throughout their life period. e.g. Rice dwarf virus (RDV) is transmitted through the eggs to about 60% of the progeny of the infective female leafhopper, *Nephotettix cincticeps*. RDV passes through the eggs to six succeeding generations. Other examples of transovarial transmission of viruses and phytoplasmas are given in table.

Table. Transovarial transmission of viruses and phytoplasmas.

Virus / Phytoplasma	Insect
<b>Virus</b>	
Rice stripe (40 generations) virus	Hopper - <i>Larodelphax striatellus</i>
Potato yellow dwarf virus	Hopper - <i>Agallia constricta</i>
(European) Wheat striate mosaic virus	Hopper - <i>Javesella pellucida</i>
Rice hoja blanca	Plant hopper - <i>Sogatodes oryzicola</i> & <i>S. cubanus</i>
<b>Phytoplasma</b>	
Aster yellows	<i>Macrosteles fascifrons</i>
Clover phyllody	<i>Euscelis plebejus</i> and <i>M. fascifrons</i>



Corn stunt	<i>Dalbulus elimatus</i> and <i>D.maidus</i>
Peach western X	<i>Colladonus montanus</i>

Clover club leaf is transmitted through 21 generations of the leafhopper vector, *Agalliopsis novella* over a span of five years.

#### **6. Survival on agricultural materials**

*C. michiganensis* subsp. *michiganensis* has been shown to survive in air-dried conditions for 7 to 8 months on the surface of wooden stakes and boxes or wires or for 15 months in air-dried tissues of diseased tomato plants.

#### **7. Survival on surface water**

*Erwinia carotovora* subsp. *carotovora* is detected from water from drains, ditches, streams, rivers and lakes in mountainous upland and arable areas of Scotland and Colorado throughout the year. .

# 3

## Dispersal of Plant Pathogens

Transport of spores or infectious bodies, acting as inoculum, from one host to another host at various distances resulting in the spread of disease, is called **dissemination, dispersal** or **transmission** of plant pathogens. It is very important for spread of plant diseases, for continuity of the life-cycle and evolution of the pathogen. The spores of some fungi are expelled forcibly from the sporophore or sporocarp by a squirting or puffing action that results in successive or simultaneous discharge of spores up to a centimetre or so above the sporophore. The seeds of some parasitic plants are also expelled forcibly and may arch over distances of several metres. These are dispersed mechanically by various means. In bacterial diseases, the bacterial cells come out on the host surface as ooze or the tissues may be disintegrated so that the bacterial mass is exposed and then dispersed by various physical and biological agencies. Viral diseases which have no such organs are transmitted by insects, mites, phanerogamic parasites nematodes and human beings.

The knowledge of these methods of dispersal is essential for effective control of plant diseases because possibilities of preventing dispersal and thereby breaking the infection chain exist.

The dispersal of infectious plant pathogens occurs through two ways,

1. Autonomous or direct or active dispersal
2. Indirect or passive dispersal

### I. Autonomous dispersal

It is also known as active or direct dispersal. In this method the dispersal of plant pathogens (fungi, bacteria, and viruses) takes place through soil and seed or planting materials during normal agronomic operations.

**1. Soil as means of autonomous dispersal:** Soil-borne facultative saprophytes or facultative parasites may survive through soil. The dispersal may be by movement of the pathogen in the soil or by its growth in soil or by movement of the soil containing the pathogen. The former is known as dispersal in soil while the latter is called dispersal by soil.

**a. Dispersal in soil :** The following are the three stages of dispersal in soil:

- i. Contamination of soil
- ii. Growth and spread of the pathogen in soil
- iii. Persistence of the pathogen

**i. Contamination of soil:** Contamination of the soil takes place by gradual spread of the pathogen from an infested area to a new area or by introduction of

contaminated soil, plant debris to a new area or by introduction of infected seed or planting materials.

**ii. Growth and spread of the pathogen in soil:** Once the pathogen has reached the soil it can grow and spread depending on the multiplication and spread. Multiplication and spread depends on the characters of the pathogen, presence of susceptible host and cultural practices. The adaptability of the pathogen to the soil environment includes saprophytic survival ability. The survival ability of the pathogen is governed by high growth rate, rapid spore germination, better enzymatic activity, capability to produce antibiotics and tolerance to antibiotics produced by other soil microorganisms. The active saprophytic survival of facultative saprophytes and facultative parasites in soil is affected by soil structure, moisture, organic matter, pH, antagonism etc.,. Specialized facultative parasites (or saprophytes) can pass their life in soil in the absence of the host plants, but they depend more on the residues of their host plant. e.g., *Armillariella mellea*, *Ophiobolus graminis*, *Phymatotrichum omnivorum* and *Fusarium*. The non-specialized facultative parasites can pass their entire life in the soil. e.g., *Pythium* sp., *Phytophthora* sp.,. The soil-borne obligate parasites such as *Plasmidiophora brassicae*, *Synchytrium endobioticum* requires the presence of active host.

**iii. Persistence of the pathogen :** The pathogens persist in the soil as dormant structures like oospores (*Pythium*, *Phytophthora* , *Sclerospora*, etc.) chlamydospores (*Fusarium*) or smut spores (*Ustilago*) or sclerotia (*Rhizoctonia*, *Sclerotium*, etc.)

**b. Dispersal by the soil:** The pathogen enters the soil, grow and spread in the soil. During the cultural operations in the field, soil is moved from one place to the nearby place through the agricultural implements and irrigation, worker's feet. Propagules of fungi or the dormant structures of fungi and the plant debris containing the fungal and bacterial pathogens thus spread throughout the field. This type of dispersal is highly erratic. The most important methods of dispersal of pathogen by the soil are transfer of soil from one place to another along with plant parts or propagating materials. e.g., transfer of papaya seedlings from a nursery infested with *Pythium aphanidermatum* (the cause of stem or foot rot of papaya) can introduce the pathogen in new pits for transplanting the seedlings. Similarly grafts of fruit trees transported with soil around their roots can transmit pathogens present in the nursery to the orchards. By this method, pathogens are not only spread from field to the field but also from district to district, State to State and often from country to country.

**2. Seed and seed materials as the source of autonomous dispersal:** The seeds serve as medium for autonomous dispersal of pathogens. Since most of the cultivated crops are raised from seed the transmission of diseases and transport of pathogens by seeds has much importance. The dormant structures of the pathogen (e.g., seeds of *Cuscuta* , sclerotia of ergot fungus, smut sori, etc., ) are found mixed with seed lots and they are dispersed as seed contaminant. The bacterial cells or spores of fungi present on the seed coat (such as in smuts of

barley, sorghum, etc.,) are transported to long distances. Dormant mycelium of many fungi present in the seed is transmitted to long distances.

There are three types of dispersal by seed viz., a. Contamination of the seed, b. Externally seed – borne, c. Internally seed- borne

- a. **Contamination of the seed:** Seed –borne pathogens move in seed lot as separate contaminants without being in intimate contact with the viable crop seeds. The seeds of the pathogen or parasite and the host are getting mixed during harvest of the crop. In many cases, the identity of the seeds of the two entities (host and the parasite) is difficult to separate. e.g., smut of pearl millet (*Tolyposporium penicillariae*), ergot of rye and pearl millet (*Claviceps purpurea* and *C. microcephala* respectively). Smut soil and ergots mix easily with the seed lots during harvest or threshing, In many smuts such as Karnal bunt of wheat (*Neovossia indica*) and bunt of rice (*Neovossia horrida*) the infected kernels containing smut sori are mixed with the seed. In leaf smut of rice (*Entyloma oryzae*) leaf pieces containing smut sori are mixed with the seed.
- b. **Externally seed-borne:** Close contact between structure of the pathogen and seeds is established in diseases like covered smut and loose smut of barley, short smut of sorghum, stinking smut of wheat and bacterial blight of cotton where the pathogen gets lodged in the form of dormant spores or bacteria on the seed coat during growth of the crop or at the time of harvest and threshing. In many pathogens the externally seed-borne structures such as smut spores can persist for many years due to their inherent capacity for long survival. The spores of *Tilletia caries* (stinking smut of wheat) remain viable even after 18 years and those of *Ustilago avenae* (oat smut) for 13 years.
- c. **Internally seed–borne:** The pathogen may penetrate into the ovary and cause infection of the embryo while it is developing. They become internally seed-borne. Internally seed borne pathogens like *Ustilago nuda tritici* are viable for many years.

Seeds of cultivated crops are distributed mainly by man. Sometimes animals and birds also help in distribution of crop seeds. Man and animals are the main agencies of dispersal of pathogen through seed. The pathogens thus mixed with the seed or on the seed are transmitted.

**Table.** Plant viruses transmitted through seeds and pollens

Sl. No.	Virus	Host species	Seed transmission (%)	Pollen transmission
1.	Abutilon mosaic	<i>Abutilon</i> spp.	14-24	-
2.	Alfalfa mosaic	<i>Medicago sativa</i>	10-50	+
3.	Arabis mosaic	<i>Chenopodium album</i>	80-100	-
		<i>Lycopersicon</i>	1.8	

4.	Barley stripe mosaic	<i>esculentum</i> <i>Hordeum vulgare</i>	15-40	+
5.	Bean common mosaic	<i>Phaseolus</i> <i>vulgaris</i>	1-93	+
6.	Bean yellow mosaic (Filamentous)	<i>Pisum sativum</i>	10-30	-
7.	Blackgram mottle	<i>Vigna mungo</i>	8	-
8.	Broad bean true mosaic	<i>Vicia faba</i>	15	-
9.	Cowpea mosaic (Common bean )	<i>Vigna unguiculata</i>	25-40	-
10.	Cowpea mosaic (Banding)	<i>V. unguiculata</i>	7-19	-
11.	Cowpea mild mottle	<i>V. unguiculata</i>	2-90	-
12.	Cowpea mosaic (chavali)	<i>V. unguiculata</i>	17-23	-
13.	Cucumber green mottle mosaic. (Transmitted on testa)	<i>Cucumis sativus</i>	1-8	-
14.	Cucumber mosaic	<i>Stellaria media</i> (in many more hosts with varying per cent )	21-40	-
15.	Cherry leaf roll	<i>Nicotiana rustica</i>	<100	+
16.	Elm mosaic	<i>Ulmus americana</i>	1-3	+
17.	Lettuce mosaic	<i>Lactuca sativa</i>	3-10	+
18.	Lychnis ringspot	<i>Lychnis divaricata</i>	58	+
19.	Mungbean & Urdbean mosaic.	<i>Vigna radiata</i>	20	-
20.	Pea early browning	<i>P. sativum</i>	37	-
21.	Pea enation mosaic	<i>P. sativum</i>	1.5	-
22.	Pea seed- borne mosaic	<i>P. sativum</i>	0-100	-
23.	Peanut (groundnut) Indian clump	<i>Arachis hypogaea</i>	12	-
24.	Peanut mottle	<i>A. hypogaea</i>	9	-
25.	Peanut stunt	<i>A. hypogaea</i>	0.1	-
26.	Prunus dwarf	<i>Prunus cerasus</i>	3-30	+
27.	Prunus necrotic ringspot	<i>Prunus</i> sp.	<70	+

28.	Raspberry ringspot		<i>Fragaria</i> spp.	50	+
29.	Southern bean mosaic		<i>V. unguiculata</i>	3-7	+
30.	Soybean mild mosaic		<i>Glycine max</i>	22-70	-
31.	Soybean mosaic		<i>G.max</i>	30	+
32.	Soybean stunt		<i>G.max</i>	39	-
33.	Squash mosaic		<i>Cucumis melo</i>	6-20	-
34.	Tobacco mosaic (Transmitted on testa)		<i>Lycopersicon esculentum</i>	2-94	-
35.	Tobacco ringspot		<i>G. max</i>	78-82	+
36.	Tobacco blackring		<i>Rubus</i> spp.	5-19	+
37.	Urdbean leaf crinkle		<i>Vigna mungo</i>	18	-
38.	Vicia cryptic		<i>Vicia faba</i>	Varying	+
39.	White clover mosaic		<i>Trifolium repens</i>	6	-

## II. Passive dispersal

Passive dispersal of plant pathogens happens through

1. Animate agents
  - a. Insects
  - b. Mites
  - c. Fungi
  - d. Nematodes
  - e. Human beings
  - f. Farm and wild animals
  - g. Birds
  - h. Phanerogamic parasites
2. Inanimate agents
  - a. Wind
  - b. Water

### 1. Animate Agents

a. **Insects:** Insects carry plant pathogens either externally or internally. Gaiiman (1950) used the terms **epizoic** and **endozoic** respectively for these two types of transmission. The external transmission of plant pathogens is of special interest in those fungi, which produce their conidia, oidia and spermatia in sweet or honey secretions having attractive odours. Some of the well known diseases of this type are the ergot, the *Sclerotinia* brown rot of pear and apple, the honey dew stage in the 'sugary disease' of sorghum and pearl millet in parts of India

and the pycnial nectar in the cluster cup stage of rusts. The spermatial oozing at the mouth of spermatogonia in the Ascomycetes attract various types of insects, flies, pollinating bees and wasps which play a dual role viz., pollination and transmission of pathogens. The fire blight organism (*Erwinia amylovora*) pathogens and citrus canker bacterium, (*Xanthomonas axonopodis* pv. *citri*) are also carried in this manner, the former by flies (bees) and ants and the latter by the leaf miner. The black leg of potato caused by *Erwinia carotovora* is disseminated by maggots, wilt of corn caused by *X.stewartii*, gummosis of sugarcane caused by *X.vasculorum* are the other examples for bacterial diseases transmitted by insects.

Ingenious transmission of pathogens, of an internal nature (endozoic) is provided by the Dutch elm disease (*Ceratostomella ulmi*) and the olive canker (*Bacillus savastanoi*). The former is transmitted by the elm bark beetles and the latter by the olive fly (*Olea europaea*). These insects, unlike the epizootic group, appear to have a close biologic relationship with the pathogens, as they have not been reared without the contaminating pathogens.

Few important plant pathogenic bacteria are spread by insects. The cucumber wilt bacterium, *Erwinia tracheiphila* is spread by the striped cucumber beetles (*Acalymma vitata*) and the spotted cucumber beetle (*Diabrotica undecimpunctata*). When the beetles are feeding on the diseased plant, the bacterium contaminates the mouth parts and passes into the gut of the insect. During the winter season, the bacterium overwinters inside the beetle. Thus the beetle helps the bacteria in two ways, i.e in their transmission and survival.

More than 80 per cent of the viral and phytoplasmal diseases are spread by different types of insects. The insect which act as specific carriers in disseminating the diseases are called insect vector.

Both aphids (Aphidae) and leaf hoppers (Cicadellidae or Jassidae) in the order Homoptera contain largest number and the most important insect vectors of plant viruses. Certain species of mealy bugs and scale insects (Coccoidae), whiteflies (Aleurodidae) and treehoppers (Membracidae) in the same order (Homoptera) also transmit virus diseases. Insect vectors of plant viruses are few in true bugs (Hemiptera), thrips (Thysanoptera), beetles (Coleoptera) and grasshoppers (Orthoptera). Aphids, leafhoppers and other groups of Homoptera and true bugs have piercing and sucking mouth parts. Thrips have rasping and sucking mouth parts. All other groups of insect vectors have chewing mouth parts and they transmit only very few viruses.

**Aphids:** Aphids are the most important insect vectors of plant viruses and transmit the great majority of all stylet - borne viruses. As a rule several aphid species can transmit the same stylet - borne virus and the same aphid species can transmit several viruses, but in many cases the vector-virus relationship is quite specific. Aphids generally acquire the stylet-borne virus after feeding on a diseased plant for only a few seconds (30 seconds or less) and can transmit the virus after transfer to and feeding on a healthy plant for a similarly short time of a few seconds. The length of time aphids remain viruliferous after acquisition of

a stylet-borne virus varies from a few minutes to several hours, after which they can no longer transmit the virus. In few cases of aphid transmission of circulative viruses, aphids cannot transmit the virus immediately but must wait several hours after the acquisition feeding, but once they start to transmit the virus, they continue to do so for many days following the removal of the insects from the virus source. In aphid transmitting stylet-borne viruses, the virus seems to be borne on the tips of the stylets, it is easily lost through the scouring that occurs during probing of host cells, and it does not persist through the moult or egg. The examples of aphid transmitted plant viruses are given in the following tables:

**Table :** Aphid - transmitted viruses

Sl. No.	Virus	Vector	Type of transmission
1.	Bean common mosaic	<i>Acyrtosiphon pisum</i> *	Non – persistent
2.	Bean yellow mosaic	<i>A.pisum</i> *	Non – persistent
3.	Beet mosaic	<i>Myzus persicae</i> *	Non – persistent
4.	Citrus tristeza	<i>Toxoptera citricida</i> *	Non – persistent +
5.	Cucumber mosaic	Various species	Non – persistent
6.	Lettuce mosaic	<i>M.persicae</i> *	Non – persistent
7.	Potato virus Y	<i>M. persicae</i> *	Non – persistent
8.	Soybean mosaic	<i>A. pisum</i> *	Non – persistent
9.	Sugarcane mosaic	<i>Dactynotus ambrosiae</i> *	Non – Persistent
10.	Turnip mosaic	<i>M.persicae</i> *	Non – persistent
11.	Barley yellow dwarf	<i>A.dirhodum</i> *	Persistent
12.	Lettuce necrotic yellows	<i>Hyperomyzes lactucae</i>	Persistent
13.	Pea enation mosaic	<i>A.pisum</i> *	Persistent
14.	Chickpea stunt	<i>Aphis craccivora</i>	-
15.	Beet yellows	<i>M. persicae</i> *	Semi – persistent

\* one of several important aphid vectors of this virus.

+ may also be circulative.

Table: Aphid - transmitted viruses that are dependent on a second (helper) virus for transmission

Sl. No.	Virus	Helper virus	Vector	Type of transmission
1.	Potato aucuba mosaic	Potato virus A or Y	<i>Myzus persicae</i>	Non – persistent
2.	Potato virus C	Potato virus Y	<i>M. persicae</i>	Non – persistent



3.	Tobacco etch (NAT strain)	Potato virus Y	<i>M. persicae</i>	Non – persistent
4.	Barley yellow dwarf (MAV strain)	Barley yellow dwarf (RPV strain)	<i>Rhopalosiphum padi</i>	Persistent
5.	Carrot mottle	Carrot red – leaf	<i>C. aegopodii</i>	Persistent
6.	Tobacco mottle	Tobacco vein – distorting	<i>M. persicae</i>	Persistent

**Leafhoppers:** Leafhoppers are phloem feeders and acquire the virus from the phloem region. All leafhopper, transmitted viruses are circulatory. Several of these viruses multiply in the vector (propagative) and some persist through the moult and are transmitted through the egg stage of the vector. Most leafhopper vectors require a feeding period of one to several days before they become viruliferous, but once, they have acquired the virus they may remain viruliferous for the rest of their lives. Usually there is an incubation period of 1 to 2 weeks between the time a leafhopper acquires a virus and the time it can transmit it for the first time.

Table. Leaf, plant and tree-hopper transmitted viruses.

Sl. No	Virus	Vector	Type of transmission
<b>a. Leaf hopper</b>			
1.	Beet curly top	<i>Circulifer tenellus</i>	Persistent
2.	Maize chlorotic dwarf	<i>Graminella nigrifrons</i>	Semi– persistent
3.	Oat blue dwarf	<i>Macrostes fascifrons</i>	Persistent
4.	Potato yellow dwarf	<i>Agallia constricta</i> , <i>Aceratagallia sanguinolenta</i>	Persistent
5.	Rice dwarf	<i>Nephotettix cincticeps</i> , <i>N.dorsalis</i> , <i>N.nigropictus</i>	Persistent
6.	Rice tungro	<i>Nephotettix impicticeps</i> , <i>N.malayanus</i> , <i>N.nigropictus</i> , <i>N.parvus</i> , <i>N.virescens</i> , <i>Recilia dorsalis</i>	Semi – persistent
7.	Rice ragged stunt	<i>Nilaparvata lugens</i>	?
8.	Rice grassy stunt	<i>Nilaparvata lugens</i>	?

9.	a. Maize streak	<i>Cicadulina</i> spp.	?
	b. Ragi mottle streak	<i>Cicadulina bipunctata</i> , <i>C.cinai</i>	
	<b>b. Plant hopper</b>		
10.	Wound tumour	<i>A.constricta</i>	Persistent
11.	Maize mosaic	<i>Peregrinus maidis</i>	Persistent
12.	Rice hoja blanca	<i>Sogatodes oryzicola</i>	?
13.	Sugarcane Fiji	<i>Perkinsiella saccharida</i>	?
14.	Maize rough dwarf	<i>Delphacodes propinglla</i>	?
15.	Maize rough dwarf	<i>Laodelphax strialellus</i>	Persistent
	<b>c. Tree hopper</b>		
16.	Tomato pseudo-curly top	<i>Micrutalis malleifera</i>	Persistent

The insect vectors of other groups and the diseases transmitted by them are given below.

#### White flies

<i>Trialeurodes vaporariorum</i>	-	Beet pseudo - yellows
<i>Bemisia goldingi</i>	-	Cotton leaf curl
<i>Bemisia gossypiperda</i>	-	Cotton leaf curl
<i>Bemisia tabaci</i>	-	Abutilon mosaic, Bhendi enation, Bhendi yellow vein mosaic, Bhendi leaf curl, Cassava mosaic, Chilli leaf curl, Cotton leaf curl, Cowpea mild mottle, Jasmine chlorotic ringspot, Mungbean yellow mosaic, Papaya leaf curl, Soybean leaf curl, Sweetpotato mild mottle, Tobacco leaf curl and <i>Zinnia</i> leaf curl.

#### Thrips

<i>Frankliniella schultzei</i>	-	Tomato spotted wilt virus
<i>Scirtothrips dorsalis</i>	-	"
<i>Thrips tabaci</i>	-	"

#### Mealy bugs

<i>Planococcoides njalensis</i>	-	Cocoa swollen shoot
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<i>Pseudococcus saccharifolii</i>	-	Sugarcane spike (Phytoplasma)
<b>Grasshoppers</b>		
<i>Melanoplus differentialis</i> (Mechanical transmission)	-	Potato virus X, Tobacco mosaic virus
<b>Earwigs</b>		
<i>Foficula auricularia</i>	-	Turnip yellow mosaic
<b>True bugs</b>		
<i>Lygus protensis</i>	-	Spinach blight
<b>Lace bugs</b>		
<i>Piesma quardratum</i>	-	Beet leaf curl virus
<i>Stephanites typicus</i>	-	Root (wilt) disease of coconut (Phytoplasma)
<b>Lygaeid bugs</b>		
<i>Nysius spp</i>	-	<i>Centrosema</i> mosaic
<b>Leaf miner flies</b>		
<i>Liriomyza langei</i>	-	Sowbane mosaic virus (Mechanical transmission)
<b>Beetles</b>		

Beetle	Virus	Nature of transmission
<i>Acalymma thiemei</i>	Squash mosaic	-
<i>Acalymma trivitata</i>	Squash mosaic	-
<i>Ceratoma trifurcata</i>	Cowpea mosaic	Persistent
<i>Diabrotica balteata</i>	Squash mosaic	-
<i>D. howardi</i>	Squash mosaic	-
<i>D.longicornis</i>	Brome mosaic	-
<i>D. undecimpunctata</i>	Radish mosaic	-
	Squash mosaic	Persistent
<i>Epitrix hirtipennis</i>	Radish mosaic	-
<i>Ootheca mutabilis</i>	Cowpea mosaic	-
<i>Phyllotreta sp.</i>	Turnip yellow	Persistent
<i>Podagra sp.</i>	Bhendi mosaic	-
<i>Psylloides sp.</i>	Turnip yellow mosaic	-
<b>Lady bird beetle</b>		
<i>Epilachna sp.</i>	-	Cowpea mosaic
<b>Weevils (Snout beetles)</b>		
<i>Apion spp.</i>	-	Broad bean stain
<b>Sitonia lineata</b>	-	"
<b>Blister beetles</b>		
<i>Epicanta vitata</i>	-	Bean pod mottle

**b. Mites:** Mites belonging to the class Arachnida and the order Acarina have puncturing and sucking mouth parts. Mites belonging to the families Eryophiidae (eryophiid mite) and Tetranychidae (spider mite) transmit plant viruses. The genera *Abacarus*, *Aceria* and *Eriophyes* in Eryophiidae and the genus *Brevipalpus* in Tetranychidae are important. The list of plant virus diseases transmitted by mites is given in the following table.

Table. Plant viruses transmitted by mites.

**Viruses transmitted by mites**

<i>Abacarus hystrix</i>	-	Ryegrass mosaic
<i>Aceria cajani</i>	-	Pigeonpea sterility mosaic
<i>A.fici</i>	-	Wheat spot mosaic
<i>A. tulipae</i>	-	Wheat streak mosaic
<i>Eriophyes insidiosus</i>	-	Peach mosaic
<i>Brevipalpus obovatus</i>	-	Citrus leprosis virus
<i>B. phoenicis</i>	-	Coffee ringspot virus

**C. Fungi :** Some soil - borne fungal plant pathogens transmit plant viruses. *Olpidium brassicae*, *Polyomyxa graminis*, *P. betae* and *Spongospora subterranea* are the fungi involved in transmission of virus disease. The viruses are apparently borne in or on the resting spores and the zoospores, which upon infection of new host plants introduce the virus and cause symptoms characteristic of the virus they transmit. All these fungi are pathogens of the host which carry of viruses. The zoospores of the fungi are released from the host and the zoospores carry the virus and transmit it to the susceptible hosts during their infection process. In some cases plant viruses are carried on the outside of the fungi. Examples are tobacco necrosis virus and cucumber mosaic virus. The viruses like lettuce big vein virus are found inside the zoospores. They persist for years in viable resting sporangia. The types of transmission by fungi can be considered as non-persistent and persistent transmission. The list of fungi and the virus diseases transmitted by them are given in the following table.

Table. Viruses transmitted by fungi

Fungal vector	Disease	Particle shape
1. <i>Olpidium brassicae</i>		
	Lettuce big vein	-
	Tobacco necrosis	Isometric
	Tobacco stunt	-
	Tobacco necrosis satellite	Isometric
2. <i>Olpidium cucurbitacearum</i> (= <i>O. radicale</i> )		
	Cucumber necrosis	Isometric
3. <i>Polyomyxa graminis</i>		

	Barley yellow mosaic	Filamentous rod
	Oat yellow mosaic	Filamentous rod
	Wheat soil-borne mosaic	Straight rod
	Wheat spindle - streak mosaic	Filamentous rod
	Peanut clump	-
<hr/>		
4. <i>Ploymyxa betae</i>		
	Beet necrotic yellow vein	Straight rod
<hr/>		
5. <i>Spongospora subterranea</i>		
	Potato mop top	Straight rod
<hr/>		
6. <i>Synchytrium endobioticum</i>		
	Potato virus	-
<hr/>		

**d. Nematodes:** Nematodes are soil borne organisms. Some of the nematodes act as agents for dissemination of pathogenic fungi, bacteria and viruses. For example, the bacterium *Corynebacterium tritici* which causes yellow ear rot of wheat is disseminated by ear cockle nematode. Similarly, some pathogenic fungi such as, *Phytophthora*, *Fusarium*, *Rhizoctonia*, etc., are carried on the body of nematodes. Nematodes help these pathogenic fungi to enter into the host through punctures for their own entry and enter into hosts along with the nematodes. Plant nematodes play a vital role as vector in transmitting certain virus diseases. Nematode vectors transmit viruses by feeding on roots of infected plants and then moving on roots of healthy plants. Larvae as well as adult nematodes can acquire and transmit viruses, but the virus is not carried through the larval molts or through the eggs. After moulting, the larvae or the resulting adults must feed on a virus source before they can transmit again. Both the polyhedral and tubular type of viruses are transmitted by *Xiphinema*, *Longidorus* and *Trichodorus*. The important viral diseases transmitted by nematodes are given in table.

Table. Nematode transmitted virus diseases

Virus group	Virus	Vector	Particle shape
<b>Tobra</b> virus (Tobacco rattle group virus)	Pea early browning	<i>Paratrichodorus</i> sp.	Tubular
		<i>Trichodorus</i> spp.	
	<i>Tabacco rattle</i>	<i>Paratrichodorus</i> sp.	Tubular
		<i>Trichodorus</i> spp.	
<b>NEPO</b>  Virus (Nematode transmitted polyhedral virus)	Arabis mosaic	<i>Xiphinema</i> <i>diversicaudatum</i>	Isometric
	Artichoke	<i>Longidorus apulus</i>	Isometric
	Italian latent		
	Grapevine chrome mosaic	<i>X. index</i>	Isometric
	Grapevine fan leaf	<i>X. index</i> , <i>X. italiae</i>	Isometric
	Mulberry	<i>L. martinii</i>	Isometric

ringspot			
Peach rosette	<i>X. americanum</i>	Isometric	
mosaic			
Strawberry	<i>X. diversicaudatum</i>	Isometric	
latent ringspot			
Tobacco	<i>X. americanum</i>	Isometric	
ringspot			
Tomato	<i>L. attenuatus</i> ,	Isometric	
blackring	<i>L. elongatus</i>	Isometric	Isometric
Tomato ring	<i>X. americanum</i>		
spot			

Nematode-borne viruses are retained in the vector in the lining of guide - sheath of the odontostyle in *Longidorus* or in the lumen of the odontophore and the oesophagus in *Xiphinema* or in the entire pharynx and oesophagus in *Trichodorus*. Nematodes have life cycles of about two years. Viruses are retained in the nematodes for long periods.

**e. Human beings:** Man is the most important factor responsible for short distance and long distance dispersal of plant pathogens. He helps in dissemination unknowingly by his usual agricultural practices. Human being's role in dissemination of plant pathogens is more direct than indirect. The ways and means by which human beings help in dispersal are as follows.

**i. Transportation of seeds (Seed trade):** Seed trade is one of the different means of dispersal of plant pathogens in which man plays an important role. The import and export of contaminated seeds without proper precautions lead to movement of pathogens from one country to another or from one continent to another. Through this way pathogens of soybean and sugarbeet hither to not prevalent in India got introduced. Human agencies of individual, official and unofficial have transported new plants and plant products, the seed, the tubers, the propagating stock and fruits, which carried the plant pathogens, many times in a latent condition and which ultimately lead to the outbreaks of new diseases in places, hither to free from them. The diseases which are amenable to such transmission are mainly those that are carried in or on the propagative parts and seed such as late blight of potato, the downy mildew of grapevine, citrus canker, chestnut blight, Dutch elm disease, *Fusarium* wilt of banana, Katte disease of cardamom and bunchy top of banana. A few such diseases together with their places of origin and years and introduction are given in table.

Table: Introduction of plant diseases in new areas

Disease	Original home	Introduced country	Year of introduction
Citrus canker	Asia	U.S.A.	1907
Fire blight of Apple	U.S.A.	New Zealand	1919
Powdery mildew of Grape	U.S.A.	Europe	1845

Downy mildew of grape	U.S.A.	France	1878
Blister rust of pine	Russia	U.S.A.	1909
Scab of apples	Sweden	U.S.A.	1834
Late blight of potato	South America	U.S.A.	1830
“	U.S.A	Europe	1845
“	Europe	India	1870
Wart of potato	Europe	U.S.A.	1918
Dutch elm Disease	Holland	U.S.A.	1928-30
Gooseberry mildew	U.S.A.	Europe	1890
Chestnut blight	Mediterranean region	U.S.A.	1904
Rice blast	South-East Asia	Madras	1918
Panama disease of banana	Panama canals	Bombay	1920
Bunchy top of banana	Sri Lanka	South India	-

Many of these diseases, not very destructive in their homelands, have brought in ruin and devastation. The sale of seeds for crops badly affected by a seed-borne pathogen is a common method of dispersal of destructive pathogens e.g. loose smut of wheat (*Ustilago nuda tritici*), grain smut of sorghum (*Sporisorium sorghi*), ergot of pearl millet (*Claviceps fusiformis*) and Karnal bunt of wheat (*Neovossia indica*).

**ii. Planting diseased seed materials (vegetatively propagated materials):**

Planting diseased bulbs, bulbils, corns, tubers, rhizomes, cutting etc. of vegetatively propagated plants such as potato, sweet potato, cassava, sugarcane, banana, many ornamentals and fruit trees etc. help in dispersal of pathogens from field to field, orchard to orchard, locality to locality or from one country to another.

**iii. By adopting farming practices:** Human beings (men and women) engaged in preparatory cultivation, planting, irrigation, weeding, pruning etc. help in dispersal of plant pathogens. The fungal spores (oospores, chlamydospores), dormant structures like sclerotia are carried by worker's clothing, shoes, hand etc. from field to field. Men or women engaged in intercultivation in tobacco field spread the dreaded tobacco.

**iv. Through clothing:** Palm workers engaged in cleaning coconut trees spread bud rot disease.

**v. By use of contaminated implements:** Pathogens are transferred from one area to another through implements used in various cultural operations (weeding, hoeing thinning etc.) in the field. e.g. root rot of pulses and cotton (*Macrophomina phaseolina*, bacterial angular leaf spot of cucumber (*Pseudomonas lachrymans*) and bacterial canker of tomato (*Corynebacterium*

*michiganensis*). Cutting knives and pruning knives help in dissemination from one plant to another e.g., Bunchy top of banana.

**vi. By use of diseased grafting and budding materials:** Grafting and budding between healthy and diseased plants is the most effective method of distribution of pathogens of horticultural crops (fruit trees, ornamentals etc.) e.g., Careless selection of stocks and scions in propagation of citrus trees.

**f. Farm and wild animals:** Farm animals (cattles) while feeding on diseased fodder ingest the viable fungal propagules (spores or oospores or sclerotia) into their digestive system. Animals which feed on downy mildew affected pearl millet or sorghum take the oospores along with the fodder. Oospores pass out as such in the dung. This dung when used as manure spread in the field and act as source of inoculum. Smut fungi like grain smut of sorghum, loose smut and head smut of sorghum are carried from field to field through the alimentary canals of farm animals. Soil inhabiting fungi especially sclerotia adhere to the hoofs and legs of animals and get transported to other places. Animals passing through the tobacco fields help in transmission of TMV.

**g. Birds:** In general, transmission by birds is of minor importance. But this method is important in dissemination of seeds of flowering parasites and certain fungi. Many migratory birds, such as mistle thrush (*Turdus viscivorus*) in the temperate region and the crows (*Corvus brachyrhynchos*) in the tropics, take active part in the transmission of giant mistletoe (*Dendrophthoe* spp.) either through external contamination of their beaks and feathers or internally through the alimentary canals. These birds feeding on the fleshy, sticky and gelatinous berries of giant mistletoe deposit the seeds on the other trees with the excreta. Stem segments of dodder (*Cuscuta* spp.) are carried by birds for building their nests. Thus the phanerogamic parasites are getting transported to new locations. Spores of chestnut blight fungus, *Endothea parasitica* are disseminated by not less than 18 species of birds. Internal transmission of this pathogen is carried out by the birds which visit such diseased plants and get contaminated by the spores. Birds are also known to carry the spores of fungi on their body.

**h. Phanerogamic parasites:** Plant viruses are transmitted from one plant to another through the bridge formed between the two plants by the twining stems of the parasitic plant dodder (*Cuscuta* spp). Dodder is yellow vine without green leaves. In this way viruses are transmitted between plants belonging to families widely separated taxonomically. The virus is transmitted in the food stream of the dodder plant, being acquired from the vascular bundles of the infected plant by the haustoria of dodder. After translocation through the dodder phloem the virus is introduced in the next plant by the new dodder haustoria produced in contact with the vascular bundles of the inoculated plant. *Cuscuta californica*, *C. campestris*, *C. subinclusa* are usually employed for dodder transmission of viruses and phytoplasmas. *C. europaea*, *C. epilinum* and *C. lupuliformis* are also employed in transmission of viruses.

## **2. Inanimate agents**



**a. Wind:** The wind dispersal of plant pathogens is known as **anemochory**. It is one of the most common methods of the dispersal of plant pathogens. It is the most dangerous and potent mode of travel for plant pathogenic fungi. It acts as potent carrier of propagules of fungi, bacteria and viruses. Usually the fungal pathogens are light in weight and are well adapted to wind dispersal. Some pathogenic bacteria are carried along with the infected material to short distances by wind. Damping-off pathogen (*Pythium* spp.), wart disease pathogen of potato (*Synchytrium endobioticum*), root rot pathogens (*Sclerotium* and *Rhizoctonia*) and seeds of phanerogamic parasites witchweed (*Striga*) are efficiently carried by wind. Viruses and phytoplasmas are not directly transmitted by wind, but the insect and mite vectors that carry the viruses move to different directions and distances depending upon the direction and speed of air. The adaptations for wind dispersal in fungal pathogens include, production of numerous spores and conidia, discharge of spores with sufficient force, production of very small and light spores so that they can move to long distances. The duration and periodicity of sporulation and discharge are also important factors for wind dispersal. Some fungal pathogens causing powdery mildews, downy mildews, rusts, smuts, sooty moulds, leaf spots, blast, apple scab etc., produce large number of very light spores and conidia on the surface of the host. Uredial stages of the rust fungi travel long distances through air currents and are thus responsible for destructive epidemics over wide areas.

Wind transmission involves the upward air currents, velocity and the downward movements of wind. All are equally responsible for the spread of infection and ultimate outbreak of diseases and have been of special significance in the rust, smut and blast fungi. Urediospores of rust fungi have been carried to long distances, both cross-wise and upwards. Christensen (1942) and Stakman (1946) determined by exposure of vaselin slides in the upper air through aeroplane flights, that urediospores and aeciospores of *Puccinia graminis tritici* could be gathered in a viable condition up to a distance of 4,200 m, above infected fields, *Alternaria* sp. at 2,400 m. and those of *Puccinia triticina* at 3,750 m. The transmission of aecial spores of *Puccinia graminis tritici* from several groups of barberry bushes to the wheat crop showed that these spores traveled successfully over a radius of 3 kms round about these bushes. The blister rust fungus, *Cronartium ribicola*, is known to travel to a distance of 500 metres or 3,750 m. inside a plantation that the range is probably more in the open. Similar observations have been made in respect of dissemination of chlamydospores of the smut fungi.

In long distance dissemination with intervening stages of infection, the retention of viability of spores is an important factor that determines the extent and severity of epidemics, over wide areas. The outbreaks of cereal rusts and blast of rice are examples of such dissemination. Spores differ widely in their ability to survive long distance travel through air. Urediospores of rusts, chlamydospores of smut fungi and conidia of *Alternaria*, *Helminthosporium*, *Pyricularia* and others are well adapted for long distance travel in a viable condition and are known to play a vital role in epidemiology. The conidia of downy mildews, powdery mildews and the aeciospores and basidiospores of the rust fungi are unable to withstand such long distance dissemination when they

are exposed to desiccation and direct sunshine and thus are only capable of producing local epiphytotics of limited magnitude.

The bacteria causing fire blight of apple and pear (*Erwinia amylovora*) produce fine strands of dried bacterial exudate containing bacteria and these strands may be broken off and they are transmitted by wind. Bacteria and nematodes present in the soil may be shown away along with soil particles. Wind also helps in the dissemination of bacteria, fungal spores and nematodes by blowing away rain splash droplets containing these pathogens. Wind carries insects and mites that may contain or are smeared with viruses, bacteria or fungal spores to short or long distances. Wind also causes adjacent plants or plant parts to rub against each other. The wound created in this manner help the spread by contact of bacteria (citrus canker), fungi, some viruses (Tobacco mosaic virus) and viroids and possibly of some nematodes.

**b. Water:** Transmission of plant pathogens by water (**hydrochory** as called by Gaiimann, 1950) is not as significant as wind transmission. Although water is less important than air in long-distance transport of pathogens, water dissemination of pathogens is more efficient, in that the pathogens land on an already wet surface and can move or germinate immediately. In case of some diseases the surface flow of water after heavy showers of rains or irrigation water from canals and wells carries the pathogens to short distances. Soil-inhabiting fungi like, *Fusarium*, *Ganoderma*, *Macrophomina*, *Phytophthora*, *Plasmodiophora*, *Pythium*, *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Sporisorium*, *Ustilago*, *Verticillium* etc., in the form of mycelial fragments, spores or sclerotia, soil-borne bacteria and nematodes carrying viruses are transmitted through the above process. They are transmitted through rain or irrigation water that moves on the surface or through the soil.

All bacteria and the spores of many fungi are exuded in a sticky liquid and depend for their dissemination on rain or (overhead) irrigation water which either washes them downward or splashes them in all directions.

Raindrops or drops from overhead irrigation pickup the fungal spores (uredospores of *Homilies*, *Puccinia* and *Uromyces* and bacteria (bacterial blight pathogen of rice, *Xanthomonas oryzae* pv. *oryzae*; bacterial leaf streak pathogen, *X.oryzae* pv. *translucens*; citrus canker pathogen, *X.axonopodis* pv. *citri*; tomato bacterial blight pathogen, *Corynebacterium michiganese* and cotton bacterial blight pathogen, *X.axonopodis* pv. *malvacearum* present in the air and wash them downward where some of them may land on susceptible plants.

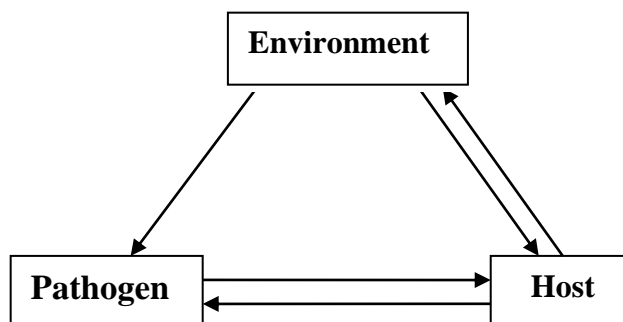
# 4

## Epidemiology

Epidemiology or epiphytology is the study of the outbreak of disease, its course, intensity, cause and effects and the various factors governing it. Disease may affect isolated individuals within a crop and is called **sporadic**. On the other hand, if the disease assumes destructive nature over widespread areas, involving sudden outbreaks it is known as **epidemic** or **epiphytotic**. When a host and the pathogen reach a state of biologic equilibrium and assumes apparently harmless character, it is termed endemic. Citrus canker is endemic in Asia but epidemic in the introduced place, Florida (U.S.A). The downy mildew of corn is a endemic disease in India but became epidemic in the Philippines. When an epidemic disease spreads over continents or subcontinents and involves mass mortality it is considered as **pandemic**. The outbreak of black stem rust of wheat in India during 1947 is best example for a pandemic disease.

An epidemic may cause widespread and mass destruction of crop in a short time or may persist for long periods depending upon the three following factors responsible for the disease:

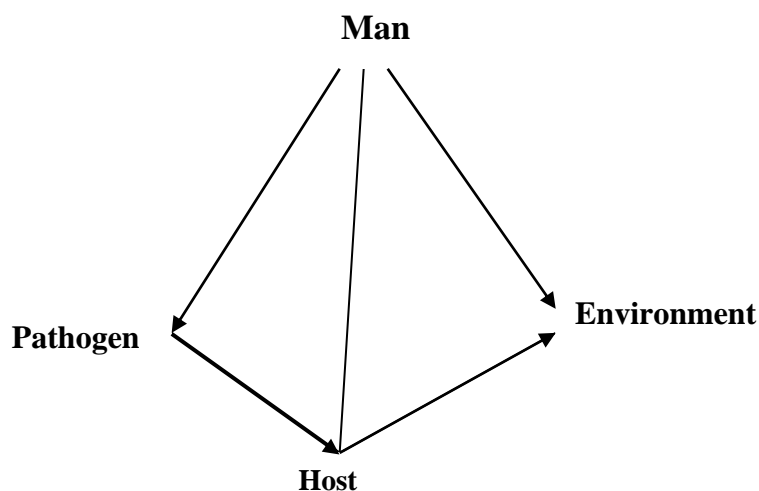
1. Host
2. Pathogen and
3. Environment



A course of epidemic in nature differs with the nature of the host, the pathogen and the environment. In arecanut the Koleroga fungus, *Phytophthora arecae* become destructive during monsoon period (July- Sep) and wanes away with rising temperatures and dry conditions. The above disease once again become destructive during rainy season. This type of epidemic is known as **seasonal epidemic or annual epidemic**. Outbreak of *Phytophthora* wilt of betelvine occurs during rainy season in South India. In temperate zone peach leaf curl and apple scab follow the similar course.

Epidemics caused as a result of introduction of new pathogens in the locality hither to free from them, appear in two phases viz., destructive phase and innocent phase (due to biologic equilibrium reached between new comer pathogen and the original inhabitant). The well known epidemics of late blight of potato in Europe and blast disease of rice in South East Asia, powdery mildew

and downy mildew of grapevine in Europe, leaf rust of coffee in Sri Lanka and anthracnose of grapevine in India are examples of this category. In the above diseases the pathogens after taking heavy toll of the crops have settled down.



#### **Factors governing epidemic or essential conditions for an epidemic**

A disease is sometimes sporadic and assumes epidemic proportions under special circumstances. The essential conditions for an epiphytotic or the factors governing epidemics can be grouped under the three heads.

1. Nature of host
2. Nature of the pathogen and
3. Environment

An epidemic can only result from the cumulative effects of all the three factors mentioned above, acting simultaneously. Few pathogens are capable of assuming epiphytotic conditions while others are sporadic. The former group consists of late blight of potato, blast of rice, downy mildew diseases and rust diseases.

<b>Host</b>	<b>Pathogen</b>	<b>Environment</b>
Susceptibility of the host	Introduction of a new pathogen	Temperature
Aggregation and distribution of susceptible hosts	Presence of aggressive strain of the pathogen	Moisture and humidity
Introduction of new hosts	High birth rate of the pathogen	Rainfall
	Low death rate of the	Light and shade
		Wind

Introduction of new collateral or alternate host	pathogen  Easy and rapid dispersal of the pathogen  Adaptability of the pathogen	
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## A. Host Factors

### 1. Susceptibility of the host

Plants have ability to combat disease which manifests itself as susceptibility or resistance. Plants are predisposed to the attack depending on their nature, environment and stage of growth. Presence of susceptible varieties in an area may act as one of the causes of epidemic. For example, late maturing varieties of groundnut are more susceptible to early leaf spot ( *Cercospora arachidicola* ) and late leaf spot ( *Phaeoisariopsis* ) than the early maturing varieties. Similarly late maturing varieties of wheat are susceptible to loose smut ( *Ustilago nuda tritici* ) than the early maturing varieties. Early sown sugarcane varieties of sugarcane are more susceptible to leaf rust in Deccan canals in Bombay area than the late sown varieties. Wheat plant becomes susceptible to black rust ( *Puccinia graminis tritici* ) at the boot stage but is resistant when young. Susceptibility of rice plants to blast disease ( *Pyricularia oryzae* ) increases with application of heavy doses of nitrogenous fertilizers. Cottons plants are susceptible to *Fusarium* wilt ( *F.oxysporum f.sp. vasinfectum* ) at soil temperatures of 26 to 28°C, brinjal to *Verticillium* wilt *Verticillium dahliae* at 20°C. But crop plants are resistant to these soil-borne diseases at relatively lower or higher temperatures. Under the above conditions, the pathogen multiplies faster, cause infection and effectively uses its propagules for quick secondary spread causing epidemic.

### 2. Aggregation and distribution of susceptible hosts

Abundance of susceptible hosts in an area is one of the major causes of the spread of epidemics. Continuous cultivation of susceptible variety or varieties in an area, that too in a large contiguous area help in the build up of inoculum and improve the chances of epidemics. Under the above conditions the pathogen increases the rate of multiplication of its propagules and repeats the disease cycles in a short span. Wheat cultivation area in the U.S.A and Canada and rice cultivation area in East Asian countries are exposed to a greater danger of epidemics by wheat black rust and rice blast respectively. Destructive epidemic of early and late leaf spots of groundnut in Bombay area (Gujarat and Maharashtra States) during 1912-1913 was mainly the result of cultivation of local varieties in a larger area. Panama wilt ( *Fusarium oxysporum f.sp. vasinfectum* ) susceptible table variety, 'Son' in banana was responsible for the destructive epidemic in parts of Bombay area (Gujarat and Maharashtra) during 1936 – 1940 Countrywide cultivation of red rot ( *Colletotrichum falcatum* )

susceptible sugarcane varieties (local varieties like *Pundya*, *Khajuria* etc.,) practically made their cultivation impossible in Bombay area.

### **3. Introduction of new host (s)**

Disease proneness in the host is induced by environment and other factors. The host is liable to vigorous attack and successful infection by the pathogen. A resistant or moderately resistant variety may become susceptible or highly susceptible. A susceptible variety may become highly susceptible when conditions favouring proneness are existing and cause severe damage. Under the above conditions the pathogen multiplies faster, cause infection and produces more propagules for secondary spread. Introduction of an exotic cotton variety (C4 (Cambodia) caused outbreak of bacterial blight (*Xanthomonas axonopodis* pv. *malvacearum*) and grey mildew (*Septoacylindrium gossypii*) in local variety, Deviraj, grown in Maharashtra area in India.

### **4. Introduction of new collateral or alternate hosts**

Alternate hosts are those plants on which the heteroecious pathogens pass part of their life cycles. Similarly, collateral hosts are some wild plants in which the pathogen survives when primary host is not available. Both alternate and collateral hosts are important in building up the primary inoculum to the next crop. They determine the course and intensity of an epidemic. Grass hosts (collateral hosts) of *Sclerospora sacchari*, *S. philippinensis* (downy mildews), *Pyricularia oryzae* (rice blast), *Ustilago scitaminea* (sugarcane smut) may produce abundant inoculum which aid in building up of epidemics. Outbreak of heteroecious blister rust of pine (*Cronartium ribicola*) in Europe and the U.S.A happened due to import or introduction of *Pinus strobus* from the USA.

## **B. Pathogen Factors**

### **1. Introduction of new pathogen**

Some pathogens, epidemic in certain area, may become quite aggressive and outbreak as epidemic when introduced to new area. For example late blight of potato caused by *Phytophthora infestans* was epidemic in South America. This disease became epidemic when the infected tubers were introduced in Europe (in 1843-45). Fire blight (*Erwinia amylovora*) in North America is endemic. Fire blight spread to Pacific coast fruit-growing areas of the U.S.A in 1884 and subsequently it reached Canada. It reached New Zealand in 1919 and it appeared in England in 1957. The mode of introduction had been through fruit boxes. Coffee rust (*Hemileia vastatrix*) is indigenous in Ethiopia, where *Coffea arabica* is native. The disease spread to Sri Lanka in 1869, India in 1870, Sumatra in 1876, Java in 1878 and the Philippines in 1889. It also spread from Kenya to the Congo by 1918 and reached the Cameroons. From 1950 onwards, it spread to the remainder of West Africa. The mode of long distance transport of *H. vastatrix* is wind. Spores have been trapped at up to 1000 m above sea level up to 150 m from infected sites. Dutch elm diseases (*Ceratocystis ulmi*) first reported in 1919 in Holland, spread throughout Europe and reached Great Britain in 1927. It was introduced to the eastern United States on elm logs imported from Europe.

## 2. Presence of aggressive strain of the pathogen

All the strains of a pathogen are not aggressive. Only the aggressive strains are capable of causing infectious diseases which spread as epidemic. They are characterised by rapid cycle of infection and causing successful infection in new hosts. Rapid cycle of infection is essential for successful infection and it happens only by aggressive strain of the pathogen. e.g., *Puccinia graminis tritici* (wheat black rust) in India, stripe rust, bunt and loose smut of wheat in the U.S.A. and Europe. The possibility of outbreak of epidemics increases with the number of physiologic forms or pathogenic strains of the pathogen present in a locality.

## 3. High birth rate of the pathogen

Pathogen with high reproductive capacity and capable of rapid dissemination over wide areas mostly cause epidemics. The fungal members causing powdery mildews, downy mildews, rusts, blasts, blights etc., produce enormous amount of spores. These spores are easily dispersed by using water or insects and cause infections to new plants. The high degree of fecundity and the enormous amounts of inoculum produced by some common plant pathogens are given in table.

Table. Fecundity rates of plant pathogens.

	Pathogen	Extent of fecundity
1	Wheat stem rust ( <i>Puccinia graminis tritici</i> )	Twenty five trillion uredospores in one hectare of wheat crops.
2	Wheat stem rust ( <i>Puccinia graminis tritici</i> )	64,000 million aeciospores from aecial cups in a single barberry bush
3	Cedar rust of apple	Two billion teliospores in a single gall.
4	A corn plant infected with downy mildew	225 million sporangia in one night
5	A. grapevine infected by downy mildew	32,000 sporangia per sq.cm
6	Bunt of wheat	6 to 12 million smut spores in a single kernel
7	Smut of corn	125,000 billion smut spores in one hectare
8	Chestnut blight	150,000,000 spores in a single spore horn

9	<i>Fomes applanatus</i>	5,460 billion spores in a single fruiting body.

#### 4. Low death rate

Epiphytotics may also be caused by low death rate diseases. These diseases are caused by agents of systemic nature which are protected by plant tissues. As they are protected by plant tissues the chances of high mortality is reduced to the minimum. In these diseases the chief source for accumulation of inoculum for epiphytotics is the diseased plant organ used for vegetative propagation (corms, setts, tubers, etc.). Here the build up of epidemics is comparatively low compared to high birth rate diseases. When a particular area is planted and covered with diseased planting material the chances of occurrence of epiphytotics are very high. e.g., virus and phytoplasma diseases in crops propagated through vegetative plant parts.

#### 5. Easy and rapid dispersal of the pathogen

The ability of the pathogen to cause epidemic depends both on the high birth rate and dispersal. The propagules of the pathogen produced should be dispersed for development of an epidemic. It may happen by external agencies like wind, water, insects, mites and nematodes. Fungal spores / conidia are minute and light and resistant to adverse conditions. Fungal spores are mostly disseminated by wind. Bacteria are mostly disseminated by water or insects. Virus and phytoplasma diseases are mostly transmitted by insects, mites or nematodes. Epidemics are determined by the velocity of wind, direction of wind, moisture, relative humidity, temperature, presence and number of vectors and their rate of reproduction.

#### 6. Adaptability of the pathogen

Pathogens have the capacity to adapt to adverse conditions. Fungi produce different types of spores like oospores, ascospores and smut spores (chlamydospores) which help in tiding over adverse conditions. Bacteria survive in diseased plant parts. Viruses and phytoplasmas live in collateral hosts or insect vectors in the absence of the suitable crop hosts.

#### C. Environmental Factors

The environmental conditions such as temperature, relative humidity, rainfall, duration and intensity of light, etc. play very important role in causing epidemics. These are actually the deciding factors and influence almost all the stages of disease cycle. Favourable environmental conditions are needed for sporulation, liberation of spores, dissemination of pathogen, germination, infection and establishment of pathogen in the host. For example, persistent optimum temperature and moisture are needed for spore germination and entry of germ tube in the host. Similarly optimum temperature, moisture, light and



specific nutrition is required for the development of the disease and sporulation of pathogen.

### **Compound interest diseases and simple interest diseases**

The terms compound interest and simple interest are for explaining rate of increase of pathogen. These terms were introduced by Van der Plank in 1963 in the book '**Plant Diseases-Epidemics and Control**'. Based on the mode of multiplication of pathogen, the diseases are classified of two types:

1. Simple interest diseases
2. Compound interest diseases

#### **1. Simple interest diseases**

In simple interest diseases the increase is mathematically analogous to simple interest in money. There is only one generation of the pathogen in the life of the crop. The primary inoculum is seed-borne or soil-borne. The secondary infection rarely occurs during the crop season. That is, the pathogens do not spread from plant to plant in one growing season.

Simple interest diseases are caused by seed-or soil-borne smuts, like loose smut of wheat, covered smut of barley and soil borne fungi which attack roots, like wilt (*Fusarium oxysporum*) and root rot (*Rhizoctonia* spp.) diseases. Most of the smuts infect the seedlings, grow along with the growth of the plant and produce spores in the inflorescence on maturity of the crop. There is no secondary spread from the smutted heads. These smut diseases are mostly systemic in nature. They do not produce propagules external to the host during the active season of the crop. Dispersal of propagules of these fungi is restricted by existing climatic and biotic conditions.

#### **2. Compound interest diseases**

In compound interest diseases the rate of increase is mathematically analogous to compound interest in money. The pathogen produces enormous amount of spores at a very rapid rate. These spores are disseminated rapidly by wind and infect the other plants. Both the inoculation and sporulation period are short so that the pathogen spreads from plant to plant during the same growing season. New crop of spores is produced, disseminated and the cycle is repeated fast. Thus more generations of the pathogen are produced in the life of a crop. e.g., late blight of potato, powdery mildews and rust diseases. If we consider wheat stem rust caused by *Puccinia graminis tritici* as an example, the fungus produces uredospores in very large numbers (50,000 to 4,00,000 uredospores per uredosorus). These spores are spread by wind and infect other plants. Each of the freshly infected wheat plant produces uredo-pustules within 5 to 7 days at 24°C. Thus within a week of appearance of the first pustule in the crop several thousand new pustules are formed which could repeat the process within a week. If the climatic conditions of about 24°C temperature and relative humidity remain for only few weeks, the entire crop is severely affected by the disease.

### **Course of epidemic**

The course of epidemic follows two distinct phases viz.,

- i. Progressively destructive phase and
- ii. the decline phase

i. **Progressively destructive phase**

Some epidemics develop slowly (tardive) while others develop rapidly. Slow epidemics (or epiphytotics) usually occur among population caused by systemic pathogens. The pathogen multiplies slowly following the characters of simple interest disease. They belong to low death rate category and have less incubation period and sporulation period. However, the rapid epiphytotics are greatly influenced by environmental factors.

ii. **Decline phase**

During early stage, an epidemic spreads vigorously causing diseases in new hosts. After development of a saturation stage it shows a decline by itself. No epidemics may be due to non-availability of susceptible stages of the crop, unfavourable weather conditions and reduction in aggressiveness of the pathogens. Generally the hosts are prone to the disease at a specific developing stage. Once this stage is crossed in a plant its proneness to infections is reduced or completely lost. Under the conditions the epidemic declines. The decline in the epidemic may also be due to unfavourable weather conditions for disease development. As a result future spread of the disease will be checked and the epidemic will decline. Wheat crop in Northern India usually gets the attack of rusts in January to March. Epidemics develop during these months. Although the plant remains prone to attack afterwards also, further development of the disease is checked because of rise in temperature which is favourable for the pathogen. Due to the above mentioned and other causes, the aggressiveness of the pathogen may be reduced. When all susceptible individuals are destroyed by the pathogen, it may try to parasitize the remaining resistant individuals of the same species. In these adverse conditions, the pathogen may lose its power of successful infection, its reproduction may slow down and the pathogen becomes less aggressive.

**Slow and rapid epiphytotics**

The form of epidemic is decided by the nature of the pathogen, host and the weather. Epidemic may develop slowly and is called '**tardive**'. Epidemic which develops rapidly is called '**explosive**'. In between these intermediate forms of epidemic may occur.

i. **Slow epiphytotics**

Slow epiphytotics occur among perennial (tree) populations. Infected host survives for several years before dying. Most of the characters of a simple interest disease are found in slow epiphytotics. The causal agent is mostly systemic. The pathogen multiplies slowly. Their movement from plant to plant is much slower. They are low death rate pathogen. In slow epiphytotics, crop sanitation is the best method. e.g., Swollen shoot of cocoa. This disease spreads very slowly from tree to tree and still less from one garden to another garden. For instance, the incidence of 31 % swollen shoot increased to 75 % over a period of 2.5 years. As stated by Van der Plank (1959) the rate of multiplication of a systemic disease of trees is about ten fold a year whereas it is 10,000 fold in

respect of herbaceous plants and it is of higher rates for local lesion pathogens e.g., late blight of potato, wheat stem rust, etc.,

**ii. Rapid epiphytotics**

Rapid epiphytotics occur among annual crops. It is caused by non-systemic pathogens with high birth rate. Several generations of the pathogen is produced within a short time. Rapid epiphytotics are largely governed by environmental factors compared to slow epiphytotics. Disease increase is rapid and the disease rises to a peak in short time and then show sharp decline when the weather turns unfavourable or when the host becomes resistant due to maturity or due to restricted dispersal of propagules of pathogen. e.g., apple scab. This type of epiphytotic is controlled by protective spraying or dusting with chemicals.

# 5

## Disease Forecasting

Forecasting of plant diseases means predicting for the occurrence of plant disease in a specified area ahead of time, so that suitable control measures can be undertaken in advance to avoid losses. Disease forecasts are predictions of probable outbreaks or increase in intensity of disease. It involves well organized team work and expenditure of time, energy and money. It is used as an aid to the timely application of chemicals. Among the first spray warning services to be established for growers, were the grapevine downy mildew forecasting schemes in France, Germany and Italy in the 1920s. Disease forecasting methods are available for the following plant diseases.

Sl. No	Plant disease	Countries
1.	Grapevine downy mildew	Australia, France, Germany, Greece, Italy, Romania, Spain, USSR, Yugoslavia
2.	Cucurbit downy mildew	U.S.A.
3.	Potato late blight	Australia, Brazil, Finland, France, Germany, Greece, Japan, the Netherlands, Norway, Peru, U.K, the U.S.S.R.
4.	Tobacco blue mould	Canada, U.S.A.
5.	Apple and pear scab	Australia, Canada, Netherlands, New Zealand, the U.S.A.
6.	Sugarbeet root rot ( <i>Aphanomyces</i> sp.)	U.S.A
7.	Wheat brown (Leaf) rust	U.S.A.
8.	Corn bacterial wilt ( <i>Erwinia stewartii</i> )	U.S.A
9.	Sugarbeet curly top	U.S.A.

### **Informations needed for disease forecasting**

Forecasting diseases is a part of applied epidemiology. Hence, knowledge of epidemiology (development of disease under the influence of factors associated with the host, pathogen) is necessary for accurate forecasting. The factors of epidemic and its components should be known in advance before forecasting is done. The informations required for forecasting are:

#### **1. Host Factors**

- a. Prevalence of susceptible varieties in the given locality
- b. Response of host at different stages of the growth to the activity of pathogen e.g. Some diseases are found during seedling stages while others attack grown up plants and
- c. Density and distribution of the host in a given locality. Dense populations of susceptible variety invite quick spread of an epidemic. Growing susceptible varieties in scattered locations and that too in a limited area are less prone to epiphytotic.

#### **2. Pathogen factors**

- a. Amount of primary (initial) inoculum in the air, soil or planting material
- b. Dispersal of inoculum
- c. Spore germination
- d. Infection
- e. Incubation period
- f. Sporulation on the infected host
- g. Re-dispersal / Dissemination of spores
- h. Perennating stages
- i. Inoculum potential and density in the seed, soil and air

#### **3. Environmental factors**

- a. Temperature
- b. Humidity
- c. Light intensity
- d. Wind velocity

### **Requirements or conditions for disease forecasting**

There are five main requirements which must be satisfied before a useful and successful disease forecast is made.

1. The disease must cause economically significant damage in terms of yield loss or quality. Damage assessment is essential to develop strategy for controlling a disease. e.g., Annual estimation of yield loss caused by barley powdery mildew (*Erysiphe polygoni*) in England and Wales had ranged from 6 to 13 %. Potato late blight can cause a yield loss of 28% if the disease reaches the 75% stage by mid-August. Diseases like apple scab and potato common scab reduces the quality of the produce lower the value of the harvested crop and cause considerable financial loss to the growers.
2. Control measures must be available at an economically acceptable cost.
3. The disease must vary each season in the timing of the first infections and its subsequent rate of progress. If it does not, there is no need for forecasting.

4. The criteria or model used in making a prediction must be based on sound investigational work carried out in the laboratory and in the field and tested over a number of years to establish its accuracy and applicability in all the locations where its use is envisaged.
5. Growers must have sufficient man power and equipments to apply control measures when disease warning is given. Long-term warnings or predictions are more useful than short-term warning or predictions.

### **Methods of disease forecasting**

Disease forecasting requires field observations on the pathogen characters, collection of weather data, variety of the crop and certain investigations and their correlations. Usually the following methods are employed in disease forecasting.

1. Forecasting based on primary inoculum: Presence of primary inoculum, its density and viability are determined in the air, soil or planting material. Occurrence of viable spores or propagules in the air can be assessed by using different air trapping devices (spore traps). In the case of soil-borne diseases the primary inoculum in the soil can be determined by monoculture method. Presence of loose smut of wheat, ergot of pearl millet and viral diseases of potato can be detected in the seed lots at random by different seed testing methods. Seed testing methods can be used to determine potential disease incidence and enable decision to be made on the need for chemical seed treatment. The extent of many virus diseases is dependent on the severity of the preceding winter which affects the size of vector population in the growing season. e.g., Sugarbeet yellows virus.
2. Forecasting based on weather conditions: Weather conditions *viz.*, temperature, relative humidity, rainfall, light, wind velocity etc., during the crop season and during the inter crop season are measured. Weather conditions above the crop and at the soil surface are also recorded.
3. Forecasting based on correlative information: Weather data of several years are collected and correlated with the intensity of the diseases. The data are compared and then the forecasting of the disease is done. Forecasting criteria developed from comparisons of disease observation with standard meteorological data have been provided for diseases like *Septoria* leaf blotch of wheat, fire blight of apple and barley powdery mildew.
4. Use of computer for disease forecasting: In some advanced countries forecasting of disease is made by the use of computers. This system gives the results quickly. One such computer based programmes in the USA is known as '**Blitecast**' for potato late blight. Examples of well developed forecasting systems are given below.
  - a. Early and late leaf spots of groundnut: A technique has been developed for forecasting early and late leaf spots of groundnut in the U.S.A. When the groundnut foliage remains wet for a period greater than or equal to

10 h and the minimum temperature is 21°C or higher for two consecutive days or nights, the disease development is forecasted.

A computer programme has been developed in the USA. This is accurate and is widely used in the USA. The data on hours for day with relative humidity (RH) of 95% and above and minimum temperature (T) during the RH observations for the period, for the previous 5 days are fed to the computer. Calculations are rounded to whole numbers. The T/RH index for each of the five days is calculated e.g., when hours of the RH 95% equal 10 and the minimum temperature during the period equals 21.1°C the T/RH index is 2.0. The T/RH indices for days 4 and 5 are summed. If the total index exceeds 4 disease is forecasted. If the index is 3 or less no disease is forecasted.

- b. Late blight of potato: In the USA a forecasting programme has been developed for late blight of potato (*Phytophthora infestans*). The initial appearance of late blight is forecasted 7 to 14 days after the occurrence of 10 consecutive blight favourable days. A day is considered to be blight favourable when the 5 days average temperature is 25.5°C and the total rainfall for the last 10 days is more than 3.0 cm.

A computerized version (**Blitecast**) has also been developed in the U.S.A for forecasting potato late blight. Blitecast is written in Fortran IV. When a farmer desires blight cast (blitecast) he telephones the blight cast operator and reports the most recently recorded environmental data. The operator calls for the blight cast programmes in the computer viz., typewriter terminal and feeds the new data into the computer. Within a fraction of second the computer analyses the data and series of a forecast and spray recommendations to the operator who relays it to the farmer. The entire operation can be completed during standard three minutes telephone call. The system makes one of the four recommendations viz., no spray, late blight warning, 7 days spray schedule or 5 days spray schedule. The last 5 days spray schedule is issued only during severe blight weather.

In West Germany, '**Phytoprog**' is the programme used. It is based on measurements of temperature, relative humidity and rainfall. Phytoprog provides a negative prognosis (an indication of when the usual routine spray application should be dispensed with).

- c. Blister blight of tea: A system for predicting epidemics of blister blight of tea (*Exobasidium vexans*) has been developed based on the number of spores in the air in the tea plantation and the duration of surface wetness on the leaves. The duration of sunshine is negatively correlated with the duration of surface wetness. The following prediction equation has been developed.

$$Y = 1.8324 + 0.8439 X_1 + 0.9665 X_2 - 0.1031 X_3$$

where,  $X_1 = \log \% \text{ infection } t_2$

$X_2 = \log \% \text{ infection } t_2 - \log \text{ infection } t_1$

$Y = \log \text{ of the number of spores in the air and } t_1 - t_2 \text{ three weeks}$

$X_3 = \text{mean daily sunshine for a 7 days period preceding } t_2$

- d. Southern corn leaf blight: 'Epimay' is a system for forecasting Southern corn leaf blight (*Bipolaris maydis*) based on conceptual model.

e. Rice blast: In India, forecasting rice blast ( *Pyricularia oryzae* ) is done by correlative information method. It is predicted on the basis of minimum night temperature 20 to 26°C in association with high relative humidity of 90% or above. Computer based forecasting system has also been developed for rice blast in India.

f. Wheat stem rust: Forecasting wheat stem rust epidemic is done by analysing the rain samples which give precise data for inoculum present in the air. Moreover several wind trajectors are also prepared to survey the air-borne primary inoculum and its deposition. It has been observed that primary inoculum comes from South India, to the plains of Central and North India.

g. Brown stripe downy mildew of corn: The forecasting of brown stripe downy mildew of corn ( *Sclerophthora raysise* var. *zeae* ) which is restricted to India is done on the basis of average rainfall 100 to 200 cm or more accompanied by low temperature ( 25°C or less).

### **Spore trapping**

Techniques of acquisition of biological data for consecutive forecasting models are important. Spore traps have been widely used in to complete disease with weather conditions. Spore trapping is useful for understanding epidemiology of a disease and behaviour of the pathogens. This helps in developing models on dispersal of pathogens or on epidemiology of the disease and to formulate methods of management. . Methodology of spore trapping depends on the following objectives of the worker.

1. Biology of the pathogen
2. Spore dispersal gradients
3. For infection forecasting
4. Management of the disease

In epidemics of air-borne plant diseases the number of spores of the pathogen landing on the plant which depends on the number of spores in the atmosphere above the crop is an important factor for the quantitative sampling of the atmosphere (number of spores per unit volume of air).For trapping and estimating these studies different types of traps are used. The following spore traps are usually employed in trapping of fungal spores.

1. Cylindrical rods or microscopic glass slides: It helps to gather data on the spore arrival in a locality. In this, the surface of microscopic slide is smeared with grease and made sticky. In the method, quantitative estimation is not possible as number of spores collected is very low.

2. Hirst's volumetric spore trap (Hirst 1952): In this instrument, air is sucked into at a controlled rate and impinged on to a glass slide moved by a clockwork mechanism past the orifice. It gives continuous count of spores in 24 h. The number of spores per unit volume of air at any given time can thus be calculated.

3. Rotorod sampler or rotorod spore trap (Sutton and Jones 1976): It comprises of a 'U' shaped rod attached at its mid point to the shaft of a small battery operated electric motor. In this equipment the surface of the rod is covered with



a vaseline strip of transparent cellophanes to catch spores which can be taken off and mounted on a glass slide. From the area of the strip and the speed of rotation, the volume of air samples can be calculated.

4. Anderson cascade spore sampler: It is a device where Petri plates with nutrient agar are used to collect the spores.

5. Bourdillon slit sampler: Air is sucked in a chamber by vacuum pump which strikes the rotating Petri dish containing agar medium. The agar medium retains the spores sucked in the air. Concentration of viable spores is calculated after counting germinated spores in the medium.

6. Burkard's 7 day volumetric spore trap: This device records spores in the air drawn by a pump on 7 days basis on a cellophane strip wrapped on a drum rotating inside a chamber.

7. Jet spore trap: In the above sampling methods, the viability of the spores cannot be determined. To overcome this, living plants have been used as spore traps. A jet spore trap in which spores are impacted in an air jet into a column of still air, through which they fall, to settle on leaf segments exposed at the base of the chamber. In this trap, suitable cultivars of host plants can be employed to determine number of viable spores.

# 6

## Disease Survey, Surveillance and Assessment

Survey on the plant diseases is one of the important aspects in the disease management. It is useful

- i . To know the prevalence of the disease in a season in a crop of a particular area.
- ii. To correlate the disease incidence with the weather factors, biological factors and soil factors.
- iii. To forecast the disease and inform about the outbreak of the disease to the crop growers.
- iv. To assess the damages caused by diseases.
- v. To find out the occurrence of new diseases.
- vi. To workout the management practices in the control of the diseases.

Pest and disease surveillance programmes are in existence and the Indian Government has set of staff for disease surveillance. Among different states, Tamil Nadu has an organized pest and disease surveillance programmes which is functioning effectively throughout the state. In this programme the officials in the Departments of agriculture and department of horticulture and plantation and the plant pathologists and entomologists in Tamil Nadu Agricultural University are involved. The departmental staff collects the data from different villages in respective districts, as the scientists (plant pathologists and entomologist) collect the data in the respective research station/ Agricultural College. Weekly reports are prepared for each district in consultation with the pathologist, entomologists in the respective research station - Agricultural College and send them to the Tamil Nadu Agricultural University for analysing the pest/ disease data and compilation. The pest/ disease surveillance report should accompany the details on the daily weather data *viz.*, minimum temperature, maximum temperature, relative humidity, rainy days, rainfall, wind velocity, etc.

### Methods of survey

There are two types of survey. They are fixed plot survey and roving survey

1. Fixed plot survey: In each agricultural division for each crop, two villages are selected and each village, two fields of one acre are fixed. In each field of one acre five sampling plots are selected and they should be three metres away from the bunds. The sampling plot should be of 1m x 1m size. In each plot twenty representative plants / leaves are selected and observed for the disease incidence

utilizing the standard score chart having 0-9 grades for leaf spot, rust, leaf blight, downy mildew, powdery mildew etc. The per cent disease index is worked out using standard formula. For systemic diseases like viral and MLO diseases, root rot, wilt, damping-off, sugarcane smut, green ear etc, percentage of disease incidence is worked out using the specific formula. In the case of vector-borne diseases, the number of vectors per tiller / plant has to be observed and included in the report. Survey on the crop diseases are done every week, the report is prepared.

2. Roving survey: In roving survey, for each Panchayat Union / Block four representative villages for individual crops are selected. In each village two fields are fixed. In each field, observe 100 plants / leaves and score them for the disease intensity by walking across starting from South-West corner to North-East corner. The percentage of disease / per cent disease index is calculated by using the standard formula. As in the fixed plot roving survey is also made at every week and prepared.

### **Disease surveillance report**

The weekly data on disease incidence (including vectors if any) along with the weather data collected from fixed plot survey and roving survey by the Assistant Director of Agriculture are sent to the Joint Director of Agriculture in respective districts with any one of the following.

**a. White report:** It contains disease / pest surveillance details of a particular block / division / district.

**b. Yellow card:** It should be sent when the disease / pest occurrence reached half of the level of economic threshold level (ETL)

**c. Red card:** It should be sent when the disease / pest occurrence exceeded the economic threshold level (ETL). Simultaneously data on fixed plot survey are collected by the scientists in the research station/Agricultural college. The data collected from the farmers' field in the districts and the research centre in the respective / nearby district is pooled analyzed and interpreted every week jointly by the pathologist / entomologist and the Deputy Director of Agriculture (Plant Protection) in the JDA's office in the district. After analysing the weekly survey reports and the weather data, the message on the severity level of the disease and management measures to be adopted are disseminated to the farmers through mass media like newspaper and All India Radio. Leaflets are also prepared on the disease(s) and their management and distributed to the farmers for adoption. If the disease is in epidemic form, materials are mobilized by staff for immediate and effective control of disease in a short span of time.

### **Assessment of plant diseases**

Methods of measuring disease should meet the following requirements,

- i. Growth stages of the plant
- ii. Study of course of the disease.
- iii. Standard diagrams for the assessment of disease for the survey.
- iv. Observation of the disease with the diagrams and the yield of the crop.
- v. Calibration of the disease severity with crop loss.

Assessment of amount of a disease on a plant is essentially required to study quantitative epidemiology. Assessment is essential for the fungicide manufacturing firms, plant breeders and academicians for studying treatment efficacies and evaluation of resistant varieties. Assessment of a specific disease in a crop over several years provides factors governing its incidence and severity. It can be used to device forecasting system for different crops in any location.

## Methods of disease assessment

**1. Assessment for systemic diseases (Virus, phytoplasmas, smut, green ear and root diseases like root-rot, wilt and damping-off) :** In this, plant diseases which kill plant outright or which cause about the same amount of damage to the infected plants are assessed. They include, all viral, phytoplasmal diseases, loose smut of wheat, sugarcane smut, green ear, damping-off, vascular wilt and root rot of different crops. The total number of crops / plants in an area is counted. The total number of diseased plants is also counted. The percentage of disease incidence is calculated by using the following formula,

$$\text{Per cent disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total number of plants observed}} \times 100$$

The per cent scale has the following advantages viz., the upper and lower limits of scales are always well defined and the scale is flexible and can be divided and subdivided conveniently.

**2. Assessment using disease grades: (for downy mildews, powdery mildews, rusts, cankers, anthracnose, leaf spots, leaf blights, blast, ergot, etc.):** For assessing most of the foliar diseases the standard disease score charts are available with pictorial diagrams. The diagrams for each disease will be based on the percentage of leaf area affected. In earhead diseases the number of grains affected are indicated. For the leaf or earhead diseases, the grade ranging from 0 to 9 are internationally used

The grades and description for each grade for important diseases are given below.

a. Leaf spot, leaf blight, anthracnose and blast

Grade	Description
0	No visible symptoms
1	<1% of leaf area affected
3	1-10% of leaf area affected
5	11-25% of leaf area affected
7	26-50% of leaf area affected
9	> 50% of leaf area affected

b. Downy mildew, powdery mildew and sooty mould

<b>Grade</b>	<b>Description</b>
0	No visible symptoms
1	<1% leaf area with mildew growth
3	1-10% leaf areas with mildew growth
5	11-25% leaf areas with mildew growth
7	26-50% leaf areas with mildew growth
9	>50% of leaf area with mildew growth and severe infection on fruits

c. Rusts

<b>Grade</b>	<b>Descriptions</b>
0	No symptoms
1	<1% leaf area affected with pustules
3	1-10% leaf areas affected with pustules
5	11-25% leaf areas affected with pustules
7	26-50% leaf areas affected with pustules
9	>50% leaf area affected with pustules.

d. Ergot

<b>Grade</b>	<b>Descriptions</b>
0	Earheads free from infection
1	<1% grains in earhead replaced by sclerotia
3	1-10% grains in earhead replaced by sclerotia
5	11-25% grains in earhead replaced by sclerotia
7	26-50% grains in earhead replaced by sclerotia
9	>750% grains in earhead replaced by sclerotia

For assessment of the above diseases, plants / leaves are observed individually for the area/ number (ergot) affected and severity grades are assigned to each diseased plant / leaf. Sampling unit and sample size vary with the host and disease involved. In assessing foliar diseases in a field it is not possible to assess all the plants in a plot / field. Hence usually 10 per cent of the representative population is selected at random for assessing the disease. In the case of tree crops five trees are selected at random and 20 representative leaf / fruit samples are assessed. For example, if 20 representative samples are to be taken assign the grade for individual leaf / plant/ fruit and enter the grade as detailed below.

Plant / Leaf number	Severity grade	Plant / Leaf number	Severity grade
1	9	11	0
2	9	12	3
3	3	13	1
4	1	14	5
5	9	15	9

6	3	16	7
7	5	17	0
8	7	18	3
9	9	19	1
10	1	20	3
Total			<b>88</b>

The per cent disease index is worked out using following formula:

$$\begin{aligned}
 \text{Per cent Disease Index (PDI)} &= \frac{\text{Sum of individual ratings}}{\text{Total number of plants / leaves observed}} \times \frac{100}{\text{Maximum grade}} \\
 &= \frac{88}{20} \times \frac{100}{9} = \frac{8800}{180} = 48.89
 \end{aligned}$$

# 7

## Plant Quarantine

The term '**Quarantine**' means simply forty i.e., 40 days period. This was more commonly referred to the period of detention for ships arriving from countries subject to epidemic diseases such as the Bubonic plague, cholera and yellow fever. The crew and the passengers used to be compelled to remain isolated on board for sufficient period to permit the diseases to develop and be detected. The purpose of the health authorities was to establish adequate detention period. Later on, the term 'Quarantine' came to be only used for the detention and the practices connected with it. The term got associated from the human disease field to the animal disease field and later on adopted to cover protective methods for the exclusion of pests and diseases of agricultural and horticultural crops.

In strict sense '**Plant Quarantine**' refers to the holding of plants in isolation until they are believed to be healthy. Now, broader meaning of the plant quarantine covers all aspects of the regulation of the movement of living plants, living plant parts/plant products between politically defined territories or ecologically distinct parts of them. Intermediate quarantine and post entry quarantine are used respectively to denote the detention of plants in isolation for inspection during or after arrival at their final destination.

### Importance

The entry of a single exotic insect or disease and its establishment in the new environment continues to cause great, national loss (table ) till such time it is brought under effective control. In certain cases a country has to spend a few million rupees before success in controlling the introduced insect pest or disease is achieved.

Table. Losses caused by introduced plant diseases

Disease	Host	Country	Introduced from	Losses caused
1. Canker	Citrus	U.S.A.	Japan	\$ 13 million; 19.5 million trees destroyed
2. Dutch elm disease	Elm	U.S.A.	Holland	\$ 25 million - \$ 50,000 million
3. Blight	Chestnut	U.S.A.	Eastern Asia	\$ 100-1000 million
4. Powdery mildew	Grapevine	France	U.S.A	80% in wine production
5. Downy mildew	Grapevine	France	U.S.A	\$ 50,000 million

6.	Bunchy top	Banana	India	Sri Lanka	Rs.4 crores
7.	Wart	Potato	India	Netherland	2500acres infected
8.	South American leaf blight	Rubber	Dutch – Guiana	Brazil	40,000 trees destroyed
9.	-do-	-do-	North Columbi a	Brazil	78% trees destroyed
10.	Blue mould	Tobacco	Europe	U.K.	\$ 50 million
11.	-do-	-do-	Sweden	U.K.	1.2 million Kroner

### History

The first plant quarantine law was promulgated in Rollen, France in 1860 to suppress and prevent the spread of common barberry, the alternate host for wheat stem rust. Among other countries, the first few to establish plant quarantine services were Germany, France, Australia and the U.S.A. In India, legislative measures against crop pests and diseases was initiated under the Destructive Insects and pests Act of 1914 (DIP act) and it was passed by Governor General of India on 3<sup>rd</sup> February, 1914. Under this Act, rules governing the import and movement of plants and plant materials, insects and fungi are framed. The Act provides

1. It authorizes the Central Government to prohibit or regulate the import into India or any part there of any specific place therein, of any article of class of articles.
2. It authorizes the officers of the Customs at every port to operate, as if the rules under the D.I.P. Act is made under the Sea Customs Act.
3. It authorizes the Central Government to prohibit or regulate the export from a State of the transport from one State to another State in India of any plants and plant materials, diseases or insects likely to cause or infestation. It also authorizes the control of transport and carriage and gives power to prescribe the nature of documents to accompany such plants and plant materials and articles.
4. It authorizes the State Governments to make rules for the detention, inspection, disinfection or destruction of any insect or class of insects or of any article or class of articles, in respect of which the Central Government have issued notifications. It also authorizes the State Governments for regulating the powers and duties of the officers whom it may appoint on this behalf.
5. It provides penalty for persons who knowingly contravene the rules and regulations issued under the Act.
6. It also protects the persons from any suit or prosecution or other legal proceedings for anything done in good faith or intended to be done under the Act.



Consequent to Bengal famine 1943, a Central Plant Protection organization was established in 1946 under the then Ministry of Food and Agriculture.

Often a new pest, disease or weed has accidentally entered a country where it did not exist before and has multiplied, spread and caused enormous damage to the crops of that country. For instance powdery mildew of grapevine (*Plasmopara viticola*), introduced into France from America, was responsible for the destruction of the vine industry of that country until hybridization with resistant American stock offered a solution. The blight disease of chestnut (*Endothia parasitica*) which was introduced into U.S.A. from Asia in 1904, completely wiped out chestnut trees.

Coffee rust (*Hemileia vastarix*) which came into India in 1879 from Sri Lanka is now widespread in all coffee growing areas. Fire blight (*Erwinia amylovora*) of pear and other pomes which was introduced from England in 1940 is well established in Uttar Pradesh. Late blight (*Phytophthora infestans*) of potato introduced into India in 1889 from Europe is now present in many parts of the country. Flag smut (*Urocystis tritici*) of wheat introduced from Australia is now well spread in Madhya Pradesh, Punjab, Rajasthan and Uttar Pradesh. Rubber powdery mildew (*Oidium heavea*), which was introduced from Malaysia in 1938, is also causing great concern in Kerala. Black rot of crucifers (*Xanthomonas campestris* pv. *campestris*) believed to have been introduced to India with seeds imported from Holland, and other European countries after World War II, prevailed for some years on the hills and then spread to the plains and became established in Indian seed stocks, especially in West Bengal.

Among the more important plant disease introductions, mention may be made of bunchy top virus of banana introduced from Sri Lanka in 1940 which has since spread widely in Kerala, Orissa, West Bengal and Assam. The wart disease (*Synchytrium endobioticum*) of potato was first noticed in Darjeeling district of West Bengal having been introduced with seed potatoes from Holland. By 1962, the disease spread over nearly 1000 ha and has recently been reported from Nepal also. The mosaic disease of banana is another introduced disease which is only confined to Gujarat and Maharashtra states. Recently the apple scab (*Venturia inaequalis*) which was only confined to small area in Jammu and Kashmir has now appeared in severe form in many locations in Himachal Pradesh, and is posing a problem to apple industry.

The establishment of a plant quarantine regulation should rest on the following fundamental pre-requisites.

- i. the pest/disease under consideration must be one that will offer actual or expected threats to substantial interests (Agricultural and / or commercial)
- ii. the quarantine regulation or degree must represent a measure for which no substitute action involving less interference with normal activities is available.

Table. Diseases believed to have been introduced into India from foreign countries

Disease	Host	Date of first record	Introduction from
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1. Leaf rust ( <i>Hemileia vastarix</i> )	Coffee	1879	Sri Lanka
2. Late blight ( <i>Phytophthora infestans</i> )	Potato, Tomato	1883	Europe
3. Rust ( <i>Puccinia carthami</i> )	Chrysanthemum	1904	Japan or Europe
4. Flag smut ( <i>Urocystis tritici</i> )	Wheat	1906	Australia
5. Downy mildew ( <i>Plasmopara viticola</i> )	Grapevine	1910	Europe
6. Downy mildew ( <i>Pseudoperonospora cubensis</i> )	Cucurbits	1910	Sri Lanka
7. Downy mildew ( <i>Sclerospora philippinensis</i> )	Maize	1912	Java
8. Foot rot ( <i>Fusarium moniliforme</i> var. <i>majus</i> )	Rice	1930	South East Asia
9. Leaf spot ( <i>Phyllachora sorghi</i> )	Sorghum	1934	South Africa
10. Powdery mildew ( <i>Oidium heveae</i> )	Rubber	1938	Malaya
11. Black shank ( <i>Phytophthora parasitica</i> var. <i>nicotianae</i> )	Tobacco	1938	Dutch East Indies
12. Fire blight ( <i>Erwinia amylovora</i> )	Pear and other pomes	1940	England
12. Crown-gall and hairy root ( <i>Agrobacterium tumefaciens</i> A. <i>rhizogenes</i> )	Apple, Pear	1940	England
14. Bunchy top	Banana	1940	Sri Lanka
15. Canker ( <i>Sphaeropsis malorum</i> )	Apple	1943	Australia
16. Wart ( <i>Synchytrium endobioticum</i> )	Potato	1953	Netherlands

Despite every precaution of inspection, certification and treatment, it is not always possible to guarantee that a consignment is completely free from pathogens. In doubtful cases it is advisable to subject plants to a period of growth in isolation under strict supervision in the importing country (post-entry quarantine). The plants are grown at a quarantine station. When direct importation of plants to a country's own quarantine station is considered very dangerous, quarantine during transit from the country of origin (intermediate quarantine) may be required. The requirements of an intermediate station are similar to those for a post-entry station. Intermediate quarantine inspection must always be followed by post-entry quarantine after arrival of the consignment at

its final destination. During post-entry or intermediate quarantine plants must be kept under close supervision, so that any pest or disease which appears may be immediately detected and grown under optimum conditions, so that symptoms are not marked by physiological disturbances.

### **International plant protection convention**

The first effort towards international agreement on Plant Protection was made in 1914 under the auspices of the International Institute of Agriculture in Rome. This was followed by an International Convention of Plant Protection by over 50 member countries of the Institute in 1919 and certain Agreements regarding the issue and acceptance of phytosanitary certificates were finalized. The project received a set back due to Second World War and was later on revived by the FAO.

In post-war period International action in Plant Protection and particularly in plant quarantine was encouraged by FAO with the establishment in 1951 of the International Plant Protection Convention. This agreement was constituted with the purpose of securing common and effective action to prevent the introduction and spread of pests and diseases of plants and plant products as to encourage Governments to take all steps necessary to implement its prevention (Ling, 1953).

The following regional Plant Protection Organizations are now in operation.

1. The European and Mediterranean Plant Protection Organization (EPPO)
2. The Inter-African Phytosanitary Council (IAPSC)
3. Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA)
4. The Plant Protection Committee for, the South East Asia and Pacific region.
5. Comit'e Interamericano de Protection Agricola. (CIPA)
6. The Caribbean Plant Protection Commission (CPPC)
7. The North American Plant Protection Organization (NAPPO).

Under article 3 of that International Plant Protection Convention, the Plant Protection Agreement for South East Asia and Pacific Region was sponsored by F.A.O in 1956, and India became in party to this Agreement in the same year the along with Australia, Sri Lanka, the U.K., Laos, Netherlands, Indonesia, Portugal and Vietnam. Our Government agreed to adopt legislative measures specified in the Convention for the purpose of securing common and effective action to prevent the introduction and spread of pests and diseases of plants and plant products and to promote measures for their control and also agreed to assume all responsibilities for the fulfillment within its territories of all requirements under the Convention. It was agreed that the Government shall make provision for:

a. An official plant protection organization, with the following main functions:

1. The inspection of growing plants, of areas under cultivation and of plants and plant products in storage and in transportation with the object of reporting the existence, outbreak and spread of plant diseases and pests and of controlling those pests and diseases.

2. The inspection of consignments of plants and plant products moving in international traffic, the inspection of consignments of other articles or commodities moving in international traffic under conditions where they may act incidentally as carriers of pests and diseases of plants and plant products and the inspection and supervision of storage and transportation facilities of all kinds involved in international traffic whether of plants and plant products or other commodities, with the object of preventing the dissemination across national boundaries of pests and diseases of plants and plant products.
  3. The disinfection or disinfection of consignments of plants and plant products moving in international traffic, and their containers, storage places, or transportation facilities of all kinds employed.
  4. The issue of certificates relating to phytosanitary condition and origin of consignments of plants and plant products (Phytosanitary certificates).
- b. The distribution of information within the country regarding the pests and diseases of plants and plant products and the means of their prevention and control
- c. Research and investigation in the field of plant protection.

A revised text of convention was approved in 1979. As of December 1980, the number of states party to the convention is 81. Besides this world-wide convention, other regional agreements and organizations have been created to safeguard the interests of groups of neighbouring countries with similar plant protection problems. Regional action is needed to prevent a pathogen or pest absent from a whole area from being introduced into any part of the area, as its entry into one territory will endanger neighbouring countries.

### **Plant quarantine methods**

There are number of plant quarantine methods which are used separately or collectively to prevent or retard the introduction and establishment of exotic pests and pathogens. The components of plant quarantine activities are:

1. Complete embargoes: It involves absolute prohibition or exclusion of specified plants and plant products from a country infected or infested with highly destructive pests or diseases that could be transmitted by the plant or plant products under consideration and against which no effective plant quarantine treatment can be applied or is not available for application.
2. Partial embargoes: Partial embargoes, applying when a pest or disease of quarantine importance to an importing country is known to occur only in well defined area of the exporting country and an effectively operating internal plant quarantine service exists that is able to contain the pest or disease within this area.
3. Inspection and treatment at point of origin: It involves the inspection and treatment of a given commodity when it originates from a country where pest/disease of quarantine importance to importing country is known to occur.

4. Inspection and certification at point of origin: It involves pre-shipment inspection by the importing country in cooperation with exporting country and certification in accordance with quarantine requirements of importing country.
5. Inspection at the point of entry: It involves inspection of plant material immediately upon arrival at the prescribed port of entry and if necessary subject to treatment before the same related.
6. Utilization of post entry plant quarantine facilities: It involves growing of introduced plant propagating material under isolated or confined conditions.

### **Plant quarantine organizations in India**

The first recorded plant quarantine measure in India dates back to 1906 when, perceiving the danger of introducing the Mexican boll weevil, the Government of India directed that all cotton imported from the New World should only be admitted to India after fumigation with carbon disulphide at the port of entry.

In India two categories of regulatory measures are in operation for controlling pests, diseases and weeds. In the first category regulatory measures are aimed to prevent the introduction of exotic pests and diseases into the country or their spread from one State or Union Territory to another (Plant Quarantine). The second pertains to suppression or prevention of spread of pests and diseases in localized areas within a State or Union Territory. The former derives its authority from the Destructive Insects and Pests (DIP) Act 1914 of the Central Government and the latter from Agricultural Pests and Diseases Acts of the various States.

The legislative measures against crop pests and diseases were initiated under the DIP Act of 1914 which was passed by the then Governor General of India in Council on 3 February 1914. Prior to the establishment of the Directorate of Plant Protection, Quarantine and Storage in 1946, under the Ministry of Food and Agriculture, the various rules and regulations of the DIP Act were enforced by the customs department. The quarantine regulations are operative through The Destructive Insects and Pests Act, 1914 (which has been revised 8 times from 1930 to 1956 and amended in 1967 and 1992).

The provisions of the DIP Act are

1. It authorizes the Central Government to prohibit or regulate the import into India or any part thereof or any specific place therein of any article or class of articles.
2. It authorizes the officers of the Customs at every port to operate, as if the rules under DIP Act are made under the Sea Customs Act.
3. It authorizes the Central Government to prohibit or regulate the export from a State or the transport from one State to another State in India of any plants and plant material, diseases or insects, likely to cause infection or infestation. It also authorizes the control of transport and carriage and gives power to prescribe the nature of documents to accompany such plants and plant materials and articles.
4. It authorizes the State Governments to make rules for the detention, inspection, disinfection or destruction of any insect or class of insects or any article or class of articles, in respect of which the Central Government has

issued notification. It also authorizes the State Governments for regulating the powers and duties of the officers whom it may appoint on its behalf.

5. It provides penalty for persons who knowingly contravene the rules and regulations issued under the Act.
6. It also protects the personnel from any suit or prosecution or other legal proceedings for anything done in good faith as intended to be done under this Act.

The quarantine regulations are operative through “The Destructive Insects and Pests Act, 1914 (which has been revised and time from 1930 to 1956 and amended in 1967 and 1992.

The Act also empowers the State Governments to frame suitable rules and issue notifications for inter-state movement of plant and plant material. Those rules are known as plant quarantine rules.

Under the Act, Central Government frames rules prescribing the seaports, airports and land frontiers through which plants and specified plant material can enter India, and the manner in which these can be imported. The DIP Act operates under the National Sea Customs Act and the points of entry are located within the jurisdiction of State on the advice of Central Government, the State frames rules for detention, inspection, disinfection and destruction (as against entry) of material, if required, and delegates powers in this regard to concerned authorities with the enforcement of rules.

The plant quarantine service is centrally organized and administered through the Directorate of Plant Protection, Quarantine and Storage established under the Ministry of Agriculture (Department of Agriculture and Co-operation) which is headed by the Plant Protection Adviser to the Government of India and having its headquarters at N.H. IV, Faridabad, Haryana State.

### **Import regulations**

When plants are imported the following principles should be followed. Some plant pathogens and pests are generally distributed in most parts of the world but others are more or less restricted in their occurrence. In some cases this limitation is due to such factors as unsuitable environmental conditions or lack of the required host plant, but in many other cases the absence of a pathogen. Most countries are aware of the desirability of delaying for as long as possible the arrival of exotic pathogens and take action to prevent their spread by introducing legislation and setting up organizations to prevent their entry. Plant quarantine legislation varies from country to country but in most cases it restricts or prohibits the importation of the pests or pathogens themselves, plants on which they might be living, soil which might be infested, foodstuffs which might carry them, and packing materials, particularly those of plant origin. Good legislation is as brief and clear as possible, at the same time being easy to interpret, gives adequate protection without interfering more than is essential with trade, and contains only restrictions which are scientifically justifiable.

When plants are imported there are certain principles which, if followed ensure that as few risks as possible are taken.

1. Import from a country where, for the crop in question, pathogens which are particularly to be guarded against are absent.

2. Import from a country with an efficient plant quarantine service, so that inspection and treatment of planting material before despatch will be thorough, thus reducing the likelihood of contaminated plants being received.
3. Obtain planting material from the safest known source within the selected country.
4. Obtain an official certificate of freedom from pests and diseases from the exporting country. Treatment of the material in the country of origin may be done; this should be noted on the certificate.
5. The smaller the amount the less the chance of its carrying infection, and inspection as well as post-entry quarantine.
6. Inspect material carefully on arrival and treat (dust, spray, fumigate, heat treat) as necessary.
7. Import the safest type of planting material, e.g. seeds are usually safer than vegetative material, unrooted cuttings than rooted. The use of axenic cultures of meristem tip tissues (micropropagation) for the international exchange of germplasm material has outstanding advantages, as such tissues can be expected to be free from latent infections by viruses, phytoplasmas etc., as well as other pathogens which are more readily detectable by visual means.
8. If other precautions are not thought to be adequate, the consignment for import should be subject to intermediate or post-entry quarantine. Such quarantine must be carried out at a properly equipped station with suitably trained staff.

Seed was not originally included in the DIP Act, but because of the changing situation and to meet the current requirements, the Government of India passed the Plants, Fruits, Seeds (Regulation of Import into India) Order 1984 which came into effect in June 1985. The conditions for the import of 17 crops are stipulated in this order. The main features of the order are:

1. Seed has been brought under the purview of the DIP Act.
2. No consignment can be imported into the country without valid import permit issued by the Plant Protection Adviser to the Government of India.
3. No consignment can be imported without an official phytosanitary certificate issued by the plant quarantine agency of the exporting country.
4. Post-entry growth of the specified crops at approved locations.

#### **A. Conditions for import**

In India, there are general and specific conditions for the import of plants (including bulbs, tubers, rhizomes, corms, cuttings, buddings, grafts, layers, suckers, roots and flowers) and plant materials (including plant products such as ginned cotton, unmanufactured tobacco etc.).

#### **General conditions**

1. Import permits are essential for : a. Seeds and fruits for consumption, b. Seeds and plants for sowing or planting, c. Soil, earth clay for microbiological, soil-mechanics or mineralogical investigations, d. Peat for

horticultural purposes, e. Live insects and f. Living fungi in pure culture, including *Rhizobium cultures*.

2. All plants should be accompanied by Phytosanitary certificate from the country of origin.
3. All plants on arrival at port, shall be inspected and if necessary fumigated, disinfested or disinfected by Plant Protection Adviser to the Government of India or any other officer authorized by him on his behalf.
4. Plants and seeds which require post-entry quarantine inspection shall be grown in post-entry quarantine facilities approved by the Plant Protection Adviser to the Government of India.
5. Import of hay or straw or any material of plant origin used for packing is prohibited.
6. Import of soil, earth, compost, sand, plant debris along with plants, fruits and seeds is prohibited.

**Note:** Cut flowers, garlands, bouquets, fruits and vegetables weighing less than 2 kg for personal use may be imported without a permit or phytosanitary certificate, but are subject to inspection.

### Special conditions

In addition to the general conditions, there are special conditions for certain notified plants as follows.

1. Prohibition from certain areas

Name of the plant	Countries from where prohibited
1. Cocoa and all species of Sterculiaceae and Bombaceae	Africa, Sri Lanka, West Indies
2. Coffee beans	Africa, South America, Sri Lanka
3. Rubber	South America, West Indies
4. Sugarcane	Australia, Fiji, Papua New Guinea
5. Sunflower	Argentina, Peru

2. Prohibited for general public: Coconut plants and seeds, coffee plants and seeds, cotton seeds and unginned cotton, forest tree seed (*Castanea*, *Pinus*, *Ulmus*), groundnut seeds and cuttings, potato, sugarcane, tobacco seeds and wheat seeds.
3. Plants/seeds which require post entry quarantine: Cocoa, citrus, coconut, groundnut, potato, sugarcane, sunflower, tobacco and wheat.
4. Additional declarations required for notified plants (see Table below)

Table: Plant and plant materials requiring additional declarations for freedom of pests

Plant/seed	Additional declarations for freedom of pests
All species of <i>Allium</i> (onion, garlic, leek, chive, shallot, etc.)	Smut ( <i>Urocystis cepulae</i> )



Cocoa and all species of the family Sterculiaceae and Bombaceae	Pod rot ( <i>Monilia rorei</i> ), Mealy pod ( <i>Trachysphaeria fructigena</i> ), Witches' broom ( <i>Crinipellia perniciosus</i> ) and Swollen shoot virus.
All species of <i>Citrus</i> (lemon, lime, orange etc.,)	Mal Secco ( <i>Deuterophoma tracheiphila</i> )
Coconut seeds and all species of <i>Cocos</i>	Lethal yellowing, Cadang, Bronze leaf wilt, Guam Coconut disease, Leaf scorch
Coffee – plants, seeds	American leaf spot ( <i>Omphali flavida</i> ), virus diseases
Cotton seeds	Bacterial blight ( <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> and <i>Glomerella gossypii</i> ).
Forest tree seeds (all species of <i>Pinus</i> , <i>Ulmus</i> , <i>Castanea</i> )	<i>Cronartium ribicola</i> , <i>Endothea parasitica</i> , <i>Ceratocystis ulmi</i> , <i>Dothiostroma pini</i> .
Groundnut seeds (all species of <i>Arachis</i> )	i. production of seeds in areas free of <i>Puccinia arachidis</i> and <i>Sphaceloma arachidis</i> . ii. Inspection of parent crops in active growing seasons and certification for freedom from peanut mottle, peanut stunt, marginal chlorosis and peanut stripe viruses
Lucerne (all species of <i>Medicago</i> )	Bacterial wilt ( <i>Corynebacterium incidiosum</i> )
Potato (all species of <i>Solanum</i> )	Wart ( <i>Synchytrium endobioticum</i> ) and freedom of parent crop from virus diseases
Rubber (all species of <i>Hevea</i> )	South American leaf blight ( <i>Microcyclus ulei</i> , <i>Sphaerostilbe repens</i> )
Sugarcane (all species of <i>Saccharum</i> )	Leaf scald ( <i>Xanthomonas albineans</i> ), Gummosis ( <i>Xanthomonas vascularum</i> ), Sereh, downy mildew, chlorotic streak and Fiji disease.
Sunflower (all species of <i>Helianthus</i> )	Downy mildew ( <i>Plasmopara halstedii</i> )

Tobacco (all species of Blue mold(*Peronospora tabacina*)  
*Nicotiana*

a. Tobacco seed

Wheat (all species of *Triticum*) Ergot (*Claviceps purpurea*), Dwarf bunt  
a. For sowing and planting (*Tilletia controversa*), Spikelet rot  
(*Pseudomonas atrofaciens*).

b. For consumption Ergot (*Claviceps purpurea*), Dwarf bunt  
(*Tilletia controversa*).

### Agencies involved in plant quarantine

The authority to implement the quarantine rules and regulations framed under DIP Act rests basically with the Directorate of plant Protection, Quarantine & Storage, under the Ministry of Agriculture. This organization handles bulk import and export of seed and planting material for commercial purpose. Under this organization 9 seaports, 10 airports and 7 land frontiers are functioning. These are the recognized ports for entries for import of plant and plant material. The names and places of the ports and stations are as follows.

#### A. Seaports

	Place	State / Union territory
1.	Bhavnagar	Gujarat
2.	Calcutta	West Bengal
3.	Chennai	Tamil Nadu
4.	Cochin	Kerala
5.	Mumbai	Maharashtra
6.	Nagapattinam	Tamil Nadu
7.	Rameswaram	Tamil Nadu
8.	Tuticorin	Tamil Nadu
9.	Visakhapatnam	Andhra Pradesh

#### B. Airports

1.	Amritsar	Punjab
2.	Calcutta	West Bengal
3.	Chennai	Tamil Nadu
4.	Hyderabad	Andhra Pradesh
5.	Mumbai	Maharashtra
6.	New Delhi	New Delhi
7.	Patna	Bihar
8.	Tiruchirappalli	Tamil Nadu
9.	Trivandrum	Kerala
10.	Varanasi	Uttar Pradesh

#### c. Land frontiers

1.	Amritsar Railway Station	Punjab
2.	Attari Railway Station	Punjab
3.	Attari-Wagah Border	Punjab

4.	Bangaon Benapol Border	West Bengal
5.	Gede Road Railway Station	West Bengal
6.	Kalimpong	West Bengal
7.	Sukhia Pokhri	West Bengal

The Government of India has also approved three other national institutions to act as official quarantine agencies, especially for research material.

1. National Bureau of Plant Genetic Resources (NBPGR): The NBPGR in New Delhi and its regional station at Hyderabad in the agency involved in processing of germplasm, seed, plant material of agricultural, horticultural, and silvicultural crops of all the institutions of Indian Council of Agricultural Research (ICAR) functioning in the country. It is also responsible for quarantine clearance of seed and plant material received from International Agricultural Research Centers viz., ICRISAT, ICARDA, CIMMYT, etc. ICRISAT was established in 1972 at Patancheru (near Hyderabad) to work on improvement of sorghum, pearl millet, chickpea, pigeonpea and groundnut. The quarantine clearance of all its exchanges was handled by Central Plant Protection Training Institute of Directorate of Plant Protection, Quarantine & Storage, until July 1986. This authority was later passed on to NBPGR in August 1986.
2. Forest Research Institute (FRI), Dehra Dun, for forestry plants, and
3. Botanical Survey of India (BSI) for other plants.

### **Quarantine inspection, treatment and certification procedures**

**Inspection:** Inspection of plant material is an important part of plant quarantine procedure, and may be done both in the exporting country, before issue of a health certificate and after arrival to detect any pest or disease which may have become evident during transit. Publications like manuals, hand books on individual organisms of quarantine importance are prepared with illustration by each country / region to help inspectors. The following series published by Commonwealth Mycological Institute will be useful for all countries.

1. CMI descriptions of pathogenic fungi and bacteria
2. CMI/AAB descriptions of plant viruses and
3. CMI distribution maps of plant diseases.

The various steps involved in import quarantine clearance of seed and propagating plant material is outlined below

- i. Scrutinization of import application filed along with attached documents such as phytosanitary certificate (original), permit (importer's copy), shipping bill, invoice, packing list and customs bill of entry etc., to ensure the import is in order and that no prohibited plant material is imported.
- ii. Assessment of inspection fees and registration of application.

- iii. Inspection and sampling of the consignment at port warehouses or container terminal. Sampling of seed usually carried out as per the provisions of ISTA Rules and Regulations. Whereas in case of bulk import of vegetative planting material such as cuttings/saplings/ budwoods/bulbs/tubers etc., at least a minimum of 0.1% of propagules are sampled variety and examined to ensure free from exotic pests or pathogens. In case of quarantine pests suspected, 100 per cent inspection is carried out for critical assessment of the risk.
- iv. Detailed laboratory testing
  - a. Visual inspection: The samples of seed/ propagating plant material is examined with the help of illuminated magnifier to record live insect infestation, contamination by soil and weed seeds, nematode galls, sclerotia, smut/bunt balls etc. Sometimes inspections are carried out under U.V. lamp to facilitate detection of specific seed-borne inspection by characteristic fluorescence.
  - b. X-Ray test for detecting hidden insect infestation such as bruchids and weevils that bore into seed.
  - c. Washing test to detect surface-borne oospores of downy mildew/smud spores/ bunt spores etc. and nematode cysts. Seed samples of onion, clover and lucerne are soaked for 24 to detect stem and bulb nematode and also root washings are examined for ectoparasitic nematodes.
  - d. Incubation tests such as blotter test or agar plate test carried out for detecting seed-borne pathogens such as fungi. Fluorescent pseudomonas agar used for selective detection of seed-borne bacteria.
  - e. Grow-out test coupled with indicator inoculation tests for detecting seed-borne viruses and bacteria.

Besides this, special diagnostic tests such as Electron Microscopy (dip method), Enzyme Linked Immunosorbent Assay (ELISA) are used for detection of specific viruses in the imported seed / planting material penicillase based DAC-ELISA is widely used for the detection of virus in imported seed/plant material. The detailed testing procedures for the detection of seed-borne pathogens are outlined in the seed health testing chapter.

v. Fumigation and treatment techniques: Fumigation is the versatile technique used for eliminating insect infestation. Methyl bromide is the most commonly employed for controlling insect infestation and readily adopted in quarantine programmes as the exposure time involved is short and affect all stages of insect pests and high penetrating power. Two types of fumigation viz., i. atmospheric fumigation under gas-proof sheets or chambers and ii. vacuum fumigation in vacuum chamber is widely employed. The other chemical treatments include insecticidal/fungicidal drippings or spraying or seed dressings are invariably associated with growing under post-entry quarantine conditions. The temperature treatments such as hot water treatment/ hot air treatment or vapour heat treatment are carried out to control internally borne infection/infestation and the latter particularly employed to control fruit fly infestation. Cold treatments such as refrigeration to control insect infestation in fresh fruits and vegetables. Of late, irradiation is used to control insect

infestation and spoilage of food products during storage and as well as application of high intensity electronic beams through an accelerator is under experimentation.

### **Certification**

Phytosanitary or health certificate is a certificate which should accompany a plant or plant material or seed which is to be moved from one place to another place. This certificate indicates or certifies that the material under transit is free from pests or diseases.

A model phytosanitary certificate proposed at the Government consultation on the International Plant Protection convention at Rome in 1976 (Chock, 1977) and approved by F.A.O. in 1979 is given below.

#### **MODEL PHYTOSANITARY CERTIFICATE**

(to be typed or printed in block letters)

Plant Protection Organization \_\_\_\_\_ No. \_\_\_\_\_  
of \_\_\_\_\_

To: Plant Protection Organization(s) \_\_\_\_\_  
of \_\_\_\_\_

#### **DESCRIPTION OF CONSIGNMENT**

Name and address of exporter \_\_\_\_\_  
Declared name and address of consignee \_\_\_\_\_  
Number and description of packages \_\_\_\_\_  
Distinguishing marks \_\_\_\_\_  
Place of origin \_\_\_\_\_  
Declared means of conveyance \_\_\_\_\_  
Declared point of entry \_\_\_\_\_  
Name of produce and quantity declared \_\_\_\_\_  
Botanical name of plants \_\_\_\_\_

This is to certify that the plants or plant products described above have been inspected according to appropriate procedures and are considered to be free from quarantine pests and practically free from injurious pests; and that they are considered to conform to the current phytosanitary regulations of the importing country.

#### **DISINFESTATION AND/OR DISINFECTION TREATMENT**

Date \_\_\_\_\_ Treatment \_\_\_\_\_  
Chemical (active ingredient) \_\_\_\_\_ Duration and temperature \_\_\_\_\_  
Concentration \_\_\_\_\_ Additional information \_\_\_\_\_

Additional declaration: \_\_\_\_\_

Place of issue \_\_\_\_\_  
(Stamp of Organization) Name of authorized officer \_\_\_\_\_  
Date \_\_\_\_\_

(Signature)

Note: No financial liability with respect to this certificate shall attach to.....  
(name of plant protection organization)... or to any of its officers or  
representatives.

### **DOMESTIC QUARANTINE**

Under the DIP Act, the Directorate of Plant Protection, Quarantine and storage has the responsibility to take the necessary steps and regulate the inter-state movement of plants and plant material in order to prevent the further spread of destructive insects and diseases that have already entered the country. The sole object of enforcing domestic quarantine is to prevent the spread of these diseases from infected to non-infected areas. Currently, domestic plant quarantine exists in four diseases, wart (*Synchytrium endobioticum*) of potato from 1959, bunchy top (virus) of banana from 1959, mosaic (virus) of banana from 1961 and apple scab (*Venturia inaequalis*) from 1979.

Most of the states in India have plant quarantine laws to avoid entry of plant pests and diseases.

1. Bunchy top of banana: The export and the transport from the States of Assam, Kerala, Orissa, West Bengal, Tamil Nadu to any other State of Banana plant or any other plant of the genus *Musa*, including sucker, stem, leaf, flower, and any other part thereof which may be used for propagation, or the materials of banana plant or any other plant of the genus *Musa*, which are used for packing and wrapping, excluding the banana fruit is prohibited.

2. Banana mosaic : The export and transport from the States of Maharashtra and Gujarat of any plant of Banana or any other plant of genus *Musa* including the sucker, stem, flower and any other part thereof, but excluding leaf and fruit thereof is prohibited; vide Government of India notification No.F. 6-10-PPS dated the 11<sup>th</sup> April, 1961.

3. Potato wart: The export to potato tubers from the State of West Bengal to any other State or territory of India is prohibited.

4. Apple scab: The Directorate of Horticulture, Himachal Pradesh worked out a detailed scheme for the eradication of scab, and also issued a notification No.NIC.20/76 dated 28 December 1978, prohibiting the export of planting material of apple outside the State.

In Tamil Nadu as per Madras pests and Diseases Act of 1919, quarantine regulations are periodically enforced. e.g., cardamom mosaic prevalent in Anamalai area of Coimbatore District and is free from Nelliampatti area. Hence

the movement of diseased plant material from Anamalai to Nelliampatti area is prevented.

### **Limitations**

There are many limitations to implementing domestic plant quarantine in India due to the vastness of the country and the unrestricted movement of plant material from one state to another. As a result the diseases like bunchy top and mosaic of banana have spread to several other states. However, the wart disease, golden nematode of potato, and scab of apple are restricted in the states where they were initially noticed.

### **Export regulations**

In India the plant quarantine measures for exporting plants and material including seeds have been streamlined and rigid inspections are enforced before the material is allowed to be landed into the country. At present plant quarantine regulations differ with different countries for major agricultural commodities that are being exported out of India. The Central Government has authorized officers of the Directorate of Plant Protection, Quarantine & Storage, ICAR Research Institutes, National Institutes like Forest Research Institute, Botanical Survey of India, and the Directorates of Agriculture of all States.

The quarantine authorities have also framed terms and conditions pertaining to inspection, fumigation or disinfection of the exportable plants and plant material in India including the following schedule/or fee for inspection and issue of phytosanitary certificate, and/or fumigation or disinfection in respect of plants, plant material, seeds, and plant products to issue phytosanitary certificate. All the plants and plant material are subjected to inspection by officials issuing certificate. Infested materials are given necessary treatment with chemicals and fumigated if necessary.

The list of plant quarantine and fumigation stations in India is given below.

#### ***Punjab***

1. Plant Quarantine and Fumigation Station, Hussainiwala, Ferozepur District.
2. Plant Quarantine and Fumigation Station, Attari – Wagah Border, near Attari Bus Stand, Attari, Ferozepur District.
3. Plant Quarantine and Fumigation Station, Civil Aerodrome, Rajasansi, Amritsar.

#### ***New Delhi***

4. Plant Quarantine and Fumigation Station, Palam Airport, New Delhi – 10.
5. Plant Quarantine and Fumigation Station, Garden Reach Road, Calcutta– 24.
6. Plant Quarantine and Fumigation Station Sukhiapokri, Darjeeling District.

#### ***Gujarat***

7. Plant Quarantine and Fumigation Station, Haryana Plot No.75, Behind Yusuf Bagh. Bhavnagar.

***Maharashtra***

8. Plant Quarantine and Fumigation Station, Haji Bunder Road, Sewri, Mumbai – 15.
9. Plant Quarantine and Fumigation Station, The Harbour, Visakhapatnam – 1.

**Tamil Nadu**

10. Plant Quarantine and Fumigation Station, 6, Clive Battery, Chennai – 1.
11. Plant Quarantine and Fumigation Station, 335, Beach Road, Tuticorin – 1.
12. Plant Quarantine and Fumigation Station, Tiruchirappalli Airport, Tiruchirappalli.
13. Plant Quarantine and Fumigation Station, 110, Railway Feeder Road, Rameswaram.

***Kerala***

14. Plant Quarantine and Fumigation Station, Willingdon Island, Cochin – 3.



# 8

## Seed Health Testing

Seeds are complex, biologically dormant entities and contain a living embryo. Fungi, bacteria or viruses may be associated with them. A number of plant pathogens are transmitted through seeds either externally or internally. Detecting the presence or absence of the pathogens help in protecting field performance of seed samples in relation to emergence and subsequent disease development. Heavily infested seed samples are rejected before sowing after seed health testing is completed. Certification of seed samples free of seed borne pathogens is an important criterion for high quality seeds. Seed health testing methods have been standardized by International seed Testing Association (ISTA). Seed health testing determines whether or not a seed lot should be treated with a fungicide or other treatment based on the nature and degree of infection associated with the seeds. It helps to develop more precise seed treatment and to avoid unnecessary seed treatment of healthy seeds.

The following are the important methods of seed health testing.

### **I. Fungi**

1. Dry seed examination
2. Examination of seeds after softening
3. Seed washing technique
4. Incubation method
  - a. Blotter method
  - b. Agar plate method
5. Seedling symptom test: Rolled paper towel test.

### **II. Bacteria**

1. Dry seed examination
2. Isolation on agar media
3. Seedling symptomatology test
4. Infectivity test
5. Serological methods
6. Phage-plague method

### **III. Viruses**

1. Dry seed examination
2. Growing-on test
3. Infectivity or Indicator – inoculation test.
4. Serological methods
  - a. Microprecipitin test
  - b. Gel-diffusion test
  - c. Agglutination test

- d. Labeled antibody
  - e. Immuno fluorescent test
  - f. Radio isotope – labeled antibody test
  - g. Enzyme Linked Immunosorbent Assay (ELISA)
  - h. Enzyme Linked Fluorescent Assay (ELFA)
  - i. Serologically Specific Electron Microscopy (SSEM)
5. Electron microscopy test

## I. DETECTION OF SEED BORNE FUNGI

**1. Dry seed examination:** Certain fungi can be detected by direct observation of dry seeds or by using a stereobinocular bright-field microscope or hand lens to detect. A sample of 400 seeds (in replication of 100 seeds each) may be drawn and examined.

- a. Seed discolouration
- b. Morphological abnormalities
- c. Fungal fruiting bodies associated with the seeds.

Seed infection can result in abnormalities in seed coat, colour, seed shape, reduction in seed size and production of fruiting structures.

**a. Seed discolouration:** Purple discolouration of soybean seed and guar seed coat show infection of seeds by *Cercospora kikuchii*. Grey or brown discolouration of soybean seeds is observed in seeds veggia. Indefinite black spot and blemishes are observed on soybean seeds infected with *Macrophomina phaseolina*. *Phomopsis sojae* causes discolouration, fissures, flatter, and greyish white mycelial growth on the seeds. White seeded *Phaseolus* bean seeds show olivaceous green discolouration when they are infected with *Alternaria alternata*. Wheat seeds infected with *A. tritici* are shriveled and entire seed surface is discoloured. Wheat seeds infected with *Drechslera sorokiniana* are smaller, light weight and discoloured. Dark brown to black spots on the seed surface in rice is mainly due to *D. oryzae*, such discolouration may also be due to *Curvularia*, *Alternaria*, *Fusarium*, *Phnom* and *Pyricularia oryzae*. Wheat grains infected by Karnal bunt fungus, *Neovossia indica* are converted partially into a black powdery mass. Grey to brown wrinkled and deformed mungbean seeds are due to *Macrophomina phaseolina* and *Fusarium equiseti*.

**b. Morphological abnormalities:** Abnormal shape and reduced seed size are caused by fungal infections. In stem gall of coriander (*Protomyces macrosporus*), the fruits become partially or completely hypertrophied and they carry chlamydospores. *Septoria nodorum* and *S. tritici* cause shriveling of wheat seeds.

**c. Fruiting structures:** Fruiting structures, sclerotia of *Claviceps fusiformis* (ergot of pearl millet), *C. purpurea* (ergot of rye) and *Sclerotinia sclerotiorum* (white rot of legume and sclerotial rot of cabbage) are found unite with healthy seeds.

**d. Observation under a bright field microscope:** It reveals the presence of chlamydospores, oospores, spores etc., on seed surfaces. Seed borne infection of the following diseases is determined and chlamydospores (smut spores) are produced in them

Wheat Karnal bunt	-	<i>Neovossia indica</i>
Wheat hill bunt -		<i>Tilletia caries &amp; T.foetida</i>
Wheat dwarf bunt	-	<i>T. controversa</i>
Rice bunt	-	<i>N. horida</i>
Wheat flag smut	-	<i>Urocystis agropyri</i>
Barley covered smut	-	<i>Ustilago hordei</i>
Oat loose smut	-	<i>U. avenae</i>
Oat covered smut	-	<i>U. hordei</i>
Corn smut	-	<i>U. maydis</i>
Corn and sorghum head smut	-	<i>Sporosporium reiliana</i>
Sorghum loose smut	-	<i>S. cruenta</i>
Sorghum grain Smut	-	<i>S. sorghi</i>
Sorghum long Smut	-	<i>Tolyposporium ehrenbergii</i>
Pearlmillet smut	-	<i>T. penicillariae</i>

Pycnidia are observed on the seed surfaces in the following diseases:

Black leg of sugarbeet	-	<i>Phoma betae</i>
Leaf spot of celery	-	<i>Septoria apii</i>
<i>Ascochyta</i> blight of pea -		<i>Ascochyta pisi</i>
Pasmo of flax	-	<i>S. linicola</i>

Oospores are observed on the pearlmillet seeds infected by *Sclerospora graminicola* causing downy mildew disease. Soybean seeds carry oospores of *Peronospora manshurica* and safflower seeds carry teliospores of *Puccinia carthami*.

**e. Observing under near ultraviolet (NUV):** Observing the seeds under NUV light can detect seed borne infection in the case of pea seeds infected with *Ascochyta pisi* (yellow green fluorescence) and *Stemphylium botryosum* (dull orange fluorescens).

**2. Examination of seeds after softening:** This method is useful for detecting fungal infections in which spores are liberated into water after soaking, e.g. *Polyspora lini* in flax seed. Infection of *P. lini* in flax seed shows small, hyaline, oval shaped conidia under X 100 magnification.

**3. Seed washing technique:** It is used to detect seed borne fungi carried as a spore on the seed surface. This method provides quick result. This method is only useful for detection of pathogens which adhere to seed surface in the form of identifiable spores. Hyphal infection or spore viability is not revealed. If spores are within the seeds or glumes and not on seed surfaces they may not be detected.

Two grams of seeds are placed in a flask containing detergent and sufficient water (5 ml) for soaking. Then the flask is shaken on a mechanical

shaker for 5 to 10 minutes. The suspension is centrifuged at 2,500 to 3,000 rpm for 10 to 15 minutes. The pellet is resuspended in water and examined under a bright field microscope for fungal structures. This method is quick. It can be adopted for detecting conidia, chlamydospores, oospores and smut spores of some fungi like *Alternaria*, *Curvularia*, *Drechslera*, *Fusarium*, *Pyricularia*, *Cephalosporium*, *Septoria*, *Peronospora* and *Tilletia*.

**4. Incubation method:** The two methods used for examination of crop seeds for fungal infections viz., blotter method and agar plate method. These methods are suitable for infections accompanied by hyphae, fruiting structures or spores. They are effective for detecting most of the seed borne fungi. Identification of the fungi is made based on the fungal morphology on the seed surface on blotters or on colony characters on an agar medium.

**a. Blotter methods:** This is a simple and inexpensive method. The basic principle in this method is to provide a high level of relative humidity and optimum light and temperature conducive for fungal growth. Blotters (three discs) are soaked in sterile distilled water and placed in sterilized Petri dishes after draining of excess water. Moistening the filter paper with a solution of antibacterial agent reduces bacterial growth. Generally 400 seeds are drawn for this test with replication of 100 seeds each. Twenty five seeds (sixteen in the outer circle, 8 in the middle and one seed in the centre) are placed in the blotters at equal distances. The Petri plates are incubated at under near ultraviolet (NUV) or fluorescent light with a 12 h cycle light and dark at  $20 \pm 2^\circ\text{C}$ . Seeds are examined 7 days after incubation using a stereobinocular microscope. Then the fungal species is identified based on the mycelial, conidiophore and conidial characters and production and appearance of fruiting bodies such as acervuli, pycnidia and sporodochia. In this method *Colletotrichum*, *Curvularia*, *Drechslera* and *Fusarium* on the seeds are identified. This test helps to evaluate severity of infection on each seed and seedling which is important in the epidemiological studies in the field. In blotter method, fast germination and lifting of seed coat are observed in crops like cabbage and rice. This results in difficulties in evaluating and identifying the microorganisms. To overcome this, 0.2% solution of 2, 4 – D (Dichlorophenoxy acetic acid) is added to blotters and incubated at  $22^\circ\text{C}$  for 7 days.

Deep freeze blotter method is used for seed health testing of maize kernels for *Fusarium moniliforme* and *Cephalosporium acremonium*, carrot seeds for *Alternaria* and *Stemphylium* and cabbage seeds for *Plenodomus lingam*. The Petri plates containing the seeds are incubated for one day at  $20^\circ\text{C}$ , then at  $-20^\circ\text{C}$  the second day and then for 5 days under 12 h dark and light at  $20^\circ\text{C}$ . This method is easy, time saving and gives higher counts compared to routine blotter method.

**b. Agar-plate method:** This method is used for identification of microorganisms associated with seeds based on growth and colony character on nutrient medium. Usually Malt extract agar (MEA) and Potato dextrose agar (PDA) are employed.

Seeds are treated with 1% sodium hypochlorite ( $\text{NaOCl}$ ) solution (to prevent saprophytic fungi) for 5 to 10 min. Usually 10 seeds are placed on the medium in the Petri dish and incubated at  $20 \pm 2^\circ\text{C}$  for 7 days. The plates are

examined periodically and the fungi are identified based on the cultural characteristics. Selective medium may be used for specific fungi.

**5. Seedling symptom test – Rolled paper towel test:** This method is used for measuring seed germination in seed testing laboratories. In this method, the seeds are placed between layer of filter paper, blotters or paper towels. The towels are rolled and placed flat or in an upright position on germinator trays in incubators with a relative humidity between 90 to 95%. Normal seedlings, abnormal seedlings, ungerminated seeds and hard seeds are recorded. Pathogens which cause seed decay, seedling blight or seedling abnormalities are detected.

Pathogen		Symptoms
1. <i>Ascochyta</i> sp. on pea	-	weak, decayed seedling with stunted primary roots.
2. <i>Alternaria linicola</i> and <i>Botrytis cinerea</i> on flax	-	decay of seedlings.
3. <i>Alternaria dauci</i> on carrot	-	severe rotting of seedlings.
4. <i>Septoria nodorum</i> on wheat	-	malformed coleoptile with brown spots and swellings.
5. <i>Drechslera oryzae</i> , <i>Alternaria padwickii</i> , <i>Pyricularia oryzae</i>	-	decayed roots and shoots. on rice.

## II. DETECTION OF SEED- BORNE BACTERIA

**1. Dry seed examination:** Bacterial infection on the seed can be visually observed. In this method symptomless seeds carrying bacteria in them cannot be found out. This method is employed in the following cases.

Host	Pathogen	Symptom
Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	dark tan
Rice	<i>Pseudomonas syringae</i> pv. <i>oryzicola</i>	glume blotch
Bean	<i>X. axonopodis</i> pv. <i>phaseoli</i>	hilum discolouration
Bean	<i>X. axonopodis</i> pv. <i>phaseoli</i>	darkened areas.
Bean	<i>Corynebacterium flaccumfaciens</i> pv. <i>violaceum</i>	purple discolouration and shriveling
Soybean	<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	cream colouration of seed coat
Pepper	<i>C. michiganense</i> pv. <i>michiganense</i>	brown discolouration and smallness of

		seeds
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**2. Isolation on agar media:** Certain bacteria are capable of fluorescing, hydrolyzing starch or growing on selective media and these characters are used to isolate them from seeds. Differences observed among colony characters on agar media of different species are not reliable. Examples are *X. oryzae* pv. *oryzae* on rice *X. axonopodis phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* on beans, *Pseudomonas syringae* pv. *phaseolicola* on beans, *P. syringae* pv. *pisi* on pea, *X. campestris* on *Brassica*.

**3. Seedling symptomatology test:** This is primarily used to detect the following bacterial pathogens. The incubation conditions of light and temperature vary with the seed – borne bacteria. *P. syringae* pv. *glycinea* on soybean, *P. syringae* pv. *lactrymans* on cucumber, *X. campestris* pv. *campestris* on cabbage, *X. oryzae* pv. *oryzae* on rice, *X. campestris* pv. *vesicatoria* on pepper and *X. campestris* pv. *zinniae* on zinnia.

**4. Infectivity test :** Indicator or susceptible host plants are inoculated with seed washings and seed extracts for detection of some bacteria. The seeds are assayed by macerating dry seeds in warring blender and then generally shaking them in water. The washings are inoculated to healthy leaves following specific methods and their symptoms are observed after a specified period. E.g., *Pseudomonas syringae* pv. *glycinea* on soybean, *X. campestris* pv. *campestris* on cabbage. *P. syringae* pv. *phaseolicola* on beans and *Corynebacterium michiganense* pv. *michiganense* on tomato.

**5. Serological methods:** Microprecipitin test is more accurate and rapid than the tube agglutination or gel-diffusion tests. Serological testing of bean seeds is done as a routine in some countries to detect *P. syringae* pv. *phaseolicola* on bean seeds, *X. campestris* pv. *manihotis* is detected in cassava seed embryos by ELISA.

**6. Phage-plague method:** The basic principle lies in the increase in the number of specific bacteriophage particles in the presence of susceptible bacteria. This method is followed in detecting *C. michiganense* pv. *michiganense* in tomato, *P. syringae* pv. *atrofaciens* in wheat, *P. syringae* pv. *phaseolicola* in beans, *X. oryzae* pv. *oryzae* in rice, *X. axonopodis* pv. *phaseoli* in beans and *X. Campestris* pv. *vesicatoria* in tomato.

### III. DETECTION OF SEED-BORNE VIRUSES

Viruses are obligate parasites and they differ in their genetic make up and require special techniques for detection. Seed borne nature of the virus is found by either raising seedlings from infected seeds or observing symptoms or by using assays of seed extract.

**1. Dry seed examination:** Visual observation of seeds may reveal abnormalities like discolouration, shriveling, reduced seed size, staining and seed coat necrosis.

**a. Discolouration :** Mottling, hilum colour break or hilum bleeding of seeds with light-coloured seed coats can indicate that such seeds have come from virus-infected plants, eg., seed coat mottling caused by soybean mottling virus

and soybean stunt virus on soybean seeds and necrosis and deformation of tomato seeds by TMV.

**b. Reduced seed size:** Small seeds are produced in barley due to barley streak mosaic virus (BSMV)

**c. Shriveled and wrinkled seeds:** A high percentage of small and shriveled seeds are produced in cowpea due to cowpea aphid-borne mosaic virus. (CABMV)

**2. Growing on test:** Growing on test is used to determine virus seed transmission. Seeds are planted in blotter paper, sand, soil, vermiculite or in other growth medium. Seedlings are examined at regular intervals for the virus symptom. The test should be conducted under insect proof condition. Light and temperature conditions should be provided for optimum plant growth and symptom expression. Blotter method is used for detection of Tomato blackring virus in *Petunia*, Tobacco ringspot virus, Arabis mosaic virus and BSMV in barley. Seeds are planted in pots containing soil or sand to detect Bean common mosaic virus (BCMV) in blackgram, BSMV in barley, lettuce mosaic virus (LMV) in barley cowpea aphid-borne mosaic virus in cowpea, SMV in soybean and squash mosaic virus in muskmelon. The reliability of this test depends upon symptom expression. The test is not reliable when distinct symptoms are not produced.

**3. Infectively or Indicator – Inoculation test:** Viruses can be detected in seeds by assaying the extracts of different parts of seeds and seedlings raised from infected seeds on suitable indicator plants. Susceptible hosts which produce local lesions or systemic symptoms are used as indicator plants. This test has been used to detect BCMV in bean and urdbean, LMV in lettuce, TMV in tomato and tobacco ring spot virus in soybean.

**4. Serological methods:** Serological methods are used for characterization and determining relationship between viruses. The tests are based on the reaction between an antiserum, a blood serum containing specific antibodies produced by injecting laboratory animals with a pure virus preparation, and an antigen-virus protein. The tests are specific since an antibody combines only with the antigen which contains similar grouping of amino-acid sequences. The union of antigen and antibody can be detected in the form of precipitation and agglutination. Common serological tests used for detection of seed borne viruses are as follows.

**a. Microprecipitin test:** The microprecipitin test is a simple serological test in which drops of crude or purified seed extracts combine with their specific antibody and result in a macro or microscopically visible precipitate. The test can be carried out on microscope slides, in test tubes, or in culture plates. BCMV in urdbean seeds can be detected by this test.

**b. Gel- diffusion test:** Gel diffusion test are performed in semi-solid media. It is a serological testing which the antibody and antigen reactants diffuse towards each other in gel and react to form a visible precipitation line. There are two types of tests viz. single or radial diffusion and double diffusion.

**i. Single or radial diffusion test:** In single diffusion, the antigen diffuses into the agar containing the antiserum. The test is performed in culture plate or test tubes. Seed or seedling (from infected seeds) extracts are tested by mixing the corresponding antiserum in agar prior to solidification. The virus diffuses

radially in culture plates and the procedure is referred to as a “radial diffusion test”. The tests are used to detect BSMV in barley and wheat seed.

**ii. Double diffusion test:** In double diffusion both antibody and antigen diffuse toward each other in a gel medium. At the site where they meet, a positive reaction results in a precipitin line. This test is suited for small, isometric (spherical) viruses which diffuse easily through agar, such as arabis mosaic, blackgram mosaic (a strain of broadbean mottle), cowpea ringspot, cucumber mosaic (mungbean strain), squash mosaic, tobacco ringspot and tomato blackring. The double-diffusion test is suitable for the detection of BSMV in barley and TMV in tomato.

**c. Agglutination test:** In the agglutination test the antibody or virus antigen is absorbed on to larger particles. A positive reaction causes these larger particles to clump and so the antibody-antigen reaction is visibly amplified. Chloroplast agglutination test is used in detection of BCMV in bean seeds. Latex agglutination test is specific for diagnosis of BSMV and SMV in young seedlings in barley and soybean seeds respectively.

**d. Labeled antibody test:** The use of the labeled antibodies antiserum has been used to detect seed borne viruses in individual seeds with accuracy and specificity.

**e. Immuno fluorescent microscopy test:** This test is used for detecting SMV in soybean seeds.

**f. Radio isotope-labeled antibody test:** Antiserum containing radiolabel antibodies is used for detecting squash mosaic virus in cantaloupe seeds.

**g. Enzyme Linked Immuno sorbant assay (ELISA):** It is also known as double antibody sandwich. It is a serological test in which the sensitivity of the antibody antigen reaction is increased by attaching an enzyme to one of the two reactants. It is useful for the identification of large number of plant samples. This method has been used to detect SMV in soybean seeds, BSMV in barley seeds, LMV in lettuce seeds, BCMV in bean seeds and TRSV in soybean. This method is relatively complex and expensive but it is the most sensitive and reliable test of all the plant virus serological tests.

**h. Enzyme linked fluorescent assay (ELFA):** It involves the utilization of fluorescent substrate such as A-methyl umbelliferyl phosphate (MUP) to enhance the sensitivity of ELISA technique.

**i. Serologically specific electron microscopy (SSEM) :** This method is used to detect seed borne viruses like BCMV in bean; LMV in lettuce, TRSV in soybean, pea seed borne mosaic virus in pea and SMV in soybean.

**5. Electron microscopy test:** It is used to study the biophysical properties of plant viruses. Viruses can be detected in seed extracts of ultra thin sections of infected seed parts or seedlings. Bean common mosaic virus in bean, BSMV in barley, SMV in soybean and LMV in lettuce are detected utilizing this method.



# 9

## Disease Management Through Host Resistance

A physiological deviation from the normal functioning of the organism (i.e., the crop plant) caused by pathogenic organisms is a disease and may be caused by fungi, bacteria or viruses. The inherent ability of an organism (i.e., the crop plant) to resist or withstand the pathogen is called resistance. Disease resistance commonly met with in the plant kingdom relative in nature, total immunity being too rare. Its hereditary transmission from parent to off-spring is essentially “Mendelian”, but often polygenic. The earliest demonstration of the behaviour of “disease-resistance” as a character transmissible from parent to off-spring in the “Mendelian” fashion was given by Biffen (1905) in his work on yellow rust of wheat. Since then, intensive work has been done on this aspect which has proved the value of applying genetical principles in developing disease-resistant varieties of plants for effective control of diseases.

Resistant varieties can be the simplest, practical, effective and economical method of plant disease control. The use of resistant varieties cannot only ensure protection against diseases but also save the time, energy and money spent on other measures of control. In addition to these advantages, resistant varieties, if evolved, can be the only practical method of control of such diseases as viruses, phytoplasmas wilts, and rusts etc. in which chemical control is very expensive and impractical. In crops of low cash value, chemical and other methods of control are often too expensive to be applied. In such crops development of varieties resistant to important diseases can be an acceptable recommendation for the farmer.

Pathogenicity is the ability of a pathogen to attack a host. Pathogenicity includes virulence and aggressiveness. Virulent strains of pathogen cause much severe symptoms of the disease and they carry the virulence gene that enables it to attack a particular host genotype. Virulence is due to the action of one or a few genes. An aggressive strain of a pathogen causes severe disease on all the host genotypes which they are able to attack and aggressiveness is polygenically inherited.

### **Host – Pathogen relationship**

A disease is the result of an interaction of genes governing resistance in the host with those governing pathogenicity in the pathogen. The resistance of a crop to a physiological race of the pathogen depends not only on the genotype of the host for resistance, but also upon the genotype of the pathogen for virulence or aggressiveness. Flor (1942) proposed the gene-for-gene hypothesis, according

to which, for every gene for resistance in the host, there is a corresponding gene for pathogenicity in the pathogen. It means that there are atleast two alleles at a locus controlling resistance/susceptibility in the host (R-r) and two alleles at a corresponding locus in the pathogen (V-v) controlling virulence / aggressiveness. Out of the four possible interactions between these alleles, only one combination leads to the expression of resistance. The demonstration of gene-for-gene relationship requires genetic studies of both the host and the pathogen.

#### Pathogen

	V <sub>I</sub>	v <sub>I</sub>	+ Pathogen can infect; the host is
R <sub>I</sub>	-	+	susceptible
r <sub>I</sub>	+	+	- Pathogen cannot infect; the host is
			resistant

The demonstration of gene-for-gene relationship requires genetic studies of both the host and the pathogen

#### Vertical resistance (VR) and horizontal resistance(HR)

Van der Plank (1960) has discussed the whole issue of disease resistance in a different perspective. He calls the unstable and often complete type of resistance as vertical resistance and the more stable but somewhat incomplete resistance as horizontal resistance.

If resistance to some races of a pathogen is more than to other races, it is called **Vertical resistance**. It is also called Perpendicular resistance, Physiological resistance, seedling resistance, hypersensitivity, race specific resistance or qualitative resistance. As it is conditioned by one or a few genes, it is called major gene or monogenic or oligogenic resistance.

Resistance to more than one race of the pathogen or to many or all races of the pathogen is called **Horizontal Resistance**. It is non-specific resistance governed by polygenes. It is severally termed as non-specific, general, polygenic, minor gene, mature plant, adult, quantitative resistance, partial or field resistance or tolerance. HR causes reduction in the number and rate of sporulation of the pathogen on the host and slows down the infection rate. HR includes tolerance slow development of disease, escape and exclusion mechanisms besides hypersensitive reaction.

The difference between vertical resistance and horizontal resistance are given in table.

Table: Differences between vertical and horizontal disease resistance

Feature	Vertical resistance	Horizontal resistance
1. Pathotype-specificity	Race specific	Race nonspecific
2. Nature of gene action	Oligogenic	Polygenic; rarely oligogenic
3. Response to pathogen	Usually, hypersensitive	Resistant response

4. Phenotypic expression	Qualitative	Quantitative
5. Stage of expression	Seedling to maturity	Expression increases as plant matures (Adult plant)
6. Selection and evaluation	Relatively easy	Relatively difficult
7. Risk of 'boom and bust'	Present (rarely durable)	Absent (durable)
Suitable for: a. Host  b. Pathogen	Annuals but not perennials  Immobile pathogen, e.g., Soil pathogens, but for mobile air-borne, pathogens	Both annuals and perennials  All pathogens
8. Need for specific deployment of resistant varieties	Critical for success with mobile pathogens	None
9. Need for other control measures	Likely	Much less likely
10. Host-pathogen interaction *	Present	Absent
11. Efficiency	Highly efficient against specific races	Variable, but operates against all races

\* Detectable by analysis of variance of a suitable experiment

Vertical resistance to specific races is generally governed by a single (monogenic) dominant gene or by a few dominant genes. Some of these genes may be multiple alleles as in leaf rust gene, **Lr2** that accords resistance to *Puccinia recondita tritici*. In that locus, four genes designated as **Lr2a**, **Lr2b**, **Lr2c** and **Lr2d** are present and are tightly linked. Each of these genes accord resistance to a different spectrum of races and hence can be differentiated from one another. Such multiple alleles exist on **Sr9** locus of wheat for *P. graminis tritici* and gene **Pi-k** in rice for resistance to *Pyricularia grisea*. The tight linkage between the multiple alleles permits an efficient transfer of all these genes in one attempt.

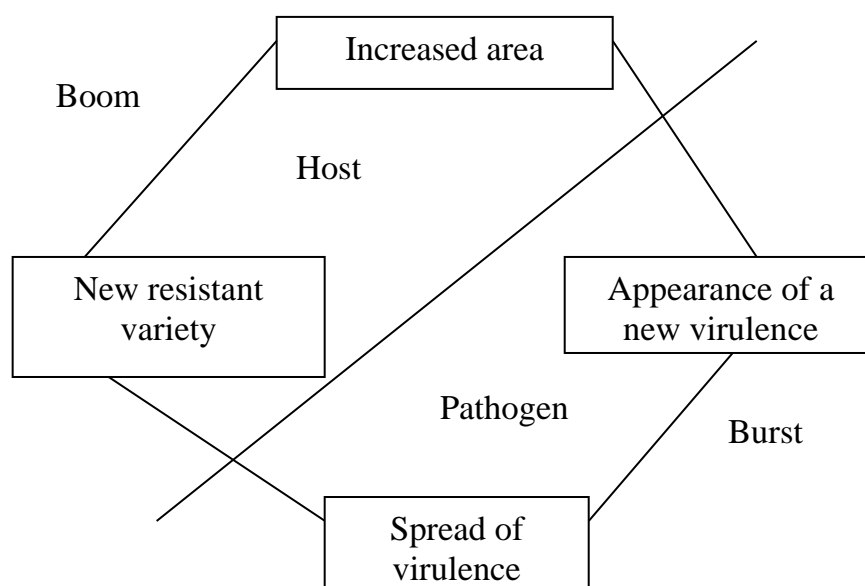
'Horizontal resistance' (HR) reduces the rate of disease spread and is evenly spread against all races of the pathogen. The low terminal disease severity in HR is assumed to result from polygenic resistance. Morphological features such as size of stomata, stomatal density per unit area, hairiness, waxiness and several others influence the degree of resistance expressed. Partial resistance, dilatory resistance, lasting resistance are some other terms coined for denoting horizontal resistance. The phenomenon of slow rusting manifested as lesser number of pustules per unit leaf area, smaller size of uredosori and

increased latent period in some wheat cultivars is a typical example of this type of resistance.

Although it is preferable to use varieties that have both vertical and horizontal resistance, most of the resistant varieties carry only one or few (2 or 3) major genes of vertical resistance. If varieties are resistant only to some of the races of pathogen and if the pathogen is airborne, then new races evolve easily, as happens with cereal rusts, the powdery mildew and *Phytophthora infestans*. Appearance of new races led to breakdown of resistance of the popular, ruling genotype. As a result, varieties with vertical resistance need to be replaced at frequent intervals.

### Boom and burst cycle

In varietal improvement programmes, it is easy to incorporate the monogenic vertical resistance genes. But the success of exploiting the monogenic host resistance invariably does not last long. Whenever a single gene-based resistant variety is widely adopted, the impact would be the arrival of new matching pathotypes. These pathotypes soon build up in population to create epidemics and eventually the variety is withdrawn. This phenomenon is generally called “boom and burst”.



### Boom and burst cycle- a characteristic of vertical resistance

To avoid the implications of boom and burst phenomenon, use of durable host resistance is advocated in several crops. Durable resistance remains effective even though it may be widely grown over a long period of time, in an environment that favours the disease. For example, oat variety, Red Rust Proof is still resistant against crown rust even after a hundred years. Wheat varieties, Thatcher and Lee have withstood stem rust for 55 and 30 years, respectively. Cappelle Desprez expresses at adult stage, a moderate resistance to yellow rust and this has been maintained for the last 20 years. Two of the genes like **Lr34**

for resistance of leaf rust and **Sr2** for resistance to stem rust have been recognized for durability. Wheat cultivars such as HD2189, HP1102, DL153-2, DL803-3 and DL802-2, which possess **Lr34** with other gene combinations have a good degree of resistance and have become popular with growers. So far, there is no precise way available to identify the genetic components that are associated with durable resistance. Nor does dissociation of genes for virulence totally explain the basis of varietal durability, though it is likely to be the most plausible reason.

### **Resistance to virus and virus vectors**

Resistance to plant pathogenic viruses is generally oligogenic in nature. For example, the host pathogen reaction to the barley yellow dwarf virus (BYDV) is controlled by detectable single gene. The discovery of **Yd2** gene in Ethiopian barley further confirms that against some of the viral diseases, vertical resistance is very much functional. Antibiosis is the most common phenomenon where the host plant metabolites interfere with the normal life and growth of the insects following feeding activity. Invariably, the adult body weight, fecundity and various facets of multiplication of the insects are adversely affected. The number of life cycles completed in a given period of time is also less. Therefore, in plants that exhibit antibiosis towards crop maturity, there is marked reduction in the level of pest infestation (virus vector population) and host damage.

### **Mechanism of disease resistance or Nature of disease resistance**

Disease resistance is governed by several in-built mechanisms of the host, plants against infection by the pathogen. They are disease escape, disease endurance or tolerance and true resistance.

**a. Disease escape:** It is a prevention mechanism that causes the host to escape pathogenic infection. Early or late maturity of the crop may prevent physical contact of the pathogen with the host. Mechanical and anatomical barriers such as thick cuticle, waxy bloom on leaves and stem, stomatal regulation prevent penetration of spores. Ergot, a fungal disease of inflorescence in cereals caused by *Claviceps purpurea* does not affect varieties of wheat and barley in which the flowers remain closed until pollination occurs. Erect leaves of barley avoid deposition of spores of *Erysiphe graminis tritici* in contrast to prostrate leaves. Early maturing varieties of groundnut escape early leaf spot infection (*Cercospora arachidicola*) and early varieties of wheat escape rust and loose smut infection. A change in planting season has also been successfully employed as a measure of securing escape, e.g., the leaf rust of sugarcane (*Puccinia sacchari*) in the canal areas of Bombay severely affects cane when planted in June, but is of minor importance or absent in crops sown in October. Disease escape confers pseudo-resistance.

**b. Disease endurance:** The host after being infected by the pathogen tolerates the infection and suffers less damage. It does not result in any substantial decrease in yield. This is brought about by influence of external factors. It is a well-known phenomenon that plants fertilized with phosphatic and potash manures are more tolerant to disease; this is the case in wheat against rust infection. Rice crops fertilized by silicates are “resistant” to blast (*Pyricularia*

*oryzae*) in Japan. Wheat crops fertilized by potash and phosphatic manures are highly tolerant to mildew and rust infection. The fertilizers act indirectly to arrest vegetative growth and promote early maturity, better straw and strengthening tissues to protect the plant which form a bulwark against pathogenic invasion.

**c. True resistance:** It is the ability of the host plant to resist or withstand the attack of a pathogen. True resistance is inheritable and much less subject to environmental influence. It is specific in character. The basis of resistance may be morphological, functional, structural or protoplasmic. Functional nature of resistance is determined by opening of the stomata, time of opening of flowers and time of maturity, rate of cork formation and cambial activity. Structural characters include the proportion of strengthening tissues, fibre content, nature of middle lamella, corky layers, number and structure of stomata and lenticels and their sizes. Protoplasmic factors controlling resistance are related to cell contents and include acids, tannins, anthocyanins, chemical constituents and their proportion, antibiotic activity and hypersensitivity present in the plant cells and in addition biological antagonism of the protoplasm of the host and the pathogen. True resistance, however, is of a specific character and is determined by the defence equipment and activities of the plant itself against the parasitic invasion and is therefore not subject to any appreciable modifications by external factors.

### **Methods of breeding for disease resistance**

The methods of breeding varieties resistant to diseases do not differ greatly from those adopted for other characters. The following methods are used:

1. Introduction,
2. Selection,
3. Hybridization followed by selection,
4. Back cross method,
5. Induced mutagenesis,
6. Development of multilines and
7. Tissue culture techniques.

#### **1. Introduction**

It is a very simple and inexpensive method. Varieties resistant to a particular disease elsewhere may be thoroughly tested in the regions in which they are proposed to be introduced. Their yield performance and disease resistance should be confirmed by large scale cultivation. It is possible that a variety resistant in one region need not be resistant in another region due to variation in the physiological race of the pathogen or due to a much different agroclimatic condition in the new location.

Introductions have served as a useful method of disease control. For example, Ridley wheat introduced from Australia has been useful as a rust resistant variety. Manila, a rice variety introduced in Karnataka from the Philippines, has tolerance to blast, bacterial leaf blight and sheath blight. Intan, a Javanica type rice variety introduced in Karnataka from Indonesia is highly resistant to blast. Munal, a rice variety introduced in West Bengal from the U.S.A. is tolerant to blast, bacterial leaf blight and leaf folder (pest). Some of IRRI rice varieties such as IR 20, IR.24, IR.28, IR.34, IR.36 and

IR .50 possess resistance to one or more diseases. Early varieties of groundnut introduced from U.S.A. have been resistant to leaf spot (*Cercosora arachidicola*). Kalyan Sona and Sonalika wheat varieties originated from the segregating materials introduced from CIMMYT, Mexico and were rust resistant. Introductions also serve as sources of resistance in breeding programmes. For example, African pearl millet (*P. americanum*) introductions have been used for developing downy mildew resistant male sterile lines (Tift 23A cytoplasm) for use in hybrid pearl millet production. This is an important development in the hybrid pearl millet programmes since the original male sterile lines Tift 23A and 23D<sub>2</sub>A were extremely susceptible to downy mildew. The introduction of Co.475 variety of sugarcane in Mumbai has conquered red rot but brought in leaf rust and whip smut to the fore.

## **2. Selection**

This is better method than introduction and has more chances of success in obtaining disease-resistant plants. The work of selection is carried out either in the naturally infected fields under field conditions or under artificially inoculated conditions. The resistance in such individuals will occur in nature by mutation. To ensure the resistant character of a plant, large population of crop plant may be exposed to the attack of pathogen under artificial conditions and the non-infected plants may be chosen. Suvarnamodan rice of Kerala is a pure line of ARC. 11775 and is highly tolerant to blast. Sugandh of Bihar is a selection from Basmati rice of Orissa tolerant to bacterial leaf blight. Rice varieties Sudha (Bihar), Sabita, Nalini (West Bengal), Patel 85 (Madhya Pradesh), Janaki (Bihar), Improved White Ponni (Tamil Nadu), Ambika (Maharashtra), are some of rice selections resistant to one or more diseases. MCU 1 cotton, a selection from Co 4, is resistant to Kufri Red, a potato selection from Darjeeling Red Round is a disease resistant variety.

## **3. Hybridization**

When selection of resistant varieties is not feasible, resistant varieties may be evolved by crossing the susceptible popular variety with resistant wild variety where in the resistant gene or genes transferred into the genetic make up of susceptible variety. Very often the F<sub>1</sub> from crosses may be resistant but carry the other undesirable qualities of the resistant parent. The bad qualities are removed by several back crossing of F<sub>1</sub> with the susceptible parent may ultimately yield a resistant progeny with good agronomic characteristics. Under certain circumstances pedigree or bulk method of selection is followed to obtain a resistant variety. In this method, the crosses are made till F<sub>2</sub> population is got. Selections are made in F<sub>2</sub> generation for superior genetic traits including disease resistance. By continued selfing, selections are made through F<sub>3</sub> to F<sub>5</sub> or F<sub>6</sub> generations and the best variety is selected. This method is suited for small grains and beans but unsuited to fruits and vegetables.

## **4. Back cross method**

Back cross method is widely used to transfer disease resistance from wild species. Wild species are rice sources of disease resistance. Interspecific hybridization is made to transfer the gene or genes for resistance to the cultivated

species. Resistance to grassy stunt virus from *Oryza nivara* to *O.sativa*, late blight resistance from *Solanum demissum* to cultivated potato, rust resistance from *durum* to *aestivum* wheat are some of the examples involving interspecific hybridization. Depending upon the number of genes governing resistance and the nature of the gene, whether dominant or recessive, the procedure varies. The number of back crosses to the cultivated species may be five to six. Once the back cross progeny resemble the cultivated parent, then they are selfed and segregating progeny screened for disease resistance.

### **5. Induced mutagenesis**

While following mutation breeding for disease resistance, a large number of mutation progeny should be produced and screened under artificial epiphytotic condition to select resistant plants. MCU10 cotton, a resistant variety to bacterial blight was evolved in Tamil Nadu by subjecting seeds of a susceptible variety CO4 to gamma rays followed by rigorous screening and selection

### **6. Development of multilines**

The concept of multilines was first suggested by Jensen(1952) and developed by Borlaug (1959) for evolving multiline varieties to resist stem rust in wheat. A multiline variety is a composite of genetically similar lines, except that each line possesses a different gene for resistance to the pathogen. Lines that are genetically similar, except for one gene, are called isoline. It is assumed that gene for resistance in each isoline contributes resistance to a separate physiological race or group of races. Genes for disease resistance are transferred by backcrossing from donor varieties to a common disease susceptible, but agronomically superior, recurrent parent. Isolines are generated differing only in the gene for disease resistance. The isolines are composited to synthesize a multiline variety. The isolines are maintained for resynthesizing the multiline whenever needed. A multiline variety is composed of a mixture of resistant and susceptible genotypes and provides a buffering effect against rapid development of disease. It will provide resistance or tolerance to a broad spectrum of races of a pathogen. If new races of the pathogen are identified at a later stage, additional isolines resistant to the newly arisen races may be constituted and incorporated. Care should be taken to see that there is uniformity for height, maturity and other features in the multiline.

Though multilines provide stability of yield due to reduction of damage by pathogens, the limitations of multiline varieties are that the yield level of multiline varieties is limited to that of the recurrent parent, 4 to 5 years are required to stabilize isogenic lines and the pathogen may produce new races at a faster rate than the development of a multiline. Multiline varieties have been developed for resistance to stem rust and stripe rust of wheat and crown rust of oats. The first multiline variety in wheat, 'Miramar 60' was developed and released in Columbia to combat the attack of yellow rust. 'Miramar 63' and 'Miramar 65' were resistant to stem rust and stripe rust. 'Yoqui 50', 'Crew' and 'Tumult' are a few other wheat multilines. Kalyan sona and Sonalika-based multilines of wheat resistant to different races of rust have been developed in India.



## **7. Tissue culture technique :**

Tissue culture techniques to produce somaclonal variation for disease are developed in different crops. Somaclonal variations for disease resistance are reported in *Zea mays* for *Drechslera maydis* race T-toxin resistance, in *Brassica napus* for resistance/tolerance to *Phoma lingam*, early and late blight resistance in potato, *Pseudomonas* and *Alternaria* resistance in tobacco, besides smut and rust disease resistance in sugarcane.

## **Breeding for resistance using biotechnology**

Salient achievements in each of the biotechnological approaches are given below.

**1. Gene identification and isolation:** The genes that regulate the traits of interest from the donor organism like bacteria, fungi, insects, plants and other categories of organisms are identified based on expression of desired traits. Such genes are isolated by various molecular techniques and gene constructs are made. Each such gene construct will essentially have the desired specific trait bearing expression to be transferred, depending upon the required host. Such constructs are put in the desired host (system) using appropriate equipments for gene transfer.

**2. Gene manipulation:** Genes of interest are monitored first by assessing their expression at transcriptional and translational levels. Further, by appropriate molecular technique the expression can be modified or enhanced or nullified.

**3. Gene tagging:** DNA sequences are inherited regularly from parent to progeny. Very closely placed DNA sequences show tight linkage. In case of genes of interest, it will be easy to follow them through inheritance if a known DNA sequences for which DNA probe is available, is found tightly linked to the genes of interest, then it is easy to locate or select the genes of interest. This tightly linked DNA sequence with known gene is known as gene tagging or gene labeling. Gene tagging helps to identify the trait even in very early stage of seedling development itself without having to wait till the expression stage of the trait in the plant. This is possible because of selection of genes tagged to the DNA marker probe unlike genetic markers.

**4. Enhancing the stress tolerance levels:** This is made possible by i. identifying and introducing genes or gene complexes and ii. by suitably modifying the genes to enhance the expression levels of the traits. For example, introduction of salt tolerance will help in growing crops in salt affected areas; thereby habitat expansion in low productive saline soil is possible.

**5. Embryo rescue:** The embryo/zygote is the fusion product of male and female gametes. In wide crosses, the parents are unrelated and the zygote is unable to develop in the enclosed maternal tissues. To overcome this incompatibility, the embryos are aseptically removed at appropriate stage of development of embryo, which is usually 3 to 7 days after fertilization. The rescued embryos are then cultured *in vitro* enabling the zygote of new types to come up thereby widening the germplasm base. Wheat x barley, wheat x rye, rice x *Porteracia coarctata* and maize x sorghum are some of the wide crosses in which embryo rescue technique has been employed.

**6. Somaclonal variation:** *In vitro* culture of cells, organs and tissues induces genetic and epigenetic variation in culture. The genetically induced *in vitro*

**7. Protoplast isolation and fusion:** Protoplasts are isolated by enzymatic digestion of cell wall of cells of calli, cells developed in suspension, very young developing leaf bases, soft tissues, immature embryos and the like. The protoplasts are purified and plated on to appropriate culture medium. The success in regeneration of plants from protoplast culture is much less. Plants regenerated from protoplasts also exhibit variation and such variants are termed as protoclonal. Useful protoclonal have been identified and released in tobacco, potato and in ornamental flower plants. Protoplasts can be fused and fusants can be regenerated. Such products carry  $2n+2n$  chromosome number, or less than that, when chromosome elimination occurs. Classical examples are potato+tomato, tobacco+ potato and *Brassica* + *Arabidopsis*. Cybrid is another protoplast fusion product in which protoplast of one genotype is fused with the cytoplasm of another genotype enabling a mix of two different cytoplasm with a genome from either one of the genotype alone. It is also possible for the substitution of nuclear genome of one genotype in the cytoplasm of the other. Cytoplasmic male sterility (cms) results when there is incompatibility between the cytoplasm and nucleus. A well defined cms can be obtained by protoplast fusion method, also known as asymmetric fusion, in which nuclear genome of one donor is irradiated by gamma or X-rays and killed and the cytoplasm of the other donor is inactivated by iodoacetamide (IOA) treatment. The resulting fusion consisted of nuclear genome of one donor and cytoplasm of the other donor parent.

a. A58(cms) ----- x ----- Fajiminor  
( $\gamma$ -irradiate) |  
(Iodoacetamide treated)  
|  
Production of cms line

b. Chinsurah Boro II ----- x ----- Nipponbare  
(X-irradiated) | (IOA treated)  
|  
cms line

c. Yamahoushi ----- x ----- Murasakidaikoku  
(purple colour) | (dwarf marker)  
Anther derived cell suspension  
|  
Diploid somatic hybrid.

**8. Anther culture:** Young developing immature anthers carrying microspores at uninucleate stage are pretreated at cold temperature (usually 0-10°C as in rice) for 8-10 days and subsequently cultured in vitro in specific medium. The microspores inside the cultured anther become embryogenic to develop directly into plantlets or through somatic embryos to develop into plantlets on further culturing. Well defined steps are available for different species and genera that are known to respond well. Plants developed from anther culture will be always expected to be haploids since microspores are haploids. These haploids could be diploidised by colchicine treatment. However an appreciable proportion of plants from anther culture are doubled haploids (DH). First known plants like rice, tomato and wheat, doubled haploids are evaluated and released for cultivation. DH is an excellent approach for released in to culture medium and incubated. The embryogenic microspore are then plated on to regeneration medium. Microspore culture is also used to develop uniform sized cell suspension for regeneration (eg. wheat, barley and rice). Anther culture and microspore cultures can be of wider application in crop improvement to develop homozygous recombinant DH lines that are stable and quick to obtain and thus can compact breeding cycle. Anther culture of wide hybrids can help to overcome sterility due to chromosomal imbalances and to obtain new chromosomal stocks.

**9. Transgenic plants:** Transgenic plants typically refer to plants into which genes have been introduced using recombinant DNA methodology. The introduced genes may originate virtually from any organism including bacteria, viruses, mammals and even the recipient plants. In fact, examples exist for all. The new trait introduced into the plant is often due to the presence of a novel protein or proteins. The information directing the plant to synthesize this protein is contained entirely in the introduced DNA or gene. Sometimes these new traits arise mediated by a protein. Occasionally new traits are also produced actually by elimination of a protein normally present in the plants, i.e. the new trait is not mediated by protein. In this case, gene coding for the elimination is essentially shut off through expression of antisense RNA, a RNA molecule designed to bind to a specific mRNA and ultimately lead to its degradation.

Agronomic traits, herbicide tolerance, resistance to diseases and pests, resistance to abiotic stress and lately improving post harvest characteristics of agricultural products like improving processing traits, extended shelf life and enhanced nutritional qualities have been either transferred or being attempted through this approach.

**10. Finger printing:** To understand inter and intra varietal differences and making evolutionary tree (dendrogram), this technique is employed. It involves restriction digestion of the interested candidates and probing with small stretches of DNA (called mini or microsatellites) and analysis of differences at DNA level. Locus specific probes are now-a-days used to get sure check of the differences. This technique is widely being used in genetic counseling and cataloguing the related species.

**11. Genetic engineering:** Steps in genetic engineering involve i) identification of protein which confers the desired trait, isolation of gene encoding the protein, putting the desired, isolated gene with inducible promoter under care of reporter gene (Selectable marker) and studying expression in stage specific or time specific intervals, delivering the modified gene into plant cells (gene transfer) and

regenerating of intact fertile plants from the successfully modified cells. The following are the advantages of genetic engineering:

- i. Transfer of one or a few genes from one organism to the other is precisely possible.
- ii. Highly defined DNA can be introduced without loss of characteristics, thus making the plant desirable in the first place.

#### **Important disease resistant / tolerant varieties of crop plants**

<i>Crop</i>	<i>Disease</i>	<i>Resistant/tolerant varieties</i>
1. Rice	Blast	ADT.36, 37, 39, 40, ASD 18, Co.4, 25, 36, 37, 41, 42, 43, 44, 45, IR 20, 62, 64, Paiyur-1, TKM 1, 10, TPS 3, 10, Bhavani, Bharathidasan, Jaya, Kanchana, Ponmani, Savithri, Sujatha Bhavani, Co 44, Kaveri
		Ajaya, BJ-1, IR 20, PR 110, PR 111, TKM 6
	Brown spot	Ambemohr 159, ARC 13820, 13901, Bhavani, Co 45, Co RH 1, IR 36, 50, 56 Latisail, Nidhi, Pankari 203, PTB 18, 21, Vasundhara, Vikramarya,
	Bacterial leaf blight	
2. Pearl millet	Tungro	
	Downy mildew	Co 6, 7, X 4, 5, 6, 7, WCC 75, UCH 9, 10, Pusa Bajri 266, CZP-IC 923, Hybrids – MLBH 285, JKBH 26, Nandi 30, RHRBH 8924, GK 1004(MH 662), 7685(MH 643, XM 631) GHB 316, PAC 303 ICI-903, MH 552)
		Hybrids – MH 770, 771, 812
3. Sorghum	Downy mildew and smut	
	Downy mildew	CS 3541, IS 3443, 18757, 22230, 22231, 27042
		CS 3541, IS 3443, 3547, 14322, SRT 18B, 26B
	Ergot	E 36-1, IS 8185, RS 29
	Rust	Co 20
	<i>Striga</i> sp.	RS 585, RS 615, SB 105 and 116 B

	Charcoal rot, rust, leaf blight, Chlorotic stripe stunt.	Co 25, TNS 23, 28
4.Maize	Downy mildew, ergot, rust and grain mould Downy mildew	Co1, CoH1, CoH2, UMC5 UMH 36
5.Finger millet	Downy mildew and leaf blight Blast	INDYMIT – 345 Co 10, 11, 12, 13, GE 3076, IE 2773, IE 2882, IE 2896
6.Wheat		
10.Ground nut	Powdery mildew Late leaf spot	HPT 6, TL 2780 ALR 1, PI 261893, 262090, 341879,371521,
11.Gingell y	Rust <i>Alternaria</i> leaf blight	ALR 1, ICG (FDRS) 4, 10, Tifrust 1-11, 12, 13, 14. Anand 9,74,TMV 1,2,
13.Cotton	Powdery mildew and phyllody White spot <i>Verticillium</i> wilt <i>Fusarium</i> wilt	Swetha Til HT-1, L-45, RSE-1. MCU 5-VT Jayadhar,Pratap, Varalakshmi,Verum, Vijay
	Bacterial blight	Badnawar-1, B1007, BJA-592, CDE 1, DHY- 286, HG-9, Khandwa-2, REBA- B-50 MCU 10,
	<i>Rhizoctonia</i> root rot	Laxmi, Sangam, Savitri, Sujatha, Suvin,Varalaxmi

	Grey mildew	Abadithya, Jayadhar, K7, K8, MCU 10, Paiyur-1.
		231-R, M60A/2
	<i>Alternaria</i> blight	
	<i>Myrothecium</i> blight	
14.Bhendi	Yellow vein mosaic	Parbhani Kranti, Arka Anamica
15.Cassava	Cassava mosaic	H97, H.165, H.226, ME 167

# 10

## Disease Management by Cultural Methods

### 1. Eradication

Eradication is the elimination of pathogen after it has become established in the area where host is growing. The following are the important methods followed to prevent the spread of the disease:

- i. eradication of alternate hosts,
- ii. eradication of collateral and self sown overwintering hosts
- iii. eradication of the affected plants or trees,
- iv. eradication of pathogens from infected plant parts by surgery and
- v. eradication of culled out plant materials, debris, etc., through different cultural practices

**i. Eradication of alternate hosts:** Removal of alternate hosts helps to prevent and check the spread of the disease caused by heteroecious rust pathogens in the primary hosts. Barberry bush is the alternate host for black stem rust pathogen *Puccinia graminis tritici* on wheat where the pathogen survives in the off-season. Barberry was eradicated in Canada, Denmark, France, Hungary, Norway and in the U.S.A. by passing stringent laws in each country. The eradication of barberry had two benefits i.e., it elimination of early spring primary inoculum and prevention of the formation of new physiologic races of the pathogens. In the U.S.A. white pine blister rust (*Cronartium ribicola*) was controlled by eradication of alternate host, *Ribes*. In Australia, Europe and the U.S.A. the apple rust (*Gymnosporangium juniperi-virginianae*) is controlled by eradication of alternate host, cedar.

**ii. Eradication of collateral and self sown overwintering hosts:** There are many weed hosts or wild species of cultivated plants act as collateral hosts or volunteer plants of an economic crop which act as reservoirs of pathogens of annual crop. Reservoir hosts help the pathogen to continue the infection chain. The primary inoculum is produced on and dispersed from these hosts to the cultivated crop hosts. If these wild or uneconomic host plants of the pathogen are destroyed, the sources of primary inoculum are eliminated and chances of initiation of the disease in the crop hosts are reduced. Destruction of these hosts breaks the life cycle of the pathogen and the infection chain. Reservoir hosts or indigenous plant species which are not actually involved with the life cycle of the pathogen but provide additional sites for its persistence and multiplication. In some cases such plant species act as symptomless carriers, especially for

viruses and root pathogens. Regional elimination of such hosts requires careful attention to roadside areas and other non-agricultural land also.

Crop	Disease	Pathogen	Collateral hosts
<b>a. Fungi</b>			
1. Rice	Blast	<i>Pyricularia oryzae</i>	<i>Brachiaria mutica</i> <i>Dinebra retroflexa</i> , <i>Leersia hexandra</i> , <i>Panicum repens</i> .
2. Sorghum	Ergot	<i>Sphacelia sorghi</i>	<i>Panicum</i> spp.
<b>b. Bacteria</b>			
1. Rice	Bacterial leaf blight	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	<i>Cyanodon dactylon</i> , <i>Cyperus rotundus</i> , <i>Leersia hexandra</i> , <i>Leersia oryzoides</i> , <i>Panicum repens</i> , <i>Paspalum dictum</i> .
2. Apple and pear	Fire blight	<i>Erwinia amylovora</i>	<i>Hauthom</i> bushes <i>Crataegus</i> sp.
3. Cotton	Bacterial blight	<i>X. axonopodis</i> pv. <i>malvacearum</i>	<i>Eriodendron anfructuosum</i> , <i>Jatropha curcas</i> , <i>Thurbaria thespesoides</i>
<b>c. Viruses</b>			
1. Potato	Rugose mosaic	Rugose mosaic virus	<i>Physalis</i> spp.
2. Bean	Yellow mosaic	Bean yellow mosaic virus	Sweet clover
3. Bhendi	Yellow vein mosaic	Bhendi yellow vein mosaic virus	<i>Hibiscus tetraphyllus</i>
<b>d. Phytoplasma</b>			
1. Brinjal	Little leaf	Phytoplasma	<i>Catharanthus roseus</i> , <i>Datura</i> sp.

Self sown crops / volunteer plants help the pathogen to overwinter / oversummer in the absence of economic hosts. In Sudan it was enforced through legislation to pull out the cotton plants to prevent regrowth which facilitate the carry over of the cotton leaf curl virus. Wheat streak mosaic virus has been effectively controlled by eliminating the volunteer wheat plants that served as reservoirs for the virus.

**iii. Eradication of affected plants or trees:** In some threatening plant diseases, it is essential to eradicate the host and the pathogen from an area. Citrus canker (*Xanthomonas axonopodis* pv. *citri*) is an example of success of an eradication programme. This disease was first noticed in Florida citrus trees in 1913. An eradication campaign was started in 1915. All the citrus nurseries and orchards were inspected and the infected trees were cut and burnt. The eradication



programme continued till 1927 and no citrus canker was present in that area. Peach yellows and peach rosette were also controlled by removal and destruction of diseased trees. In Tamil Nadu also there were some eradication campaigns launched under Destructive Pests and Diseases Act. Eradication programme was set up to control bud rot of palms and completed with success. At Sathyamangalam eradication of sandal wood tree affected by spike disease was also made to contain this disease.

**iv. Eradication of pathogens from infected plant parts by surgery:**

Eradication of affected plant parts (tree surgery) are also practiced in certain cases which reduces the source of primary inoculum. Lesions caused by fire blight bacterium (*Erwinia amylovora*) on pear and apple trees are removed during winter months. This not only prevents further spread in the affected trees but also reduces the amount of inoculum that can spread to other branches and trees. The cankered areas in the branch or trunk of almond and pear trees caused by *Ceratocystis fimbriata* are surgically removed and the trees are saved. Tree surgery is also practiced in coconut trees affected by stem bleeding disease (*Ceratocystis paradoxa*), citrus gummosis (*Phytophthora citrophthora*), *Dendrophthoe* spp. on citrus, bud rot of palms (*Phytophthora palmivora*) and koleroga of arecanut (*P. arecae*)

**v. Eradication of culled out plant materials, debris etc. through different cultural practices**

**2. Crop rotation**

Crop rotation is essentially a preventive measure and has its effect mainly on the succeeding crop. Crop rotation is the oldest and cheapest method adopted in agriculture for eradication of certain types of pathogens from infested soil. Continuous cropping or monoculturing provides opportunity for perpetuation of pathogenic organisms in the soil when the same crop is raised year after year in the same field. The soil-borne pathogens of that crop easily perennate in the soil and increase in their population. After sometime, the soil becomes so heavily infested that it becomes unfit for cultivation of the particular crop. Virus diseases of crop plants and their vectors are found to increase after every crop if a crop is cultivated continuously in a field. On the other hand, when immune, resistant or non-host crops are grown for a definite duration after a susceptible crop in the field it is expected that in the absence of nutrition, the pathogen will be starved off and the population of such pathogens consequently decreases. It is also possible that different crops release some biochemical substances in their root exudates which either directly kill the pathogen or encourage development of antagonistic microorganisms in the soil. In this way, crop rotation is one of the most effective methods of root disease control. Organisms which are soil inhabitant types remain in soil for a very long time, even more than five years in the absence of the host. Long-lived spores or the organisms by themselves, subsist as saprophytes and therefore their presence in soil is long term. Onion smut (*Urocystis cepulae*) and club root (*Plasmodiophora brassicae*) organisms are producing resistant type of spores while *Rhizoctonia*, *Fusarium* and some species of *Pythium* are those which could remain in soil as saprophytes for a very long time. Eradication of such organisms becomes fairly difficult. Soil also harbours soil invaders. These

organisms are not persistent and they can live as long as the host residues serve as substrate. They perish when they are forced to exist in the soil in competition with soil inhabitants and disappear gradually in due course. Bean anthracnose fungus *Colletotrichum lindemuthianum*, cabbage black rot bacterium, *Xanthomonas campestris* pv. *campestris* are some examples, which live in soil for 1 to 2 years. They can be eliminated from soil by adopting 3 or 4 year rotation with non-host crops. Crop rotation is effective in the control of brown stem rot of soybean (*Cephalosporium gregatum*). The disease incidence can be reduced to a great extent by rotating with corn for 4 to 5 years between two soybean crops.

Crop rotation with sugarcane or paddy is effective in the control of 'Panama wilt' of banana (*Fusarium oxysporum* f.sp. *cubense*) and crop rotation with paddy or green manures is effective in the control of red rot of sugarcane (*Colletotrichum falcatum*). Rotation of cereal crops like pearl millet, finger millet or fox-tail millet is recommended for the control of *Macrophomina* root rot of pulse crops. Two year crop rotation with lucerne is recommended in the control of *Verticillium* wilt of cotton. Many diseases such as *Fusarium* wilt of pigeonpea (*F. udum*), foot rot of betelvine (*Phytophthora capsici*), bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*), bacterial blight of cotton (*X.campestris* pv. *malvacearum*) etc., are controlled by this method. Soybean seed infection by *Phomopsis* sp. can be reduced by rotating soybean with maize. Pathogens are reduced or eliminated by following the crop rotations given in the table.

Table. Effect of crop rotation in reduction / elimination of plant pathogens.

Beneficial crop	Pathogen reduced or eliminated	Preceding crop
1.Rice	<i>Verticillium dahliae</i>	Cotton
2.Pea	<i>Gaeiimannomyces graminis</i>	Wheat
3.Sudan grass	<i>Pseudomonas solanacearum</i>	Tomato

### 3. Fallowing

Fallowing starves the pathogen and helps in reduction of the inoculum by elimination of the host. Diseases like *Macrophomina* root rot on different crop plants is controlled by following this method. Flood fallowing is to a depth of 0.6 to 1.5 m for 4 to 6 months markedly reduced the Panama wilt pathogen *Fusarium oxysporum* f.sp.*cubense* inoculum in banana. Soil inoculum of *Phytophthora parasitica* var. *nicotianae*, the causal organism of black shank of tobacco was destroyed by flooding the field for 3 to 4 months and by raising swamp rice in a 2 year rotation with tobacco-rice crop in Java. Flooding the soil strewn with debris infected by *Xanthomonas axonopodis* pv. *malvacearum* for 4 days reduced the inoculum level and thus the incidence of disease was only 2.1% as against 69.5% in unflooded fields. Wet fallowing makes the pathogenic propagule in or on the soil to germinate, spent them, is become susceptible attack of saprophytes. Example, *Sclerotium rolfsii* and *Verticillium dahliae*. The sclerotia or microsclerotia of these fungi are activated in the absence of root exudates of this host. They germinate quickly when there is alternate wetting and drying of the soil. When the population of *Pythium myriotylum* is not high

wet fallowing is successful in reducing the population. Wet fallowing reduces saprophytic survival of *Alternaria solani* on crop debris.

#### **4. Application of organic manures**

Addition of organic manures like farm yard manure or green manures or oil cakes to the soil increases the antagonistic microorganisms in the soil. Build up of antagonistic microorganisms reduces the population of soil borne plant pathogens and the diseases caused by them. Application of farm yard manure at the rate of 12.5 tonnes/ha reduced the incidence of *Macrophomina* root rot of cotton. Application of 5 kg of neem cake/tree three times in a year reduces the basal stem rot (*Ganoderma lucidum*) of coconut. In the control of sesame root rot (*Macrophomina phaseolina*) application of neem cake at the rate of 150 kg/ha is recommended. Application of neem cake at the rate of 2 tonnes/ha in two split doses and covering with mud reduced foot rot disease in betelvine garden.

#### **Soil amendment**

It has been proved that the organic amendments rich in carbon and deficient in nitrogen control the take-all disease (*Ophiobolus graminis*) of wheat. There is considerable liberation of CO<sub>2</sub> by soil saprophytes which suppresses the pathogenic activity of this fungus. In the process of survival also, low nitrogen content in the soil reduces the longevity of the fungus. *Phytophthora* root rot of avocado is controlled by amending the soils with alfalfa meal- a material of low C/N ratio. The other diseases are pea root rot *Aphanomyces euteichus* when cruciferous plant residues were incorporated into the soil. Alfalfa meal and barley straw application in the soil reduced the root rot of cotton and sorghum caused by *Macrophomina phaseolina*. Black scurf of potato (*Rhizoctonia solani*) is less in the field where wheat straw was incorporated.

#### **5. Summer ploughing**

Deep ploughing during summer periods buries the inocula of fungi of soil borne nature. Fungal propagules, sclerotia and different types of spores conidia on plant refuses die when exposed to sunlight due to the higher temperature prevailing during the summer. Further infected self sown plants, volunteer hosts plants, weed hosts, regrowths from the plant roots, alternate hosts and alternative hosts are also destroyed. Here, the spread of the disease is avoided. Groundnut blight (*Corticium rolfsii*) is controlled by ploughing the soil to a depth of 20 cm. The inverted plough sole soil buries the sclerotia of the fungi, *Claviceps*, *Sclerotium* and *Sclerotinia* in association with plant or alone, impedes the discharge of ascospores from perithecia. Bunt and smut spores of wheat, smut spores of sugarcane and sorghum and microsclerotia of *Verticillium* in cotton are buried deep in to the soil by deep ploughing.

#### **6. Crop growing seasons**

Rice blast becomes serious when the rice crop is raised from August to September in Tamil Nadu. Ragi blast becomes serious when sowing is made between June and August. Similarly yellow mosaic of blackgram/green gram and phyllody of sesame are serious during kharif season in South India. Incidence of powdery mildews of different crops is found to be high during rabi

when compared to kharif and summer seasons. In bhendi, yellow vein mosaic incidence is very high during summer. The seasons with high incidence of diseases should be avoided in the epidemic areas.

**a. Adjustment of sowing time:** In many diseases the incidence is more severe when the susceptible stage of the plant growth and favourable conditions for the pathogens coincides. While choosing the time of sowing it should be taken into consideration that susceptible stage of the crop growth and soil conditions and other environments favourable for maximum activity of the pathogen does not fall at the same time. Properly adjusting the sowing dates can give good dividends. Late planted wheat crops contract less infection than wheat planted on normal dates. Early and late sown crops have been found to be free from Oodhubathi disease of rice. Avoiding cool and cloudy days for planting will help to reduce red rot of sugarcane. Late sowing of winter wheat and barley is considered to be the most effective measures in reducing take all disease of wheat. Rapeseed sown in mid to late August is more liable to attack by leaf spot (*Alternaria brassicae*) than late-sown crops. Pea and gram planted soon after rains when soil temperature and moisture are at a high level, show high incidence of root rot and blight. As the soil temperature falls and moisture becomes less (Nov-Dec) these diseases are also reduced. In areas where these diseases are serious, late sowing helps in saving the crop. Stem rust of wheat damages the late sown crop more than the early sown crop. Because, time of onset of disease and ear formation coincides. Sowing from January to April or October to December is advocated to escape from the attack of neck blast of finger millet. Peas and chickpea sown in October usually suffer heavily from root rot and wilt (a complex of *Fusarium*, *Rhizoctonia* and *Sclerotium*). When these crops are sown late, the diseases are not so severe or almost absent. The groundnut rosette is transmitted by *Aphis craccivora*. In Nigeria the population of this vector is low in crops sown in June than in July. The sowing time is adjusted in cumbu and sorghum in such a way that the flowering stage does not coincide with the rainy season to avoid the sugary diseases. Early sown crops show decreased incidence of curly top and yellows on sugarbeet, rosette on groundnut and barley yellow dwarf on cereals. Delayed sowing on the other hand is beneficial to maize rough dwarf disease.

**b. Adjustment of harvesting time:** Harvesting of groundnut should not coincide with the rainy days and it helps to avoid infection by *Aspergillus flavus*. Freedom of onions and roses grown in rainless seasons from downy mildew diseases and freedom of beans, chilli and cucurbits from bacterial diseases in such seasons are the best examples for sowing of crops at correct season to avoid disease outbreaks. In the case of deciduous fruit trees and grapevines, the season of sprouting, flowering and fruit set can be advanced or delayed by pruning practices or by treatments to break dormancy. Advantage can sometimes be taken of this fact to avoid coincidence of all or one of these phases of host growth with weather periods particularly favourable to specific pathogens that attack trees in the phases.

**7. Growing of seed crops:** Coffee can be grown in the western Hemisphere usually free from coffee rust which causes heavy losses in Eastern Hemisphere.

In the case of virus diseases this will be more useful. By growing seed materials in isolated places where the population of vectors is very low and the condition is uncongenial for the vectors. Virus free potato tubers to be used as seeds are grown in cool and windy places in many parts of the world. Under tropical and subtropical countries, such conditions prevail in the hills at high altitudes. Obtaining seed from disease-free localities has been very successfully resorted to the elimination of many seed-borne diseases. In the U.S.A. seed-potatoes are invariably grown in northern snow-clad sections, where viruses are practically absent and then exported to various other sectors in the south. Similar practice has been in vogue in India, where seed-potatoes are annually imported in southern states from Simla hills for control of virus diseases and bacterial ring. In the U.S.A, the seed growing areas have been shifted to arid pacific regions for crops like cabbage, turnip, beans and peas for obtaining disease-free seed and indirectly controlling such diseases like black leg and black rot of cabbage and turnip and anthracnose of beans and peas. Similar practice is obtained in parts of Bombay, where the foot rot of ginger (*Pythium myriotylum*) prevalent in the southern parts, is controlled through the importation of seed-rhizomes from disease-free arid regions of the north, where the disease is practically non-existent on account of the dry climate, lighter soils and moderate rainfall.

#### **8. Selection of seeds and seed materials**

Seeds and seed materials carry many fungi, bacteria, viruses and phytoplasmas and may introduce these pathogens into the field, i.e., seeds and seed materials form the primary source of infection. Seed and seed materials like cuttings, tubers, grafts, setts etc., should be well matured, disease free, uninjured and have a high germinating capacity. The absence of an initial inoculum in seeds is definitely helpful in delaying or suppressing the incidence of the disease. It is a preventive method. The diseases like foot rot, brown spot, short smut of sorghum, loose smut of wheat, bacterial blight of rice, bacterial blight of cotton, leaf crinkle of blackgram etc., are transmitted through seeds. Virus diseases and black ring of potatoes, foot rot of ginger, foot rot of betelvine, Panama disease of banana, red rot of sugarcane cassava mosaic, bunchy top and virus diseases of fruit trees are transmitted through tubers, setts, rhizomes, corns, grafts and budwoods. 'Tuber indexing' is a special method to obtain disease free seed materials in potato. It is commonly practiced by nurseries and seed merchants selling potato seed tubers. Use of seeds in the place of rhizome/sucker is recommended in the control of 'katte' disease of cardamom.

#### **9. Leveling of the field and provision of drainage facilities**

Water stagnation in different patches of field favours the fungi like *Pythium*, *Phytophthora*, *Rhizoctonia solani*, etc., for which proper leveling of the field before sowing or planting is very essential. Further improving the drainage is necessary in the control of sheath blight of rice. Provision of drainage channels in orchard crops like citrus, jack, mango etc., in the garden is necessary before planting. In the control of damping-off diseases of vegetable and other crops, raising seedling in the raised beds method is followed. Foot rot of ginger (*Pythium myriotylum*) is also controlled by following the raised bed system of nursery.

### 10. Seed rate

Use of higher seed rate in the nursery creates favourable microclimate for the pathogens causing damping-off in vegetables, tobacco, chillies and forest nurseries. Hence, use of optimum seed rate should be adhered in such crops.

### 11. Burning of stubbles and crop residues

Burning of plant wastes, crop residues, stubbles, *etc.*, in the areas selected for raising nurseries for vegetable crops, tobacco, chillies and forest trees *etc.* heats the soil and kills the inoculum of the pathogens present in the top layer of the soil. When nurseries are raised in these areas incidence of damping off disease is highly reduced. This practice is also followed in pits made for planting coconut, banana, fruit trees *etc.*, Burning of wheat plant every second or third year is suggested for eradication of pathogen in the field when *Cephalosporium gramineum* infects wheat. Otherwise, debris in the field helps the perpetuation of the pathogen and the disease. Burning of rice crop residues avoid carry over of sheath blight (*Rhizoctonia solani*); stem rot (*Sclerotium oryzae*) of rice and bacterial blight of cotton.

### 12. Depth of sowing

Depth of sowing greatly influences seed transmission of smuts. Shallow planting in wet soils protects wheat plants from *Urocystis tritici* (flag smut) of wheat. Deep planting may cause delay in the emergence of seedlings, which may be vulnerable to pre-emergence damping off. Early emergence results in early lignification of tissues which become resistant to attack of soil-borne pathogens.

### 13. Spacing

Closer spacing invariably alters the microclimate underneath the canopy of the crop which may provide favourable environment for development of diseases. Boll rot in cotton is quite common in crowded crop. Defoliation of plants or skip cropping gives better control against the boll rot disease. In certain virus diseases like groundnut rosette the incidence is observed to be less when wider spacing is adopted. Closer spacing favours many air borne diseases because of high humidity in the crop canopy. Early and late blight of groundnut and blister blight of tea are more in dense canopy. Early spread of black rot of cabbage takes place in closer spacing. Crowded stands may reduce some systemic diseases. Cotton wilt caused by *Verticillium albo-atrum* will be less in closely planted crop if the fungal inoculum is less in the soil. Similarly closer spacing of rice reduces rice tungro virus infection particularly when vector population is less. Avoiding shade and providing wider spacing reduces the incidence of powdery mildew of tobacco. Late blight of potato and downy mildew of grapevine spread fast in closer spaced crops. In the case of bud necrosis of groundnut caused by tomato spotted wilt virus, seeds are sown adopting closer spacing of 15x15cm to compensate the rogued out plants with regard to plant population and yield. These are examples where dense sowing helps in disease reduction. The virus of tomato leaf curl, transmitted by *Bemisia tabaci*, is less severe in a crowded planting than in spaced planting. Same is true for cucumber mosaic, transmitted by *Aphis gossypii* and groundnut rosette transmitted by *Aphis craccivora*. The fungal diseases for which the phenomenon

of lower incidence at closer spacing of the crop has been studied most profitably is the wilt caused by *Verticillium albo atrum* and *V.dahliae* in cotton. This is ascribed to the reduction of effective inoculum per plant in proportion to the increase in the number of plants per unit area in the densely sown field. The incidence of brown rot (*Cephalosporium gragatum*) of soybean is also higher in widely spaced planting than in closer rows.

#### **14. Method of sowing/planting**

In places where water accumulation is a problem to the crop growth sowing of seeds on the sides or ridges is found effective in reducing the incidence of *Sclerotium rolfsii* on groundnut and vegetable crops and *Sclerotinia sclerotiorum* and *Rhizoctonia solani* on vegetable crops and *Phytophthora* blight of pigeonpea. High ridging prevents infection of potato tubers, by zoospores from leaf lesions in late blight diseases. Ridging is disadvantageous in water deficit areas where it encourages pathogens like *Macrophomina phaseolina*.

#### **15. High budding**

High budding is a practice to avoid infection by gummosis fungus of citrus trees. In low budded plants the bud point is close proximity to infection centre (the soil), become readily diseased. High budding is a simple device for lengthening this distance between the bud point and infected soil. In this method the soil borne pathogens (*Phytophthora palmivora* and *P.citrophthora*) have no chance of reaching the bud point, through which they enter the bark. Staking of lower most branches arising close to the soil, increases the distance between the fruits and soil inoculum and removes the chances of brown rot (*Phytophthora* sp) infection and buck-eye rot of tomato (*P. nicotianae* var. *parasitica*).

#### **16. Avoiding injury**

Injury of plant parts should be avoided in order to check the entry of pathogens. Clipping of tips of tall rice seedlings favours the entry of bacterial blight pathogen and incidence of the disease. Hence clipping should be avoided at the time of transplanting of rice. While harvesting the pods in groundnut, fruits in tree crops and vegetable crops injuries to the fruits pave the way for the pathogen and causing pod/fruit rot. It also reduces the storage life of fruits and vegetables. Hence much care should be given to avoid wounds during the harvest time.

#### **17. Altering the soil pH**

In certain soil borne diseases adjustment of soil reaction helps in the reduction of inoculum level of the pathogens. The altered pH of the environment forms a barrier against the pathogen. A very low pH less than 5.2 is unfavourable to common scab bacterium on potato (*Streptomyces scabies*). Thus, use of acid forming fertilizers (like sulphur) and avoiding lime and calcium ammonium nitrate application are effective in controlling the common scab disease. On the other hand the club root pathogen of cabbage (*Plasmodiophora brassicae*) cannot live and infect when the soil pH is 7.0 or more. Hence liming which increases the soil pH gives satisfactory control of

club root disease. In Punjab, root rot of tobacco (*Macrophomina phaseolina*) has been overcome by application of 2.5 to 5.0 tons of lime /ha to the soil.

### **18. Mixed cropping**

Mixed cropping materially helps in checking certain diseases. Blight of pulse crop (*Phyllosticta phaseolina*) has been successfully overcome by growing pulses as a mixed crop with cereals like sorghum and pearl millet.

### **19. Intercropping**

Intercropping is also a device in the control of some soil borne diseases. Intercrops should be properly chosen so that they should not have any common pathogen for e.g., *Macrophomina phaseolina* has got wide host range and hence common host should not be grown as intercrops. Intercropping with moth bean (*Phaseolus aconitifolius*) in a cotton field reduced the root rot (*M.phaseolina*) incidence. Due to reduction in the number of host plants there is sufficient spacing between them and chances of contact between foliage of roots of diseased and healthy plant are greatly reduced. Therefore, root pathogens are unable to spread from diseased to healthy roots and spread of foliar pathogens is also reduced to a great extent. Intercropping of sorghum in pigeonpea field reduced the wilt (*F. udum*) incidence. The roots of non-host plants may act as a barrier obstructing the movement of pathogens in soil. They may release toxic substances from their roots which may suppress the growth of the pathogens attacking the main crop. Hydrocyanic acid (HCN) in root exudates of sorghum is toxic to *F. udum*, the pigeonpea wilt fungus. Intercropping of sorghum or mothbean in a crop of clusterbean reduced the incidence of root rot (*R.solani*) and wilt (*F.coeruleum*) from 50 to 60% in single crop to 8 to 15% in the mixed crop. Intercropping of pigeonpea with gingelly at 1:6 ratio reduced the incidence of phyllody disease. In Jordan, intercropping tomatoes with cucumber is found to be effective and cheaper in controlling the whiteflies and lowering the incidence of tomato yellow leaf curl virus. (TYLCV) Cucumber is planted one month before tomato. Cucumber is known to be a preferred host for whiteflies and immune to TYLCV. Insecticides are applied when adult whitefly populations are at high levels, usually two weeks after planting of cucumber and the second one before tomato planting. Growing of an intercrop of cereals such as corn or sorghum between rows of peach trees is an effective method in combating Texas root rot (*Phymatotrichum omnivorum*) infection in the U.S.A.

### **20. Barrier cropping**

Taller crops can be grown to protect a crop of lesser height from virus vectors. The insects may land at the taller crops (barrier crops) and the dwarf crop may escape from virus diseases by those insects. Barrier cropping with 3 rows of maize or sorghum or pearl millet around the main crop namely blackgram or greengram is effective in reducing the vector population and incidence of yellow mosaic. Another best example is growing of 3 rows of kale or barley as barrier crops in cauliflower seed beds and undersown beet steckling against cauliflower mosaic and beet yellows diseases respectively. The incoming aphids are thought to land on the barley or kale and probe briefly,



causing them to lose the non-persistently transmitted virus they are carrying. Maize or sunflower are the other barrier crops considered for these crops.

## 21. Decoy crop and trap crop

Decoy crops (hostile crops) are non-host crops sown with the purpose of making soil-borne pathogens waste their infection potential. This is effected by activating dormant propagules of fungi, seeds of parasitic plants, etc. in absence of the host. A list of pathogens that can be decoyed is given in table.

Table. Decoy crops for the reduction of pathogen populations

Host	Pathogen	Decoy crops
1. Sorghum	<i>Striga asiatica</i>	Sudan grass
2. Cabbage	<i>Plasmodiophora brassicae</i>	Rye grass, <i>Papaver rhoeas</i> , <i>Reseda odorata</i>
3. Potato	<i>Spongospora subterranea</i>	<i>Datura stramonium</i>
4. Tomato, tobacco	<i>Orobanche</i> spp.	Sunflower, safflower, lucerne, chickpea etc.

Trap crops are host crops of the pathogen, sown to attract pathogens but destined to be harvested or destroyed before they complete their life cycle. Fodder sorghum can be raised as a trap crop to reduce downy mildew of sorghum.

## 22. Trenching

Trenching between rows of trees in orchards has been effectively utilized in arresting the growth and spread of the pathogen in the soil to the neighbouring trees. *Ganoderma lucidum* root rot infected citrus trees should be isolated by digging a trench of 30 cm wide and 60 cm to 90 cm deep around the tree at a distance of 2.5 to 3.0 m from the base to prevent the contact of diseased roots with healthy roots. Thereby the spread of the pathogen to neighbouring tree is prevented. Similar method is also followed in the control of basal stem rot (*Ganoderma lucidum*) of coconut in India.

## 23. Isolation distances

The distance between seed production and commercial plots has been worked out for reducing seed borne loose smut of barley and wheat. Barley and wheat crops should be isolated by at least 50 m from any source of loose smut infection for production of certified seeds in the U.K. The number of viruliferous insects reaching a healthy crop from a diseased one decreases with distance between them so that cultivation of susceptible crops at a distance from each other delays or reduces the severity of virus diseases. Incidence of lettuce and cucumber mosaic viruses is about 3% if the new lettuce crop is sown 0.8 km away from an old lettuce field. Much greater incidence of mosaic in sugarbeet fields occurs within 90 metres of a seed crop than in the fields at a greater

distance. Beet mosaic and beet yellows are markedly reduced by isolating beet fields by 19 to 24 km and 24 to 32 km mites respectively from a large source of infected beets.

#### **24. Yellow sticky traps**

Sticky, yellow polythene sheets erected vertically on the windward side of red pepper fields have been sown to reduce the incidence of potato virus Y (PVY) and cucumber mosaic virus (CMV) in the crop. The aphids are attracted to the yellow colour and are caught on the sticky polythene. The control obtained was so successful that the method has become a standard control procedure in red pepper crops in Israel. Similar traps have also been used to protect 'seed' potato crops, against potato leaf roll virus. Yellow sticky traps are in use to attract and kill the whitefly vectors which spread yellow mosaic of blackgram and greengram and bhendi yellow vein mosaic.

#### **25. Mulching**

Mulching or covering of top soil with organic residues often helps in reducing plant diseases. Mulches of non-host origin should be used in the field. These mulches are known to release inhibitory substances in the underlying soil and also promote development of parasites and predators of nematodes. Reflective surfaces (mulches) laid on the soil around the crop plant, have been found to be highly effective in controlling aphid vectors. Aluminium strips or grey or white plastic sheets are used as mulch and it has successfully protected red peppers against CMV and PVY in Israel and summer squash against watermelon mosaic virus in the Imperial valley of California. Straw mulches have been successfully used to control the white fly – transmitted tomato yellow leaf curl virus in tomato crops in Israel. It is believed that the colour of the straw attracts the whiteflies and they are subsequently killed by the reflective heat. The disadvantage with straw mulches is that they eventually lose their yellow colour, but prolonged control may be obtained if straw is replaced by yellow polythene sheets.

#### **26. Irrigation water management**

Irrigation to the crop in the field is to wet the soil to the extent that roots easily get water and nutrients. If excess water is added to soil, it may directly affect activity of pathogens and/or it may affect disease incidence through the effect on the host. Scab attack on potato tubers is prevented by maintaining soil moisture near field capacity during tuber formation. Bacterial flora antagonistic to *Streptomyces scabies* increases under high moisture conditions. The charcoal rot pathogen, *Macrophomina phaseolina* attacks potatoes and cotton when the soil temperature rises and there is water stress. By irrigating the field, soil temperature is brought down, stress is removed and the disease is suppressed. When excess irrigation is made the juvenile stage of plants is lengthened making it susceptible to attack of fungi like *Pythium*. Supply of frequent but low quantity of irrigation water is, therefore, recommended for reducing chances of damping off in nurseries. Under conditions of excess water, respiration of roots is inhibited and many soluble salts accumulate in toxic amounts around the roots

and base of the stem. This increases disease proneness of the roots. Irrigation increases guttation. Guttation drops on leaves serve as media for multiplication and penetration of many pathogens, such as *Helminthosporium* spp. on cereals and *Xanthomonas campestris* on *Brassica* spp. Cereal rusts usually are more severe when the crop is grown in wet soil than in relatively drier soils. Vascular wilts appear aggravated soon after irrigation. These effects are through the host. Pathogens directly taking advantage of excess water are those that need wet soil for (i) activation of their resting structures and (ii) for movement of these propagules. Thus, in presence of excess free water bacterial cells and zoospores of Pythiaceus fungi are dispersed easily. Therefore, at the plant stage when these pathogens can attack the crop irrigation should be avoided. Generally, sprinkler irrigation increases diseases by increasing leaf wetness and by dispersing propagules of the pathogens by water splashes just like rain water. At the same time, it has some advantages also such as washing off of inoculum from the leaf surface.

Irrigation especially at seed-development stage, may favour seed infection. Irrigation time and amount of water should be controlled so that the relative humidity is not raised to such an extent that it becomes conducive for seed infection. Control of seed-borne diseases favoured by wet climate can be achieved by raising the seed crop in dry areas. Some examples are anthracnose of bean and cucurbits (*Colletotrichum* spp.), *Ascochyta* blight of pea (*Ascochyta* spp.) and bacterial blight of legumes. Such crops can be grown in dry areas with the help of irrigation so that these aerial parts remain dry and do not contact infection. Virus-free potato seed tubers can be produced more successfully in areas where temperature and moisture conditions do not favour buildup of populations of the insect vectors.

Sclerotia, smut spores, chlamydospores, oospores and mycelium found in the soil are carried from one field to another through irrigation and drainage water. Stem rot, sheath blight and bacterial blight diseases of rice, damping off of vegetables and *Macrophomina* root rots of many crops spread mainly through irrigation and drainage water. Hence care should be taken not to irrigate a healthy field using drainage/irrigation water from a diseased field.

## **27. Field and plant sanitation**

Field and plant sanitation is an important method of disease control through cultural practices. The inoculum present on field plants in the field may multiply on the plant or in the soil and in due course of time may be sufficient to nullify or reduce the effect of control practices. Many pathogens overwinter or oversummer on plant debris during the off-seasons and become active when the crop is again grown in the field. Hence plants bearing pathogens or plant debris introducing inoculum into the soil should be removed as early as possible. In most of the soil borne diseases like wilt and root rot, it has been reported that as long as the dead roots and other roots and other affected parts are present in the soil, the fungus continue its growth. When such diseased plant materials are removed, there is quick decline in the population of pathogens in the soil. In this manner *Fusarium* wilt of cotton, pigeonpea and banana, *Verticillium* wilt of cotton, root rot of beans, downy mildew of pearl millet, sorghum, maize and peas, foot rot of betelvine, bacterial blight of cotton, white rust of crucifers, black spot of rose, powdery mildew of pea and cereals are reduced. In certain

areas the linseed rust fungus (*Melampsora lini*), the rice blast and brown spot fungi and the fungus causing early blight of potato also perennate through dormant stages in diseased crop debris. Destruction of crop debris by burning immediately after harvest reduces the amount of inocula which survive through debris.

It has been observed that leaf blight disease of rice particularly one caused by *Helminthosporium oryzae* is carried over in the stubbles and primary infection is evident in the self-sown tillers arising from these stubbles. Infection of *Sclerotium rolfsii* on jute is carried over in the foot and root regions in the stubbles left over after harvest of the jute plants. Sugarcane stubbles left over in the field help to carry over red rot fungus *Glomerella tucumanensis*, *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease on rice is capable of surviving for some time in rice stubbles. In many cases, diseased planting materials left in the field after discarding them, serve as sources of infection as in the case of late blight of potato where piles or refuses of rejected tubers later become an important source of infection. Left over plant parts of maize infected with the smut *Ustilago zae* constitute an important source of infection later.

Avoidance of the transfer of inoculum from one field to another by man, machine or water is one of the ground rules of cultural control. Where soil-borne diseases are concerned, anything that carries soil is suspect, this includes wheels, boots and water flowing either from adjacent fields, or through drainage ditches from distant fields. As regards sap-borne viruses, attention must be paid to disinfection of wheels and of the hands of labourers, as they pass from one field to another. Where such virus can also be carried on clothing. The work should be planned so that the labourers do not go from older to younger fields on the same day.

Many pathogens are capable of surviving on implements and materials used in sequential seasons. Tobacco mosaic virus has been shown to survive on iron stakes used for tomato trellises and disinfection of such stakes has been recommended. Soil adhering to plastic sheeting may carry sclerotia and other overseasoning bodies.

## **28. Roguing**

Roguing consists of completely removing or uprooting the diseased plants to prevent further spread of the disease. This method is widely adopted in the control of virus diseases spread by insects (cassava mosaic, yellow mosaic of blackgram and greengram, citrus tristeza, katte disease of cardamom, bunchy top of banana) and basal stem rot of coconut, green ear of pearl millet and broomrape (*Orobanche*) in tobacco. The whip smut of sugarcane (*Ustilago scitaminea*) in the canal areas of Bombay in Co.475 variety has been greatly checked by roguing carried out over wide areas and long period. In Jamaica, a country-wide campaign of destroying infected plants has succeeded in the control of Panama wilt of banana. Root rot and wilt attacked plants after their death should be as and when noticed in the field uprooted and burnt to check the inoculum build up in the soil.

## **29. Management of plant nutrients**

The plant nutrients in general when applied in excess may increase or reduce the resistance in plants to diseases. Increased application of nitrogenous fertilizers increases the incidence of many diseases. Crops fed with heavy doses of nitrogenous fertilizers grow robust with foliage and succulent tissue but become highly susceptible to the attack of diseases like rust powdery mildew, blast, tobacco mosaic and some bacterial diseases. In the case of blast of rice optimum dose of nitrogenous fertilizers are recommended and it is applied in 3 split doses viz. 50% as based at transplanting, 25% at tillering and 25% at panicle initiation stage. Late application of nitrogenous fertilizers increases wheat leaf blotch ( *Septoria nodorum*) and powdery mildew ( *Erysiphe graminis tritici*).

Some diseases are favoured by ammoniacal form of nitrogen while others are favoured by nitrate form of nitrogen. In general wilts ( *Fusarium* sp.) and root rots ( *Rhizoctonia* spp.) are favoured by ammoniacal nitrogen while *Verticillium* wilts and root rots due to *Pythium* spp. are favoured by nitrate nitrogen. In rice, blast disease is favoured by ammoniacal nitrogen while brown spot ( *Helminthosporium oryzae*) is favoured by nitrate nitrogen. In maize Northern corn leaf blight caused by *H. turcicum* is favoured by ammoniacal nitrogen while stalk rot ( *Diplodia maydis*) is favoured by nitrate nitrogen. In wheat, sharp eye spot ( *Rhizoctonia solani*) is favoured by ammoniacal nitrogen while stem rust ( *Puccinia graminis tritici*) is favoured by nitrate nitrogen. In potato, wilt ( *Verticillium albo-atrum*) and scab ( *Streptomyces scabies*) are favoured by nitrate nitrogen while ammoniacal nitrogen increases black scurf ( *R.solani*) .

Effects of nitrogenous fertilizers on major soil borne diseases have been studied. Their effect on the disease i.e., whether increased or decreased incidence by nitrogen in different forms are given in the following table.

Table. Effects of different forms of nitrogen on soil-borne diseases

Pathogen	Host	Amendment
<b>Diseases increased</b>		
<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	Tomato	NO <sub>3</sub>
<i>F.moniliforme</i>	Sorghum	NaNO <sub>3</sub> - NH <sub>4</sub> NO <sub>3</sub>
<i>F. roseum</i>	Carnation	NO <sub>3</sub>
<i>F.solani</i> f.sp. <i>phaseoli</i>	Bean	NH <sub>4</sub>
<i>Gaeumannomyces graminis</i>	Wheat	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
<i>Phytophthora nicotianae</i>	Tabacco	NO <sub>3</sub>
var. <i>nicotianae</i>	Cotton	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .Ca(NO <sub>3</sub> ) <sub>2</sub> .KNO <sub>3</sub>
<i>Verticillium albo-atrum</i>	Potato	NH <sub>4</sub> NO <sub>3</sub> +CaCO <sub>3</sub>
<i>Streptomyces scabies</i>		
<b>Disease decreased</b>		
<i>F. oxysporum</i> f.sp. <i>cubense</i>	Banana	Urea(nitrite)
<i>F.solani</i> f.sp. <i>phaseoli</i>	Bean	KNO <sub>3</sub>
<i>Gaeumannomyces graminis</i>	Wheat	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
<i>Phytophthora cinnamomi</i>	Avocado	KNO <sub>3</sub>
<i>Sclerotium rolfsii</i>	Tomato	Ca(NO <sub>3</sub> ) <sub>2</sub>

<i>S.rolfsii</i>	Sugarbeet	NH <sub>3</sub> .(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .Ca (NO <sub>3</sub> ) <sub>2</sub>
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Repeated application of phosphatic fertilizers delays the onset and lessens the severity of take-all disease of barley (*Gaeiimannomyces graminis*). Potassium application reduces the disease incidence in many crop diseases probably by increasing phenolics synthesis in plants. Application of potash induces resistance in groundnut against root rot caused by *Macrophomina phaseolina*. Calcium application suppresses the lesions due to the *R.solani* on bean roots. It is due to formation of calcium pectate, which is less available to action by polygalacturanase (PG) enzyme than is pectic acid. Calcium has also been shown to affect *Sclerotium rolfsii* by neutralizing the oxalic acid produced by the fungus. Application of molybdenum reduces infection of potato tubers by *Phytophthora infestans* and also diminishes incidence of *Ascochyta* blight on beans and peas .Manganese reduces late blight of potato, ferric chloride controls rice brown spot and silicon application reduced rice blast.

### 30. Time of harvesting

Time of harvesting affects the cleanliness of the seeds. Delayed harvesting of grain crops in temperate climatic conditions enables the pathogen more time to contaminate the seeds. The best example is grain mould of sorghum where contamination by species of *Fusarium*, *Curvularia*, *Alternaria*, *Aspergillus*, *Phoma* is seen. Potato tubers harvested when the tops are green get easily contaminated by the late blight pathogen present on the leaves. Removal of tops and making them to dry before digging the tubers kills the sporangia and avoids contamination of tubers harvested later.

### 31. Avoiding ratoons

Ratooning is a general practice in sugarcane when the incidence of grassy shoot disease and red rot are very high. Hence ratooning should be avoided.

### 32. Solar heating

When the soil is covered with white polythene sheets during hot seasons, soil temperature increases. Increased soil temperature eliminates wilt pathogens like *Fusarium oxysporum* f.sp. *lycopersici* and *Verticillium dahliae* from tomato field. High soil temperature also favours antagonistic fungi.

# 11

## Disease Management by Biological Methods

Biological control or biocontrol is employment of one or more organisms to eliminate or reduce the disease or damage caused by another. The biological methods of plant disease management include the use of antagonistic microorganisms or biocontrol agents, cross protection, hypovirulence, bacteriophages, use of plant products, *etc.*

### 1. BIOLOGICAL CONTROL

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. Otherwise it refers the use of natural or modified organisms, genes or gene products to reduce the effect of undesirable organisms and to favour desirable organisms such as crops. Biological control has been developed and extensively used against soil-borne diseases in the nursery and in the main fields. It has also been used in the control of other diseases. Management of plant disease by chemical methods is uneconomical, less effective and harmful to the beneficial microbes. In addition they cause residual problem in the soil and farm produce. Seed treatment chemicals enable protection only in the early stages of crop growth (about 15 days) but biological control is cheaper, highly effective and has no residual problem. They are not harmful to the beneficial microorganisms. When they are applied to the seed, they reach the soil, multiply in the organic matter in the soil and offer protection throughout the crop growth. In addition, they can be mixed safely with the biofertilizers and sown immediately after the seed treatment.

#### Advantages of biological control

1. Biological control is less costly and cheaper than any other methods.
2. Biocontrol agents give protection to the crop throughout the crop period.
3. Biocontrol agents not only control the disease but also enhance the growth by way of encouraging the beneficial soil microflora. It increases the crop yield. It helps in the availability of certain nutrients. For example *Bacillus subtilis* solubilizes phosphorus.
4. Application of biocontrol agents is safer to the environment and the person applying them.
5. They are highly effective against specific disease.
6. Biological agents can eliminate pathogens from the site of infection.
7. Biocontrol agents are very easy to handle and apply to the targets.

8. Biocontrol agents can be combined with biofertilizers.
9. They are easy to manufacture.
10. They do not cause toxicity to the plants.
11. They multiply easily in the soil and leave no residual problem.

### **Disadvantages**

1. Biocontrol agents can be used only against specific diseases.
2. They are less effective than the fungicides
3. Only few biocontrol are available for use and are available in few places.
4. They are unavailable in larger quantities at present.
5. They have slow effect in the control of plant disease
6. This method is only a preventive measure and not curative.
7. Biocontrol agents should be multiplied and supplied without contamination and requires skilled person.
8. Shelf life of biocontrol agents is short. *Trichoderma viride* is viable for 4 months and *P.fluorescens* is viable only for three months.
9. The required amount of population of biocontrol agents should be checked at periodical intervals and should be maintained at required level.
10. Their efficiency is decided by the climatic conditions.
11. Under certain circumstances a biocontrol agent may become a pathogen.

Biocontrol agents (fungi and bacteria) are used in the control of plant diseases and are highly effective in the nursery diseases and in fields where commercial crops are grown.

A good biocontrol agent should have the following features / characteristics.

- i. It should not be pathogenic to plants
- ii. It should have broad spectrum of its activity in controlling many types of diseases.
- iii. It should have fast growth and sporulation
- iv. It should be amenable for mass multiplication.
- v. It should be compatible with bio-fertilizers
- vi. It should have least susceptibility to the action by the seed treating chemicals.
- vii. It should not be toxic to human beings and animals
- viii. It should not be toxic to beneficial organisms in or on the target area.
- ix. It should be easily formulated
- x. It should have more shelf life

### **Mechanisms involved In biological control**

The biocontrol agents control the plant diseases by the following general mechanisms:

- i. Parasitism
- ii. Antibiosis
- iii. Competition
- iv. Lysis and
- v. Induced systemic resistance



**i. Parasitism or Predation of one organism by another:** The antagonistic microorganisms, parasitize the pathogen by coiling around the hyphae e.g. *Trichoderma viride* parasite derives its nutrients from the pathogen by puncturing the host hyphae and kill the pathogen. *Trichoderma hamatum* on *Pythium* sp. and *Rhizoctonia solani*, *Trichothecium roseum* on *Puccinia horiana* and *Sphaerotheca fuliginea* *T.harzianum* and *Trichoderma virens* on *Sclerotium rolfsii*.

**II. Antibiosis :** Antibiosis is defined as inhibition of an organism or its death by the action of toxic metabolites (antibiotics) produced by another organism. e.g.

Antagonist	Pathogen
<i>Gliocladium virens</i>	<i>Pythium ultimum</i> , <i>Rhizoctonia solani</i>
<i>Trichoderma</i> spp.	<i>Pythium</i> sp., <i>Fusarium</i> sp.
<i>Bacillus subtilis</i>	<i>Fusarium oxysporum</i> f.sp. <i>dianthi</i> <i>Monilinia fructicola</i>
<i>Pseudomonas fluorescens</i>	<i>Pythium ultimum</i> , <i>Rhizoctonia solani</i>

*Trichoderma* sp. produces three antibiotics, viz., Trichodermin, gliotoxin and viridin in culture. But they are not produced in appreciable quantities in soil. Phenazine – 1 – carboxylic acid produced by *P.fluorescens* plays an important role in suppressing the take-all disease of wheat.

iii. **Competition:** The antagonistic microorganisms compete with the pathogen for food and essential elements and space, making them unavailable for the pathogen and thereby suppress the growth of the latter. e.g.

Antagonist	Pathogen
<i>Fusarium</i> spp. (non-pathogenic)	<i>F.oxysporum</i> f.sp. <i>melonis</i>
<i>Trichoderma harzianum</i>	<i>F.oxysporum</i> f.sp. <i>melonis</i> , <i>F.oxysporum</i> f. sp. <i>vasinfectum</i>
<i>Acaligenes</i> sp.	<i>F.oxysporum</i> f.sp. <i>dianthi</i>
<i>Pseudomonas putida</i>	<i>Fusarium oxysporum</i>
<i>Pseudomonas</i> spp.	<i>F.oxysporum</i> , <i>F.oxysporum</i> f.sp. <i>cucumerinum</i> <i>F.solani</i> , <i>Pythium</i> <i>aphanidermatum</i> .

Bacteria such as *Pseudomonas*, *Chromobacterium*, *Enterobacter*, *Acaligenes* and *Arthrobacter* compete with the plant pathogenic fungi and reduce the disease.

Many bacteria produce siderophores which are low molecular-weight ferric ion-transport agent. Siderophores supply iron to the bacterial cell and selectively complex with iron with very high affinity and make it unavailable to other microorganisms including plant pathogens. *P.fluorescens* and *P.putida*

produce siderophore which controls soft rot of potato caused by *Erwinia carotovora*. The siderophores of *Pseudomonas* sp. is fluorescent and it is called pseudobactin. Pseudobactin has been found to control take-all disease of wheat and barley (*Gaeumannomyces graminis* var. *tritici*) and flax wilt disease (*Fusarium oxysporum* f.sp. *lini*). Inoculation of flax seeds with a suspension of *Pseudomonas* sp. or pseudobactin controls *Fusarium* wilt disease. Addition of pseudobactin to the soil also controls wilt diseases. The pseudobactin converts pathogen– conducive soils into pathogen suppressive soils. It suggests that suppressiveness of the soil is mainly due to the siderophores.

**iv. Lysis:** Different fungi and bacteria secrete hydrolytic enzymes and degrade the cell wall of the pathogens. *Trichoderma* sp. produces  $\beta$ -1, 3 glucanase and chitinase which degrade chitins and glucans in cell wall and causes its lysis in *R.solani*. The same fungus degrades glucans in the cell walls of *Pythium* sp. leading to its lysis. *Bacillus* sp. causes hyphal lysis of *Gaeumannomyces graminis*. The chitinolytic enzymes of *Serratia marcescens* caused cell wall lysis of *Sclerotium rolfsii*.

#### v. Induced systemic resistance

It involves inoculation of a plant with a non-pathogenic strain of microorganism becoming resistant to subsequent challenge by a pathogen.

Antagonist	Pathogen	Host
<b>Bacteria</b>		
<i>Bacillus subtilis</i>	<i>F.roseum</i> f.sp. <i>dianthi</i>	Carnation
<i>Pseudomonas</i> sp.	<i>F.oxysporum</i> f.sp. <i>dianthi</i>	Cucumber
<b>Fungi</b>		
<i>Colletotrichum lagenarium</i>	<i>Colletotrichum lagenarium</i>	Cucumber
<i>C.lindemuthianum</i> (non-pathogenic)	<i>C.lindemuthianum</i>	Bean
<i>F.oxysporum</i> (avirulent)	<i>F.oxysporum</i>	Sweet potato
<i>Verticillium albo atrum</i> (avirulent)	<i>V. albo-atrum</i>	Tomato

#### Mass multiplication of bio-control agents

##### a. *Trichoderma viride*

*T. viride* is a fungal antagonist, which is widely used to control root rot disease of pulses and oil seeds. The mass production technology of *T.viride* has been developed by Tamil Nadu Agricultural university, Coimbatore is as follows.

**Isolation:** *Trichoderma* is isolated from the soil by using *Trichoderma* selective medium (TSM) developed by Elad and Chet (1983). Collect soil samples from different parts of the field at random, mix well and make it into fine particles. The samples should be collected from *rhizosphere* region at a depth of 5-15 cm.

Ten gram of soil sample is taken and suspended in 90 ml of sterile distilled water and stirred well to get 1:10 dilution (10-1). One ml from this dilution is added to 9ml of sterile water in a test tube to get 1:100 dilutions (10-2). Make serial dilutions by transferring one ml of suspension to subsequent tubes to get dilution of 1:10,000 (10-4). Transfer one ml of the desired soil suspension (preferably 10-3 and 10-4 dilutions) to sterile Petri plates Pour 15 ml of melted and cooled TSM in the same Petri dishes. Rotate the plate gently and allow it to solidify. Incubate the plates at room temperature for 5-7 days and observe the development of fungal colonies. *Trichoderma* colonies will be white initially and turn to green afterwards. Transfer the individual colonies to Potato Dextrose Agar (PDA) slants.

**Dual culture technique:** It is a technique used to test the antagonistic activity of *Trichoderma* against plant pathogens. This method consists of growing the test organism and the pathogenic organism on the same plate. The procedure is as follows.

Transfer 15-20 ml of melted and cooled Potato Dextrose Agar medium to sterilized Petri dishes. Allow it to solidify. Transfer an 8mm disc of test organism (*Trichoderma*) to one end of the Petriplate. In the opposite end, an 8 mm disc of the pathogenic culture is placed in the same Petriplate. Incubate the plate at room temperature; observe the development of inhibition zone. Observe under microscope where both the test organism and the pathogen come in contact.

**Mass production:** Molasses-yeast medium (Molasses 30g+yeast 5g+one litre of water) is prepared in conical flasks and sterilized at 1.1kg/cm<sup>2</sup> for 30 minutes. *T. viride* culture is inoculated into the medium by taking a fungal disc from 10 day old culture. Incubate it for 10 days. This serves as mother culture. The molasses-yeast medium is prepared in a fermentor and sterilized. Then the mother culture is added to the fermentor @ 1.5 litre / 50 litres of medium and incubated at room temperature for 10 days. After incubation, the fungal biomass including the broth culture is mixed with the talc powder at 1:2 ratios (1 litre for 2 kg of talc powder). The mixture is then air dried. The sticking agent carboxy methyl cellulose (CMC) is added to the mixture @ 5 g/kg of the product. Then the product is packed in polythene bags and is used for treating the seeds. It contains approximately 107 to 108 colony forming units (cfu) per gram of product. This product can be stored of 4 months under room temperature without losing its efficiency.

#### ***Pseudomonas fluorescens***

*Pseudomonas fluorescens* is a bacterial antagonist, effectively used in controlling sheath blight and blast of paddy, wilt disease of redgram, chickpea and banana.

**Isolation:** Ten gram of rhizosphere soil is mixed in 90 ml of sterile water to give 1:10 (10-1) dilution. From this, serial dilutions are made up to 10<sup>-7</sup> by repeatedly transferring 1ml of 1:10 dilution to 9ml sterile water tubes. 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> dilutions are plated in king's B Agar medium and incubated for 24-48 hours. *P.fluorescens* appears as smooth, slimy, circular translucent colonies.

**Mass production:** *P.fluorescens* is multiplied in sterilized king's B broth for 48 hours. The pH of the substrate (talc powder) is adjusted to 7 by adding calcium carbonate @ 150 g/kg. The substrate is then sterilized at 1.1kg/cm<sup>2</sup> pressure for 30 min for two successive days. Four hundred ml of 48 h old culture suspension of *P.fluorescens* is added to 1 kg of substrate containing 5 g of carboxy methyl cellulose (CMC) and mixed well. The formulation is packed in polythene bags and can be stored for three months.

#### ***c. Bacillus subtilis***

This bacterium is used for the control of soil borne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* sp. etc. It is a rod shaped thermophilic, Gram positive, aerobic bacterium. It forms endospores during adverse conditions.

**Isolation:** Ten gram of soil sample is mixed with 90 ml of sterilised nutrient broth medium is kept under water bath at 80 c for 10 minutes. This is incubated at room temperature for 24-48 hrs. From this serial dilutions are made up to the 10<sup>-6</sup> dilutions. One ml of 10<sup>-5</sup> and 10<sup>-6</sup> dilutions are plated in nutrient agar medium and incubated for 24-48 hrs. The developing *B.subtilis* colonies are rough, opaque with irregular margins.

**Mass production:** Nutrient broth (peptone 5 g; Beef extract 3g; sodium chloride 3 g and 1 litre of distilled water, pH 7) is prepared in conical flasks and sterilized at 1.1 kg/cm<sup>2</sup> for 30 minutes. To this medium one loopful of *B.subtilis* culture is inoculated and incubated for 24 hrs. This serves as mother culture. The nutrient broth is prepared in fermentor as mentioned above. One litre of mother culture is inoculated into 100 litres of broth in a fermentor and incubated for 72 hrs. The broth culture is collected and mixed with peat soil amended with calcium carbonate (100 litres of nutrient broth culture mixed with 250 kg of sterilised peat soil amended with 37 kg of calcium carbonate). This product is air dried or shade dried and packed in polythene bags. It can be stored for six months.

#### **Methods of application of biocontrol agents**

Fungi like *Trichoderma viride* and bacteria like *Bacillus subtilis* and *Pseudomonas fluorescens* are commercially used as biocontrol agents in the management of plant diseases. They are used through the following methods.

- a. Seed treatment
- b. Seedling root dip
- c. Sucker treatment
- d. Capsule application
- e. Soil application
- f. Foliar spraying

##### **a. Seed treatment**

The fungus, *Trichoderma viride* and the bacteria such as *Bacillus subtilis* and *P. fluorescens* are used to treat the seeds of various crops.

**i. *T. viride* :** The talc based formulation (with 28 x 10<sup>6</sup> cfu/g product) of *T. viride* is used as dry seed treatment at 4 g per kilogram of seeds for the control of

root rot diseases of blackgram, greengram, chickpea, gingelly groundnut, sunflower and cotton caused by *Rhizoctonia solani*, *Pythium spp.*, *Macrophomina phaseolina* and *Sclerotium rolfsii*. Biofertilizers like *Rhizobium* and *Azospirillum* can be mixed with *T. viride* during seed treatment. The treated seeds are sown immediately unlike in fungicide treated seeds where the seeds are sown 24 hrs. after seed treatment.

**ii. *Bacillus subtilis*:** Peat based culture of *B. subtilis* is used for seed treatment. *B. subtilis* effectively controls root rot diseases caused by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* and wilt diseases caused by *Fusarium spp.* Besides, it also enhances growth and yield of crops.

**iii. *Pseudomonas fluorescens* :** Seed treatment with *P. fluorescens* is usually adopted in rice for the control of blast (*Pyricularia oryzae*) and sheath blight (*Rhizoctonia solani*). It controls wilt of pigeonpea (*Fusarium udum*) and wilt of chickpea (*F. oxysporum* f. sp. *ciceri*).

**Rice:** In rice, wet seed treatment is followed to control blast and sheath blight diseases. Rice seeds are mixed with the talc based product at 10 g per kilogram of seed soaked in 1 litre of water overnight. In the next morning excess water is decanted. The treated seeds are allowed to sprout for 24 hours and then sown in the nursery. The decanted water containing antagonistic bacteria is sprinkled over germinating rice seeds.

**Pigeonpea and chickpea:** Dry seed treatment is followed in pigeonpea and chickpea for the control of wilt diseases caused by *Fusarium spp.* Seeds are treated at the rate of 10 g for kilogram of seed.

#### **b. Seedling root dip**

Seedling root dip with *Pseudomonas fluorescens* is adopted in rice to control blast disease. Irrigation water is stagnated in an area of 25 sq. m. in the rice field. A quantity of 2.5 kg of *P. fluorescens* formulation is applied and mixed with the stagnated water. Rice seedlings required to plant one hectare are pulled out from the nursery. The root portion of these pulled out seedlings is immersed in the stagnating water containing antagonistic bacteria for a minimum period of 30 min. The seedlings after treatment are transported and transplanted.

#### **c. Sucker treatment**

In banana, suckers are treated with *P. fluorescens* before planting to control of panama wilt. Ten gram of the formulation is sprinkled on clay dipped suckers before planting in the field.

#### **d. Capsule application**

Capsules filled with formulation of *P. fluorescens* are used for the control of panama wilt of banana. Each capsule is filled with 50 mg of *P. fluorescens* and applied at one capsule to each banana sucker in the field at third month of planting. Capsule application is repeated on 6 and 9 months of planting for effective control of the disease.

#### e. Soil application

Soil application of biocontrol agent is recommended in the use of *Trichoderma viride* and *Pseudomonas fluorescens*.

i. ***T. viride*** : Soil application of *T.viride* is recommended for the control of *Macrophomina* root rot in pulses and oilseeds and *Fusarium* wilts in pigeonpea and chickpea. Talc based formulation of *T.viride* is mixed with well decomposed farm yard manure (FYM) or sand and then applied to soil. For treating one hectare of land 2.5 kg of the formulation is mixed with 50 kg of FYM or sand and then applied to the soil. Soil application is done 30 days after sowing.

ii. ***P. fluorescens***: Soil application is done in rice to control blast disease and panama wilt of banana. In rice, *P. fluorescens* formulation is mixed with well decomposed farm yard manure or sand and applied to rice crop 30 days after transplanting. For treating one hectare of land 2.5 kg of the formulation is mixed with 50 kg of FYM. In banana 2.5 kg of the formulation is mixed with 50 kg of FYM and applied at the time of planting and repeated once in three months.

#### f. Foliar spraying

Foliar spraying of *P. fluorescens* is recommended for the control of blast of rice at 0.2 per cent concentration i.e. 1 kg / ha of crop. The product is sprayed three times from 45 days after transplanting at 10 days interval. One to three sprays are recommended.

#### Commercial formulations of biocontrol agents

	Antagonist	Product	Source	Pathogen controlled
	<b>I. Fungi</b>			
1	<i>Ampelomyces quisqualis</i>	AQ 10 Biofungicide	Ecogen Inc., Isreal	<i>Erysiphe</i> spp., <i>Uncinula necator</i>
2	<i>Coniothyrium minitans</i>	Coniothyryn, Koni, BIOVED Ltd., Hungary	Russia	<i>Sclerotinia sclerotiorum</i>
3	<i>Gliocladium virens</i>	Gliogard	U.S.A.	<i>Rhizoctonia solani</i> , <i>Pythium ultimum</i>
4	<i>Peniophora gigantea</i>	Pg suspension	U.K.	<i>Heterobasidium annosum</i>
5	<i>Pythium oligandrum</i>	Polygandron	Vyskumy ustav rastlinnej, Czechoslovakia	<i>Pythium ultimum</i>
6	<i>Trichoderma</i> a	Trichodermin	Bulgaria and Russia	<i>Botrytis cinerea</i> , <i>Pythium</i> spp., <i>Sclerotinia sclerotiorum</i> , <i>Verticillium</i> spp.

		Binab-T	Sweden & U.S.A.	<i>Armillaria mellea</i> <i>Ceratocystis ulmi</i> <i>Endothia parasitica</i> <i>Rhizoctonia</i> spp.
7	<i>Trichoderma viride</i> / <i>T.harzianum</i>	Bioderma	Biotech International Ltd., India	
8	<i>Trichoderma harzianum</i>	F-Stop Trichodex	U.S.A Israel	<i>Erysiphe</i> spp., <i>Pythium</i> spp., <i>Uncinula necator</i>

## II. Bacteria

1	<i>Agrobacterium radiobacter</i> strain – 84	Galltrol –A Norbac 84 C  Diegall Nogall	Agrobiocsem Inc., U.S.A. New Bioproducts Inc., U.S.A. Bio-care Technology Pvt. Ltd. Australia U.S.A.	<i>Agrobacterium tumefaciens</i> " "
2	<i>Bacillus subtilis</i>	Quantum 4000  Gus 4000  Kodiak, Kodiak-At Kodiak	U.S.A.  U.S.A. Gustafson Inc., U.S.A.	<i>Fusarium</i> spp., <i>Rhizoctonia</i> spp., <i>Sclerotium rolfsii</i> , <i>Gaeumannomyces graminis tritici</i> <i>Aspergillus flavus</i> , <i>A. parasiticus</i>
3	<i>Pseudomonas fluorescens</i>	Conquer – HB  Dagger-G	Mauri foods, Australia  U.S.A.	<i>Pythium</i> spp., <i>Rhizoctonia solani</i>  <i>Pythium</i> spp., <i>Rhizoctonia solani</i>

## III. Actinomycetes

1	<i>Streptomyces griseoviridis</i> strain K 61	Mycostop	Kemiro Agro. Oy, Finland	<i>Alternaria brassicae</i> , <i>Fusarium</i> spp., <i>F. oxysporum</i> f.sp. <i>dianthi</i> ,
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Bulgaria

## IV. Viruses

1	Tomato mosaic virus	Tomovax (mild strain)	New Zealand	Tomato mosaic virus (severe strain)
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## 2. CROSS PROTECTION TECHNIQUE

Cross protection is a method by which some of the important viral and bacterial diseases are controlled. Cross protection is the phenomenon in which plant tissues infected with one strain of the pathogen is protected from infection by other strains of the pathogen. This phenomenon has been reported in different crops to important viral and bacterial diseases.

### Virus diseases

Mckinney was first to report the phenomenon of cross protection against TMV, Mckinney (1992) defined cross protection as a phenomenon in which plants systemically infected with one strain of a virus are protected from infection by a second related strain of the same virus.

The criteria before cross protection is contemplated as a strategy are:

- a. the disease should be endemic and impossible to eradicate.
- b. it should spread rapidly
- c. losses from the disease should be so great that some reduction in yield from a mild strain over a long period may be preferable alternative and there should be evidence that mild strains protected effectively without causing undue harm.

The plant diseases controlled by using cross protection are given below:

**Papaya ringspot virus (PRV):** This is a major disease in South America, Caribbean countries, Africa, India and Far East. It causes mottling and distortion of leaves, ringspots on fruits, water soaked streaks on stems and ringspots on fruits, water soaked streaks on stems and petioles, stunting of plants and reduced fruit size. The virus belongs to potyvirus group with filamentous particles measuring 780 x 12 nm, stylet-borne by aphid vectors and sap transmissible. Roguing of infected plants is not feasible under field conditions. Therefore attempts were made to solve the problem by cross protection. For this purpose, mild strains were produced by treating crude sap of plant infected by a severe Hawaiian strain with nitrous acid and the sap was inoculated to *Chenopodium quinoa* which is a local lesion host. After screening 663 local lesion isolates on papaya two mild mutants PRV HA5-1 and 6-1 were selected. Nitrous acid treatment resulted in point mutation. These mutants produced no conspicuous symptoms in papaya in the greenhouse and provided a high degree of protection against the severe wilting and mosaic strains prevalent in Taiwan. In Taiwan, solid blocks with plants inoculated with these mild strains were established. Roguing plants was carried out in the adjacent unprotected block every ten days upto flowering. Protected trees yielded 82 per cent more with better fruit quality resulting in 111 per increased income.

The mild strain was propagated in *Cucumis metuliferus*. The leaves were ground in 0.01 M cold potassium phosphate buffer  $p^H$  7.0 using 50 ml/g tissue. After straining the extract through cheese cloth carborundum powder was added at 40 g/lit and taken in a metal tank connected to a spray gun attached



to an air compressor. The seedling was inoculated by pressure spray (8 kg/sq.cm). One person can inoculate 10,000 seedlings in 2 h. After four weeks, all the inoculated plants showed positive presence of mild strain of the virus. In 1984, large scale field trials in 122 ha were conducted (2, 44,000 plants). Due to the success, it has now become routine practice in Taiwan. Upto 1993, papaya orchards in 1722 ha (4, 44,000 plants) have been planted with protected plants. The cost worked out to just \$ 28/ha. However, cross protection broke down under heavy disease pressure and lasted for 13 to 16 months only.

**Causes for breakdown:** The breakdown is attributed to the following causes

- i. Mild strain is introduced into young leaves around apex where its concentration is low.
- ii. High disease pressure adjacent to severely infected orchards.
- iii. Some of the mild strain inoculated papaya seedlings may not have been infected.

Severe strain different from the one for which it was tested may be prevalent in the locality.

**Tomato mosaic virus (ToMV):** It is a major problem wherever tomato is grown especially glasshouse grown tomato. A symptomless mutant (M 11-16) was produced by nitrous acid treatment of the virus. This provided a wider base for selection than heat attenuation. It is commercially produced and widely used in the Netherlands and the U.K. where it increased yield by 6-15% crude sap of heat 34 °C attenuated mild strain diluted to 1:10 or commercial preparation diluted to 1:1000 so as to contain 28-1220 g of virus/ml was effective. The inoculum was prepared by mixing 100 ml of diluted virus suspension with 1 g carborundum powder and taken in a commercial paint sprayer. The seedlings in nursery were inoculated with a pressure of 1.28 kg/cm<sup>2</sup> by keeping the spray nozzle 10 cm from the plants and moving it at 1 m/sec. For small nurseries an artists air brush can be used when the seedlings were in the third leaf stage, which gave a higher percentage of infection than paint sprayer with compressor. But the latter method is preferred because it avoided transmission of severe strains present in the seedlings. In Japan scientist produced mutant (L 11A) by exposing severe strain infected plants to high temperature (34°C). It is successfully used on a large scale and the method is called vaccination. It gave complete protection against Ohio IV strain. In China, a symptomless mutant was produced by nitrous acid treatment. The seedlings were infected by simply immersing the roots in virus suspension which is an easy method. Under field conditions it increased the yield by 60 per cent.

**Citrus tristeza virus (CTV):** This is a serious disease in Argentina, Brazil, the U.S.A., Spain, South Africa, India and Israel where it destroyed over 50 x 10<sup>6</sup> trees. In sweet orange (*C. sinensis*) it is a problem when susceptible rootstocks like sour orange are used. On the other hand it affects acid lime irrespective of the rootstocks. It is caused by a Closterovirus transmitted by budding and also aphid vector *Toxoptera citricida*. Several mild strains which protected the plants against severe strains inoculated by the aphid vector were identified.

Symptoms of severe strain: The infected trees in general do not show the seasonal new flushes or growth. The leaves show chlorosis along the main and

lateral veins with a large number of vein flecks. Defoliation occurs from tip downwards and show dieback symptoms and twigs remain barren. Sudden death of some trees may occur within a few months following infection but some may produce sparse foliage and bear fewer small fruits of inferior quality. The severe strain also produce stem pitting symptom. This is characterised by small long depressions or grooves in the wood of the branches and trunk.

Symptoms of mild strain: Few flecks are seen on the leaves only. Less number of pits develops which are smaller in size than the severe strain affected stem. The trees infected with mild strains may survive for more than 30 years with chlorotic leaves, but their productivity is considerably reduced. These trees are used as source of mild strains. In Brazil,  $8 \times 10^6$  sweet orange trees were protected by this method with no breakdown in protection. However in Australia, protection of grapefruit lasted only for three years. Since citrus is a perennial fruit plant propagated by budding it is a one time operation and hence easy to adopt. In Tamil Nadu, the tristeza problem in acid lime has been solved by a mild strain which is able to give effective cross protection against severe strain in field. From 1976, over one lakh seedlings have been cross protected without any breakdown. Two mild strains  $M_1$  and  $M_2$  gave effective protection in acid lime in Karnataka and increased tree vigour and yield.

Methods of obtaining mild strain: Mild strains can be obtained by selection from natural viral populations, induced mutation of natural population followed by selection and exposing inoculated plants to high or low temperature.

#### **Pre- immunization technique in acid lime:**

Acid lime seedlings are pre-immunized with mild strain of citrus tristeza virus to protect the trees from severe strain of the citrus tristeza virus. Patch budding is adopted in citrus. Acid lime seedlings are raised in the seed beds. Four to five month old vigorous seedlings transferred to polythene containers are selected for grafting. Scion materials (rectangular bark piece of 5mm x 2mm size) are collected from the trees with symptoms of the mild strain virus. The selected scions are stored in cool box to prevent desiccation. Scions are grafted on the seedlings within 36 hours after collection. In the acid lime seedlings to be protected a rectangular bark piece (of 5mm x 2mm size) is removed. The bark piece collected from mild strain infected trees is inserted into the seedling and covered with a polythene strip. The grafted seedlings are left undisturbed but watered at regular intervals for 15 days. Then the grafts are examined for their union. If the union remains green in colour, it indicates the successful establishment of the graft and introduction of mild strain into the seedlings. The mild strain of the virus multiplies in the seedlings and subsequently gives resistance to infection by the severe strain.

**Apple mosaic virus:** When branches of a tree showing infection by severe strains were removed and top work with a branch with mild strain the trees were effectively rescued. However, since the disease does not spread in the field, its scope for young trees is limited.

**Cocoa swollen shoot:** It is devastating and widespread in Ghana where 260 x 10<sup>6</sup> trees have been destroyed. Several mild strains of the virus occurring in nature protected the trees against transmission by the mealy bug vector *Pseudococcus njalensis*.

**TMV in chilli :** This is a rod shaped RNA virus belonging to Tobamovirus group and is transmitted by contact and mechanically but has no insect vector. An attenuated strain of the virus by heat treatment gave complete protection against the parent isolate, if inoculated ten days in advance.

**CMV in chilli:** This is a spherical virus belonging to group cucumovirus and is transmitted in a stylet borne manner by aphid vectors. Strain of CMV, S5-1 gave effective cross protection against severe strain under field conditions and increased yield.

**Soybean mosaic virus:** In Japan, Attenuated strains of Soybean mosaic virus Aa 15-M.1 and Aa 15-M were isolated. In field experiments they reduced the disease incidence to 5 and 15 per cent respectively as against 80 per cent in unprotected field.

**Drawbacks:** Though this technique has been promising under field conditions for managing some serious virus diseases of several crops, the following drawbacks have been observed.

- a. Protection might not be complete.
- b. The mild strain applied to one crop may spread to other crops in which it may cause severe symptoms.
- c. When a mild strain inoculated plant is infected by unrelated virus there may be synergistic reaction leading to a severe disease than infection by the second virus alone.
- d. The mild strain may mutate to severe form which would endanger the crop. Considering the fact that one cell and leaf of tobacco infected by TMV produces 10<sup>6</sup> and 10<sup>14</sup> viruses respectively the risk of mutation assumes added importance, and
- e. For annual crops the cost of inoculating million of seedlings every year possesses a Herculean task.

### **Bacterial diseases**

The phenomenon of cross protection has been established in some of the bacterial diseases in plants. A plant infected with a mild strain of the bacterium escapes disease from infection by severe strain(s). Virulent strain of *Agrobacterium tumefaciens* protects the apple crop from severe infection by virulent strains of *A.tumefaciens*. The same mechanism has been reported in apple to the fire blight bacterium, *Erwinia amylovora*. Heat killed cells of *Pseudomonas tabaci* protected tobacco crop from angular leaf spot or wild fire by a virulent strain.

## **3. HYPOVIRULENCE**

Hypovirulence is defined as the reduced virulence of pathogen strain as result of the presence of transmissible double stranded RNA. Inoculation of diseased plants with hypovirulent strains (pathogens with reduced pathogenicity) carrying double-stranded RNA or DNA plasmids can results in decrease virulence in the population of pathogens overall, through transfer of these extra chromosomal elements to pathogenic strains *in vivo*. If a hypha of a hypovirulent strain can be anastomosed with hypha of a virulent strain the latter loses vigour for infection and producing disease. A mixture of normal and hypovirulent strains significantly reduces damping off caused by *Rhizoctonia solani*. However, the hypovirulent strains have less longevity. Examples of Hypovirulence in fungi are listed below.

Hypovirulent strain of <i>Rhizoctonia solani</i>	- <i>R. solani</i> on different crops
Hypovirulent strain of <i>Cryphonectria parasitica</i>	- <i>C. parasitica</i> on chestnut
Hypovirulent strain of <i>Ophiostoma ulmi</i>	- <i>O. ulmi</i> on elm
Hypovirulent strain of <i>Endothia parasitica</i>	- <i>Endothia parasitica</i> on chestnut

Contagious hypovirulence is noted in bacteria and viruses. Some of the viruses which are called mycoviruses attack fungi and cause reduction in their virulence. This phenomenon is called contagious hypovirulence.

#### 4. BACTERIOPHAGES

Bacteriophages (bacterial viruses) are viruses which kill the bacteria. In 1917 d'Herelle found out bacteriophages. He presented evidences of a transmissible lytic principle that acted on the shiga bacillus, showing that bacteria have their infective diseases. A number of phages have been discovered for many phytopathogenic bacteria such as *Agrobacterium tumefaciens*, *Erwinia amylovora*, *E. aroid*, *E. atroseptica*, *E. carotovora*, *Pseudomonas glycinea*, *P. lachrymans*, *P. phaseolicola*, *P. pisi*, *P. syringae*, pv .*morsprunorum*, *P. tabaci*, *P. xanthoclona*, *Xanthomonas axonopodis* pv.*citri*, *X. axonopodis* pv.*malvacearum*, *X. axonopodis* pv. *phaseolicola*, *X. axonopodis* pv. *pruni* and *X. oryzae* pv. *oryzae*. Practical use of phages for control of bacterial plant diseases in the field has not been successful. When some control was achieved, this was brought about by inoculation with a mixture of the phage and the bacterium, or by plant or seed treatment with phage before challenge with bacteria. In practice, however, the pathogenic bacteria in plant tissues are in a dense mass and frequently surrounded by abundant extracellular polysaccharides, which prevent effective adsorption of phage particles. Another obstacle is the complexity of phage bacterium interrelationships in nature, due to the diversity of bacterial strains that differ in phage susceptibility.

## 5. MYCORRHIZAE

Mycorrhiza (*Mykes* = mushroom + *rhiza* = root) is a symbiotic association between certain fungi and the roots of higher plants. The infection of roots into unique morphological structures are called 'mycorrhiza'. There are two distinct classes of mycorrhiza. They are

1. Ectomycorrhiza and
2. Endomycorrhiza

**1. Ectomycorrhiza:** In ectomycorrhiza, the fungal symbionts penetrate intercellularly and partially replace the middle lamella between cortical cells of the roots and this hyphal arrangement around such cortical cells is called the '**Hartig-net**'. The ectomycorrhizal fungal symbionts also form a dense, usually continuous, hyphal net work, or 'fungal mantle' over the feeder root surface. Normally ectomycorrhizal association takes place on most tree species in the Pinaceae, Salicaceae, Betulaceae, Leguminosae, Ericaceae, Fagaceae and certain other families. The majority of the ectomycorrhizal fungi are Basidiomycetes, mainly in the families of Amanitaceae, Tricholomataceae, Rhizopogonaceae, and Boletaceae. From a diverse spectrum of investigations carried out, it has been postulated that the mycorrhizal interaction with root diseases may occur in following ways.

**i. Antibiosis:** Some ectomycorrhizal associations can produce antibiotics towards pathogens, such as *Phytophthora cinnamomi*. It has further been shown that a well developed fungal sheath of ectomycorrhiza is less able to be penetrated by pathogenic fungi.

**ii. Improved nutrition:** Improved nutrition caused by mycorrhizal development can indirectly affect the disease. Increased concentration of amino acids particularly arginine of mycorrhizal tobacco can suppress chlamydospore formation by root pathogen, *Thielaviopsis basicola*.

**iii. Compensation:** Roots lost by disease attack, in a deficient soil, will normally lead to heavy yield losses, but active mycorrhiza formation and resultant growth into the soil can compensate for such losses. This compensating tissue is not susceptible to the same diseases as root and this provides an ideal example of insurance systems the plants have developed during evolution. It has been shown that mycorrhizal infection of *Pinus radiata* by *Rhizopogon lutiolus* compensated for damage which would have otherwise occurred from *Phytophthora cryptogea* had the plant been non-mycorrhizal.

**iv. Avoidance:** Suberization or cutinization of newly formed roots confers some degree of disease protection, but it also causes a large decline in the ion-uptake by the part of the root. Nevertheless, if the root has become mycorrhizal before suberization it can maintain an efficient uptake function via the mycorrhizal system enjoying the disease protection of suberization.

### 2. Endomycorrhiza

In Endomycorrhiza, the fungal symbionts penetrate the cortical cells of the feeder root intracellularly. Such fungal symbionts produce large ‘vesicles’ and ‘arbuscules’ in cortical tissues and thus are called vesicular arbuscular mycorrhiza (VAM). Such fungal symbiont, however, do not form a dense fungal mantle, instead develop on the root surface a loose, intermittent arrangement of mycelium with large spores. Endomycorrhizal colonization takes place in most of the agronomic and horticultural crops that do not form ectomycorrhiza, and the fungal symbionts are Phycomycetes belonging to the family Endogonaceae.

A third class of mycorrhizae, viz., ectendomycorrhiza has also been observed. This intermediate type is present on roots of certain tree species under specific ecological situations. This mycorrhizal type resembles ectomycorrhiza in forming a Hartig-net and a fungal mantle, but they also resemble endomycorrhiza in respect of intracellular penetration cortical tissues by these fungi. It has been demonstrated that VAM infection may be able to promote or inhibit the development and consequent progression of plant diseases in several ways including.

- i. Shift in the dynamics of rhizosphere population to the benefit or detriment of pathogenic organisms and
- ii. Change in the physiology of the host plant to promote or inhibit pathogenic development.

Table. Important plant diseases managed by VAM fungi (VAMF)

Sl. No.	Host	Disease	Pathogen	VAMF
1.	Chickpea	Root rot	<i>Sclerotium rolfsii</i>	<i>Glomus fasciculatum</i>
		Wilt	<i>F. oxysporum</i> f.sp. <i>ciceri</i>	<i>Glomus</i> sp. <i>G. aggregatum</i>
		Root rot	<i>Rhizoctonia bataticola</i>	<i>G. mosseae</i>
2.	Groundnut	Root rot	<i>Sclerotium rolfsii</i>	<i>Glomus fasciculatum</i>
3.	Rice	Stem rot	<i>S.oryzae</i>	<i>G.mosseae</i>
4.	Blackgram	Root rot	<i>Macrophomina phaseolina</i>	<i>G.aggregatum</i>
5.	Greengram	Root rot	<i>M.phaseolina.</i>	<i>G.claroideum</i> <i>G.mosseae</i>
6.	Cowpea	Root rot	<i>M.phaseolina</i>	<i>G.fasciculatum</i> <i>G.etunicatum</i>
7.	Tomato	Wilt	<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	<i>G.etunicatum</i>
8.	Cotton	Wilt	<i>Fusarium oxysporum</i>	<i>G.macrocarpum</i>

			<i>f.sp.vasinfestum</i>	
9.	Soybean	Root rot	<i>Phytophthora megasperma</i> var. <i>sojae</i>	<i>G. etunicatus</i>
10	Maize	Root rot	<i>Rhizoctonia solani</i>	<i>G.mosseae</i>
11.	Rice	Sheath blight	<i>Rhizoctonia solani</i>	<i>G.mosseae</i>
12.	Tobacco	damping off	<i>Pythium</i> sp.	<i>G.fasciculatum</i>

## 6. PLANT PRODUCTS

Neem products, Mahua oil, pungam or Karanj oil and leaf extracts of various plants are used in the control of vectors of viral and phytoplasmal diseases and in the control of some fungal and bacterial diseases of crop plants. Among them neem products like neem seed kernel extract, neem oil, neem oilcake and neem extract are widely used as they are safe to ecofriendly populations. The important active principles in the neem products include azadirachtin, nimbin, nimbidin, nimbinene, nimbidic acid and azadirone and they possess antifungal and insecticidal properties.

### a. Neem products

**Neem seed kernel extracts (NSKE) 5%:** Neem seed kernel is powdered. Twenty five kg of powdered neem seed kernel is taken in a gunny bag and tied. It is soaked in 500 litres of water for 8 hours. The gunny bag is shaken thoroughly inside the water and removed. 500 ml of sticker is mixed to the extract. The neem seed kernel extract thus obtained is ready for spraying. It is used to control the green leaf hopper (GLH), the insect vector of Rice Tungro Virus (RTV). Three sprays at 15 days interval effectively controls the vector and reduces the spread of RTV. NSKE 5% foliar spray at the time of panicle emergence reduces the sheath rot disease (*Acrocyndrium oryzae*) in rice. In blackgram two sprays of NSKE 5% at 15 days interval controls powdery mildew (*Erysiphe polygoni*).

**Neem oil 3%:** In plant disease management neem oil 3% foliar spray is followed unlike in the insecticidal sprays preparation. Here Teepol (1ml / litre of water) is mixed first with water and then the insecticidal neem oil is added. The final solution will be milky white in colour. 30 ml of neem oil per litre is added to water to get 3% concentration. For one hectare 15 litres of neem oil is required to mix in five hundred litres of water. Neem oil 3% is used to control green leaf hopper, the vector of RTV. Three sprays are given at 15 days interval. For control of whitefly vector of yellow mosaic in blackgram and green gram neem oil 3% spray is done. Sheath rot of rice is controlled with neem oil 3% when it is sprayed at the time of panicle initiation. Rice blast (*Pyricularia oryzae*) is also controlled by neem oil 3%. Rust of groundnut (*Puccinia arachidis*) and powdery mildew of blackgram (*E. polygoni*) are controlled by two sprays with neem oil 3% at 15 days intervals.

**Neem cake:** Neem cake obtained after extraction of oil is used in the control of soil-borne diseases. Neem cake is powdered and directly applied to the field before last ploughing for sowing. Soil application of 150 kg of neem cake per hectare as basal dressing reduced sheath blight (*Rhizoctonia solani*) and blast (*Pyricularia oryzae*). In cotton, pre-emergence, post-emergence damping off diseases (*Rhizoctonia solani*) were reduced by soil application of neem cake at 2.5 and 5.0 tons/ha respectively. Soil application of neem cake controlled root rot of blackgram (150kg/ha), chickpea wilt (*Fusarium solani*), basal stem rot of coconut (*Ganoderma lucidum* @ 5 kg/ tree), betelvine foot rot (*Phytophthora capsici*) and Crossandra wilt (*Fusarium solani*).

**Neem cake extract:** Neem cake is powdered. Fifty kilogram of neem cake is taken in a gunny bag and is soaked in 500 litres of water for a period of 8 hrs. The gunny bag is removed after thorough shaking. To the extract, 500 ml of sticker (Sandovit or Teepol or Triton AE or Tween 20) is added and mixed well. This extract is used to control citrus canker.

Table. List of neem formulations

Trade name and formulation	Manufacturer
Bioneem	Ajay Biotech Laboratories (P) Ltd., Maharashtra
Biosol (Neem oil)	-
Econeem	P.J. Margo (P) Ltd., Karnataka
Field Marshall	Khetiwadi Corner, Vadodara – 390 001. Gujarat.
Gilmore uni-Gel Neem IGR	Gilmore Inc.USA
Godrej Achook	Bahar Agrochem and Feed (P) Ltd. Godrej Agrovat Ltd., Bombay –79.
Jawan Crop Protector	McDA Agro Pvt. Ltd., Bombay-1.
Juerken	Madurai Fertilizers and Agro chemicals pvt. Ltd., Tamil Nadu.
Kemissal	-
Margocide – CK 20EC	Monofix Agro products Ltd., Hubli 580 029.
Margocide –OK 80 EC	-do-
Neemark	West Coast Herbochem Pvt. Ltd, Bombay-25.
Neemax	Ecomax Agrosystems, Maharashtra.
Neemazal F	EID Parry (I) Ltd., TamilNadu.
Neemazal t/S	-do-
Neembicidine	T. Stanes and CO.Ltd., TamilNadu
Neem gold	Southern Petrochemical and Industrial Corporation Ltd., Madras – 32.
Neem guard	Gharda chemicals Pvt. Ltd., Bombay-50
Neemicide	-
Neem Plus	-
Neemta 2100	A.J. Chemicals, Ahmedabad – 380 002.
Nimba	IARI, New Delhi – 12.



Nimbasol	Nimba Foods and Chemicals Pvt. Ltd., New Delhi.
Nimbin	Sunline Agrochemicals , P.B.No.73, Dhulia.
RD-9 Ropellin 93EC	ITC Ltd., ILTD, Andhra Pradesh
Sukrina	Conser Chemicals Pvt. Ltd., Madras-16.
Suneem	Sunida Exports, Bombay – 400 049.
Wellgro	ITC Ltd., ILTD, Rajamundhry, Andhra Pradesh.

#### b. Other botanicals

Plant	Disease / Pathogen Controlled
Tulsi ( <i>Ocimum sanctum</i> ) (leaf extract)	Rice brown spot ( <i>Helminthosporium oryzae</i> )
Vilvam ( <i>Aegle marmelos</i> ) (leaf and pollen extract)	Early blight of tomato ( <i>Alternaria solani</i> ) Blight of onion ( <i>A. Porri</i> )
Periwinkle ( <i>Catharanthus roseus</i> ) (Flower extract)	Early blight of tomato <i>A.solani</i>
Garlic ( <i>Allium sativum</i> ) (Bulb extract)	Early blight of tomato ( <i>A. solani</i> ), Blight of finger millet ( <i>Helminthosporium nodulosum</i> ) Blast of rice– ( <i>Pyricularia oryzae</i> ).
Mint ( <i>Mentha piperita</i> ) (leaf extract)	Rice grain discolouration ( <i>Drechslera oryzae</i> )
Kolinji (Root exudates)	Basal stem rot of coconut ( <i>Ganoderma lucidum</i> )
Banana (Rhizome extract)	Basal stem rot of coconut <i>Ganoderma lucidum</i>
Pinnai ( <i>Calophyllum inophyllum</i> ) (Seed oil)	Groundnut rust ( <i>Puccinia arachidis</i> )
Nochi ( <i>Vitex negundo</i> ) (leaf extract)	Rice tungro virus

#### Antiviral principles

The use of antiviral agents as a component of the biological control is aimed to reduce the inoculum density or disease producing activities of the viral pathogens. The antiviral agents are broadly classified into two groups based on their mode of action viz.,

- i. antiviral agents restricting virus infection and
- ii. antiviral agents affecting virus multiplication.

Though different forms have been used by research workers, the term antiviral principles (AVP) is considered more appropriate for substance derived from organic sources and complex in composition. An antiviral principle (AVP) is defined as a substance, when present in the inoculum or plant tissue, is capable of either acting directly by inactivating the viral pathogen or activating in directly by increasing the level of host resistance resulting in reduction of virus infection. Antiviral principle may act both directly and indirectly. They may be present as a constituent of plant of another substance or they may be provoked in

the plants following application of another substance or a consequence of interaction between the host and the virus. Induction of resistance in susceptible plants by introducing certain substances can also be considered as a resultant of action of antiviral principles.

### **Antiviral principles for non-host plants**

**i. Distribution:** Presence of AVPs in plant extracts has been reported. Presence of potent AVPs reducing TMV has been detected in about 50 plant species. Infection with TMV was completely prevented by extracts for *Beta vulgaris*, *Bougainvillea spectabilis*, *Crassula indica*, *Chenopodium ambrosioides*, *C. murale*, *Eugenia jambos*, *Mirabilis jalapa*, *Pisonia alba*, *odononema nitidum* and *Peltophorum ferrugineum* and *Tuxnera ulmifolia*.

The AVPs can be extracted from fresh leaves or from air dried plant tissues. Aqueous extracts of flowers contain the AVPs capable of reducing the number of local lesions or systemic infections. Leaf and root extracts of *Phyllanthus fraternus* (*P. niruri*) effectively inhibited TMV, peanut green mosaic and tobacco ring spot virus. Fresh latex from fig, mulberry and *Calotropis procera* completely inhibited the development of local lesions by Radish mosaic, Zinnia mosaic and Petunia mottle viruses.

**ii. Extraction and testing of antiviral principles:** The antiviral principles may be extracted from fresh leaves in water or in organic solvents such as acetone, ethanol. etc. Solvent or water is added to plant tissue in required proportion (W/V) and the tissue is macerated and filtered. The extract is added to the virus inoculum and inoculated on the leaves of test plants to determine the antiviral activity of the AVP. The extract may be sprayed on the leaves of test plants which are inoculated with the virus at different periods after application.

**iii. Chemical nature:** The chemical nature of the antiviral principles from plants was studied. The inhibiting substance from the pokeweed (*Phytolacca americana*) is a basic protein consisting of about 116 amino acid residues with a molecular weight of 13,000. *Chenopodium album* contains an inhibitor which is also a basic protein having molecular weight of 25,000 to 38,000. The antiviral principle from sorghum leaves is indicated to be a protein. The antiviral principle from spinach (*Spinacia oleracea*) is a protein and it reduces the number of lesions / concentration of virus in plants reacting with local lesions or systemic symptoms to TMV inoculation, the reduction being proportional to the concentration of virus inhibiting protein. The AVP is a protein with a molecular weight of 29,000 and isoelectric point of 10.3. The antiviral activity is destroyed by heating to 70°C for 30 min. This protein is found to be serologically related to the AVP from *Phytolacca* sp. The AVP from the dried roots of *Boerhaavia diffusa* has been found to be glycoprotein capable of inhibiting several viruses affecting vegetable crops. In other cases AVP may be glycoproteins polysaccharides, flavones or glycoalkaloids. The list of non-host plants in which the AVPs are found, the nature of AVPs and the virus to which AVPs are effective are given in the Table.

Table. Chemical nature of antiviral principles from non-host plants

Source	Nature of AVP	Virus to which effective
<i>Dianthus caryophyllus</i>	Protein	Tobacco mosaic virus (TMV)
<i>Phytolacca americana</i>	"	"
<i>Chenopodium album</i>	"	"
<i>Cocoa nucifera</i>	"	Tomato spotted wilt virus
<i>Spinacia oleracea</i>	"	Tobacco mosaic virus
<i>Pseudoranthemum atropurpureum tricolor</i>	"	Sunnhemp rosette virus
<i>Yucca recurviflor</i>	"	TMV
<i>Punica granatum</i>	Glycoprotein	TMV
<i>Boerhaavia diffusa</i>	"	TMV
<i>Abutilon striatum</i>	Polysaccharides	TMV
<i>Beta vulgaris</i>	"	Tobacco mosaic
<i>Capsicum annuum</i>	Flavones	Potato virus Y
<i>Solanum</i> spp.	Glycoalkaloids	TMV and Sunhemp rosette virus

**iv. Biological properties:** The antiviral principles occurring *de novo* in plants have certain common biological properties. The AVPs are active against the viruses affecting other hosts but not against the viruses affecting the plant species from which the AVP is obtained. For example, the inhibiting substance from pokeweed inhibited polypeptide synthesis, when ribosomes from wheat and cowpea were used, but there was no inhibiting effect when ribosomes from pokeweed were employed. It was considered that this inhibitor blocked *in vivo* messenger activity of viral RNA on ribosomes from other host species. Synthesis of phenylalanine peptide as also inhibited by pokeweed inhibitor. The activity of pokeweed inhibitor was found to be non-specific. The antiviral activity of the inhibitors is reduced on dilution. It is suggested that most of the virus inhibitory substances affect the host but not the virus by competing with the virus for (receptor) infection sites on the host. The inhibitor from *Chenopodium album* was introduced into the cytoplasm was responsible for the failure of the cytoplasm to absorb virus particles as a result of the disorganization of cytoplasm due to its hypersensitivity to the inhibitor.

The AVPs are found to be more effective as pre-inoculation treatments than as post inoculation treatments. The persistence of antiviral activity of plants treated with AVPs may be for different periods. As pre-inoculation application, the AVP from sorghum leaves protected cowpea plants against tomato spotted wilt virus (TSWV) for 10 days. The AVP from *B. diffusa* when sprayed twice a week prevented infection of tobacco mosaic virus in tobacco. The activity of the AVP from *Spinacia oleracea* was lost after about 9 h.

**v. Uses:** AVPs are used in the disease management in crops like groundnut and tobacco (Table )

Table. Effect of AVP on disease management

Source of AVP	Crop and diseases controlled
Coconut leaf extract	Tomato spotted wilt virus causing ring mosaic in groundnut.
Sorghum leaf extract	“
<i>Basella alba</i> leaf extract	TMV on tobacco
<i>Peltophorum ferrugineum</i> leaf extract	TMV on tobacco in the nursery and main field.
<i>Pithecellobium dulce</i> twig extract	“
<i>Prosopis juliflora</i> leaf extracts (10%)	Tomato spotted wilt virus on tomato
<i>Cynodon dactylon</i> leaf extract (10%)	“

**vi. Preparation of AVP extract from sorghum leaves:** Sorghum leaf extract contains AVP effective against tomato spotted wilt virus (TSWV) causing bud necrosis or bud blight or ring mosaic in groundnut. Fresh sorghum leaves (1kg) are collected from the field and cut into pieces and air-dried. Then it is powdered using a grinder. Pure water (2 litres) is added to the leaf powder to have a proportion of 1:5 (W/V). The suspension is taken in a vessel, warmed and maintained at 60°C for one hr. Care should be taken not to exceed this temperature level as the AVP may lose its activity at higher temperature. Then the suspension is filtered through cheese cloth or cotton wool. The leaf extract is diluted with water to 10 litres i.e. AVPs in sorghum leaves is diluted to 10 per cent concentration.

For the control of bud necrosis in groundnut 500 of sorghum leaf extract 10 % is used at 500 litres per hectare (50 kg sorghum leaf powder / ha). Leaf extract is sprayed twice at 10 days interval i.e. on 10 and 20 days after sowing.

**vii. Antiviral principles from virus infected plants:** Following virus infection, antiviral factor (AVF) is found in plant tissues. Antiviral factor in potato virus Y infected plants was neither specific to viruses nor to hosts. The AVP isolated from *Nicotiana glutinosa* infected with tobacco mosaic virus was shown to be a ribonucleic acid (RNA). In the leaves of *Chenopodium amaranticolor* infected with tobacco necrosis virus, the formation of an AVP was detected 3 days after inoculation. The AVP was found to be phosphorylated glycoprotein.

Tomato plants systemically infected with TMV also contained an AVP. A host nonspecific AVP was isolated from *Capsicum pendulum* exhibiting systemic resistance following potato virus Y infection. From the bean plants (*Phaseolus vulgaris*) showing systemic resistance induced by inoculating TMV or tobacco necrosis virus (TNV), a nucleic acid fraction 3 S RNA was isolated. In hypersensitive hosts viruses are usually localized within local necrotic lesions and hence this RNA fraction is to be considered as a virus induced product involved in the development of systemic resistance in bean plants. Traumatic acid isolated from CMV infected cowpea leaves inhibited lesion production in

cowpea. Four new proteins formed in the leaves of Samsun NN tobacco locally infected by TMV were reported. These new proteins were absent in healthy tobacco leaves.

Antiviral agents have been reported to be present in culture filtrates of fungi and bacterial tissues. Among the 25 fungi tested, the culture filtrates of *Sclerotinia furcigera* and *Venturia inaequalis* inhibited TMV infection. *Aerobacter aerogenes* prevents TMV infection. The AVP from yeast reduced the infection of TMV. Antiviral activity in the culture filtrate of *Trichoderma* sp. against TMV was detected. The extract of potato leaves infected by *Phytophthora infestans* contained an AVP capable of inhibiting potato virus X. Filtrates of 3 to 7 week old cultures of *Stachybotrys chartarum* did not affect the infectivity of cucumber mosaic virus (CMV) *in vitro*, but induce resistance to the virus in cucumber *in vivo*. The degree of resistance depends on the culture filtrate concentration. The culture filtrate appears to have systemic properties and it is also effective in reducing the TMV content and distribution in treated tobacco plants.

**viii. Inducers of virus resistance:** Various kinds of substances have been used to induce resistance in plants to viruses and the induced resistance depends on the formation of some new substance (s). Rubbing the *N. glutinosa* leaves with TMV protein 4 days prior to TMV inoculation reduced the number of local lesions formed. Heat-killed bacterial cells of *Pseudomonas syringae*, when injected into the plant, afford similar protection against TMV infection. Application of heat stable polysaccharides from *Trichothecium roseum* to *N. glutinosa*, 2 days before inoculation induced local and systemic acquired resistance. The number of lesions formed on the leaves of Samsun NN tobacco could be reduced significantly by injecting yeast RNA, 3 days before TMV inoculation. Synthetic double stranded RNA, poly and polyanions also could induce resistance in plants against TMV.

Systemic resistance to infections against TMV and TNV could be induced by injecting polyacrylic acid (PA) into tissues 2 to 3 days before inoculation. Polyacrylic acid appears to activate a mechanism responsible for localizing viruses in hypersensitive plants. The development of PA induced resistance was associated with the formation of four new proteins in treated plants. The response to induction of resistance by PA is governed by a dominant gene. Salicylic acid also induced resistance to plant viruses. In tobacco plants sprayed with salicylic acid, the synthesis of "Pathogenesis related" (PR) proteins is induced leading to the development of resistance to viruses causing necrotic lesions. Spraying Samsun NN tobacco with salicylic acid induces the production of PR1 mRNAs and inhibits the systemic multiplication of alfalfa mosaic virus (AMV) by 90%. Synthesis of PR proteins in bean and cowpea occur following salicylic acid application and the production of local lesions by AMV is reduced in inoculated bean plants.

# 12

## Disease Management by Biotechnological Methods

The use of genetically modified organisms and or modern techniques (genetic engineering, tissue culture etc.) with biological systems for disease control is known as biotechnology. Genetic engineering or Genetic manipulation is the deliberate alteration of the composition of a genome by man. A growth of cells in a laboratory nutrient medium is known as tissue culture i.e. the technique of growing of plants in *vitro*. Cells of plants can be cultured in special nutrient medium and whole plants can be regenerated from cultured cells. Plant biotechnology is used for rapid clonal propagation of plants. It can help to produce industrial plant products under tissue culture conditions. Biotechnological methods are employed to control important plant diseases which are not amenable to control by usual methods.

### Genetic engineering

Genetic Engineering is the technology by which a particular gene is isolated from one organism and inserted into the genome of another organism and made to express at the right time.

**Vectors for transfer of genes:** Genetic engineering has been used to manage plant virus diseases. For transfer of genes to plants vectors are needed in which the gene to be transferred will multiply several folds. The most effective gene vector developed is the Tumour inducing plasmid of *Agrobacterium tumefaciens* from which the Tumor inducing genes have been removed. *A.tumefaciens* induces tumors (crown galls) through di-plasmid (tumor-inducing) which is a circular double stranded DNA molecule containing up to 2,00,000 base pairs organized into several genes. The Ti-plasmid is transferred from the bacterium into the cell. A specific region of the plasmid, the T-DNA, is transferred from the plasmid to the nucleus of the plant cell. It becomes integrated into the plant nuclear genome, and is transcribed. Cauliflower mosaic virus (CaMV) is the only plant virus with double-stranded DNA genome. As it has DNA genome, it is used as a possible vector in introducing foreign genes into plant. It is possible to insert a non-viral gene into CaMV genome and obtain expression of the gene in the infected plant. The viral promoter regions from CaMV are effective for obtaining expression of other genes in plant cells. The genes to be expressed is now fused to a promoter element from CaMV and a gene of *A.tumifaciens*. They are then introduced into the plants using *A. tumefaciens* Ti-DNA transformation.

**DNA construction :** Messenger RNA is extracted and exposed to an enzyme reverse transcriptase which synthesizes a complimentary single stranded DNA. The complimentary DNA (cDNA) is exposed to another enzyme, DNA polymerase, which produces the double stranded cDNA. The cDNAs are inserted into the plasmids of *A. tumefaciens*.

**Coat-protein expression in transgenic plants:** e.g., Transgenic tobacco plants expressing coat protein gene protected the plants against TMV. Transgenic tobacco plants showing resistance to alfalfa mosaic virus and tobacco rattle virus have also been developed. Transformation using a gene encoding the viral nucleocapsid protein of tomato spotted wilt virus (TSWV) has yielded transgenic tobacco plants that are resistant to TSWV. The expression of the viral genome in transgenic plants gives resistance to virus infection. Transgenic tobacco plants transformed with a DNA copy of the satellite RNA of cucumber mosaic virus (CMV) are shown to produce large amounts of satellite RNA following inoculation with CMV and symptom development is greatly reduced.

**Satellite RNA expression in transgenic plants:** Satellite RNAs are associated with several viruses. They are packaged into virus particles along with the genomic RNAs of the helper virus. They are not part of the viral genome and have no obvious sequence relationships with the helper virus. The presence of the satellite RNA suppresses the disease severity in many hosts. Hence transgenic plants which express satellite RNA have been produced to manage virus diseases. e.g., Transgenic plants of tobacco expressed the synthesis of satellite tobacco ring spot virus and reduce the virus disease incidence. Satellite RNA expressing tobacco plants against Cucumber Mosaic Virus (CMV) and Tobacco aspermy virus have been synthesized.

**MIC RNA expression in transgenic plants :** A DNA copy is made of one or more sections of the viral genome that include the initiation codon for proteins vital to virus replication. The DNA copy is inserted in the host-cell genome. Cells then produce an 'antisense RNA' called mic RNA (mRNA-interfering complementary to 5' end of the gene). The mic RNA hybridizes in vivo with the viral mRNA blocking translation. The mic RNA is inserted into the plants using the Ti plasmid of *A. tumefaciens*. Plants regenerated from the transformed cells will be resistant to the particular virus. This possibility is also being exploited for the control of virus diseases.

**Use of RFLP markers for cloning resistance genes:** Molecular markers viz., isozymes and DNA markers (Restriction Fragment Length Polymorphisms - RFLPs; Random Amplified Polymorphic DNA - RAPD and others) are being used in several areas relevant to identification of disease resistance genes. Some of the disease resistance genes using random DNA markers have been identified.

#### **Disease resistance genes mapped using RFLP markers**

<i>Plant</i>	<i>Pathogen</i>
Tomato	<i>Fusarium oxysporum</i>
Citrus	<i>Phytophthora</i> spp.

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### **Detoxification of pathotoxin**

Pathogens that produce pathogenesis-related phytotoxins usually also have the capacity to metabolize i.e. detoxify, these compounds. The search for genes encoding the enzyme(s) performing the key catabolic step(s) should thus lead to a convenient source of resistance, which can be engineered into plants to protect them from the effects of the toxin. A gene encoding a tabtoxin acetyltransferase from the pathogen, *Pseudomonas syringae* pv. *tabaci* which causes wild fire disease of tobacco was isolated and transferred into tobacco under a strong constitutive promotor. The transgenic plants expressed this gene and, when treated with either the pathogen or its toxin, did not produce the chlorotic lesions typical of wild fire disease.

**Activation of plant defense mechanism-Phytoalexins:** Phytoalexins have long been known to accumulate in certain plants upon infection by pathogens. The production of phytoalexins is also triggered by mechanical stimulation, ultraviolet (UV) irradiation, stress and a variety of chemical elicitors. Phytoalexins are part of the localized hypersensitive response at the site of damage or pathogen ingress, which involves cell trauma and death. The importance of phytoalexins in the defense response is underscored by experiments and pathogenicity in *Nectria haematococca* was correlated to its ability to detoxify the phytoalexin, pisatin, by way of demethylation. By transferring the demethylase gene from *Nectria*, *Aspergillus nidulans*, a non-pathogen on peas, was rendered insensitive to pisatin.

### **Defense related genes**

**a. Single gene defense mechanism:** There are some defense proteins which do not require any intermediate step both for their synthesis and their expression require only few steps and those genes encoding such proteins are called single gene defense mechanism. Chitinases and glucanases are those proteins belonging to single gene defense mechanism.

**Chitinases and glucanases:** Chitinases are abundant proteins found in wide variety of plants. Although the physiological function of chitinases is not known, there is strong correlative evidence that they are defense proteins with antifungal activity. Chitin is a major structural component of cell walls of many fungi. The low constitutive activity of chitinase found in many plants can be dramatically induced by wounding or by infection of the tissue with fungal pathogens. Chitinase in concert with  $\beta$ -1,3-glucanase (capable of degrading glucans present in fungal cell wall), degrades fungal cell walls and inhibits fungal growth at hyphal tips and has been shown to associate with hyphal walls in plants. The chitinase and glucanase enzymes are having direct action against several fungal pathogens compared to other defense related proteins. Since lytic enzymes are encoded by single genes, these defense should be high amenable to manipulation by gene transfer. The first reports of success with this approach was the expression of bean vacuolar chitinase gene under the control of the strong constitutive gene under the control of the strong constitutive promoter of the



cauliflower mosaic virus (CaMV) 35 S transcript in tobacco and *Brassica napus*, which resulted in decreased symptom development by *Rhizoctonia solani*, the causative agent of post-emergence damping off.

An endochitinase gene (from genomic tomato DNA library) was introduced into *Brassica napus* var. *oleifera*. The transgenic *Brassica* showed enhanced resistance against several fungal pathogens like *Cylindrosporium concentricum*, *Phoma lingam* and *Sclerotinia sclerotiorum* under field conditions when compared to non-transgenic plants. More recently, chitinase gene from *Manduca sexta*, tobacco horn worm, has been cloned into *P. fluorescens* to increase their antagonistic potential against *R. solani*.

**b. Multigenic defense mechanism:** Defense responses such as phytoalexin biosynthesis or lignin deposition in the cell wall require the action of many genes.

**Peroxidases :** Anionic peroxidases in the cell wall catalyze the production of phenolic radicals for the oxidative polymerization of lignin from cinnamyl alcohols. In tomato, there is a marked induction of two linked genes encoding highly anionic peroxidases in an incompatible interaction with an avirulent form of *Verticillium albo-atrum*, with only weak induction in the compatible interaction with a virulent form of this vascular pathogen. Expression of one of these genes in transgenic tobacco under the control of either its own promoter or the CaMV 35s promoter resulted in massive increase in anionic peroxidase activity and these plants apparently showed a significant increase in resistance to *Peronospora parasitica* as judged by symptom development and fungal sporulation.

#### **Activation of defense genes by chemicals**

Several classes of compounds have the potential to act as inducers of natural resistance mechanisms in horticultural crops and chemicals with such indirect modes of action may offer attractive alternatives or supplement to existing contact/systemic fungicides in integrated disease management. Increase was found to occur in response to salicylic acid treatment as well as oligosaccharides and glycoproteins originating from either fungal cell wall or host cell walls, the so called elicitors. Recently, chitosan seed treatment has been found to induce defense related genes like chitinase and glucanase in tomato and consequently the Fusarium crown and root rot diseases were significantly reduced. Pre-treatment with 2, 6-dichloroisonicotinic acid was highly effective in significantly reducing both anthracnose (caused by *Colletotrichum lindemuthianum*) and rust (caused by *Uromyces appendiculatus*) diseases in bean plants.

#### **Cell and tissue culture**

Tissue culture approach is one of the oldest techniques in the field of molecular biology and it is applied in several ways for the development of disease resistance cultivars in agriculture and horticulture.

**a. Somaclonal Variation:** In the past two decades, several advances have been made in culturing of isolated plant cells and tissue under controlled conditions in vitro. When plants are regenerated from cultured cells, they

exhibit new phenotypes, sometimes at high frequencies. If these are heritable and affecting desirable traits, such "somaclonal variation" can be incorporated into regular breeding programmes. However, the finding of specific traits by these methods is largely left to chance and hence inefficient. Rather than relying on this undirected process, selection in vitro aims to specific traits by subjecting large populations of cultured cells to the action of a selective agent in the petridish. For purpose of disease resistance, this selection can be done by fungal pathogens, culture filtrates of pathogens or isolated phytotoxins that are known to have a role in pathogenesis. The selection will allow only those cells to survive and proliferate that are resistant to the challenge. Plants regenerated from resistant cells often display a resistant phenotype when evaluated with either the toxin or the pathogen itself.

#### Disease resistant plants from tissue culture

Plant	Culture System	Selection	Resistance to Pathogen
Potato	Protoplasts	SCV	<i>Phytophthora infestans</i> <i>Alternaria solani</i>
	Callus	CF	<i>Fusarium oxysporum</i>
Tomato	Callus	Fusaric acid	<i>Fusarium oxysporum</i>
Banana	Meristem	SCV	<i>Fusarium oxysporum</i>
Strawberry	Callus	SCV	<i>Fusarium oxysporum</i>

(SCV- plant regeneration without selection; CF crude culture filtrate)

Although this method has obviously yielded some impressive results, it also has its drawbacks; viz, i. Many pathogens do not produce pathogenesis specific toxins useful for selection ii. Culture filtrates are rather artificial and neither pathogens nor plant cells grown together in vitro behave quite as they would in a natural environment iii. The selection approach can only detect mutations in plant genes that are expressed at the time that selection is applied. In order to be useful, new resistance traits, whether selected or not, must be heritable sexually or in the case of vegetatively propagated crops must be transmitted through vegetative propagules. The pathogens produced toxins can be used to screen calluses (cultured cells) which may regenerate resistant plants. The toxins will kill the calluses, but the mutant toxin resistant calluses will survive. The toxin-resistant calluses yield disease resistance plants. Vidhyasekaran obtained brown spot resistant rice plants using *Helminthosporium oryzae* toxin. Similarly, *H. maydis* resistant maize plants, *H. sacchari* resistant sugarcane plants and *Phytophthora infestans* resistant tobacco plants have been evolved.

**b. Anther culture** In this method, the plants are produced directly from microspores (immature pollen grains). Through anther or microspore culture,

one has immediate access to unique and rare combinations of genes representing the recombination of the genetic material contributed by the parents of the cross. Through anther culture, followed by chromosome doubling, such gene combinations can be fixed in their homozygous state as instant inbreds in a single step. Over the past two decades, anther culture has become widely accepted as a tool in cultivar development. This technique can be particularly useful for producing plants with novel combinations of resistance genes for managing fungal diseases.

**c. Protoplasmic fusion:** This generates hybrid cells by merging the total cellular components of somatic cells from which the cell walls have been removed to produce protoplasts. The incompatibility preventing sexual fertilization between species is thus avoided and viable hybrids have been created, even between unrelated distance species. Disease resistance genes have thus been transferred by protoplasts fusion from wild species into potato.

# 13

## Chemical Methods

Many fungicides have been developed for purpose of controlling crop diseases which may be used as sprays, dusts, pastes, paints, fumigants, etc. The chemicals may be salts of toxic metals or of organic acids belong to either organic group. Fungicide or fungitoxicant is an agency or chemical which has the ability to kill the fungus. Fungistat is a chemical which inhibits the fungal growth temporarily. The phenomenon of temporary inhibition is known as fungistasis. A chemical which do not affect the growth of vegetative hyphae but inhibits spore production is known as antisporeulant.

### Characteristics of a good fungicide

- Low phytotoxicity: Good fungicide should not be toxic to the plants. Certain fungicides cause phytotoxicity only under certain environmental conditions. In such cases those fungicides must be restricted only to favourable environment *e.g.*, Sulphur dust is toxic to some crops if applied during hot season Bordeaux mixture.
- Stability in storage: A fungicide should have long shelf life. Stability of the active ingredient under a variety of storage condition is a desirable quality.
- Stability after dilution to spray strength: A good fungicide should possess stability after dilution to spray strength also. Some chemicals are less effective if diluted.
- It should be less toxic to human being, cattle etc. It should not be toxic to earthworms and soil microorganisms.
- It should have high toxicity to a wide range of fungi
- Fungicidal preparation must be ready for use
- It should not cause accumulation of toxic chemicals in soil or in plants or in plant products.
- It should combine with important or commonly used insecticides or acaricides without any deleterious effects.
- It should be cheap (economical) and easily available in the market.
- It should be available in different formulations.
- It should easily spread on the host surface when applied.
- It should be easily prepared using simple procedures.
- It should be easily transportable.
- It should not cause environmental pollution.

### Classification of fungicides

Fungicides can be classified into different groups based on mode of action, general use and chemical composition

**Based on mode of action**

- a. Protectant
- b. Therapeutant
- c. Eradicant

**a. Protectant:** Fungicide which protects a plant from a pathogen if it is applied prior to infection. They are prophylactic in their behaviour. eg., Sulphur, zineb.

**b. Therapeutant :** Fungicide which eradicates a fungus after it has caused infection by curing the plant is called therapeutant. Usually chemotherapeutants are systemic and eradicates deep seated infection. eg. Carboxin, oxycarboxin, aureofungin

**c. Eradicant :** A chemical substance that destroys a pathogen after its establishment in the host plant is known as eradicator. It eradicates the dormant or active pathogen from the host. It also acts as protectant. eg. Organomercurials, lime sulphur, dodine.

**Based on general use**

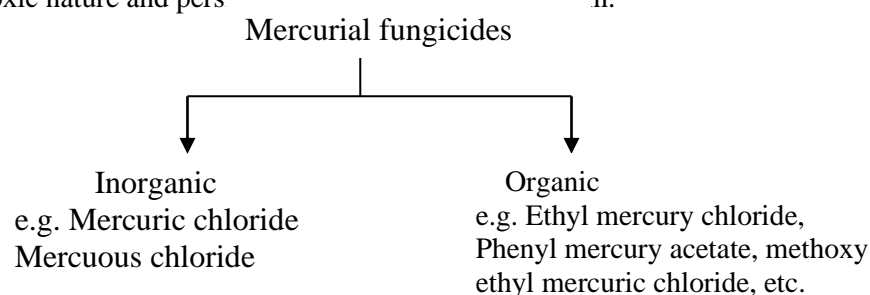
Seed protectants	e.g., Organomercurials, captan, thiram, carbendazim, carboxin
Soil fungicides - Pre-plant	e.g., Bordeaux mixture, copper oxychloride, vapam, chloropicrin, formaldehyde.
Soil fungicides - when plants are in field	e.g., Bordeaux mixture, copper oxychloride, thiram, captan.
Foliage and blossom protectants	e.g., Bordeaux mixture, copper oxychloride, ferbam, zineb, mancozeb, captan, carbendazim, chlorothalonil,
Fruit protectants	e.g., maneb mancozeb, captan, carbendazim, thiabendazole.
Tree wound dresser	e.g., Bordeaux paste, Chaubattia paste
Antibiotics	e.g., Streptomycin, streptomycin, griseofulvin

**Based on chemical composition**

1. Mercurial fungicides
2. Copper fungicides
3. Sulphur fungicides
4. Quinones
5. Heterocyclic nitrogenous compounds
6. Aromatic compounds
7. Non – aromatic compounds
8. Organotin compounds
9. Organophosphorus compounds
10. Nickel compounds
11. Miscellaneous fungicides
12. Systemic fungicides
13. Antibiotics

## 1. Mercurial fungicides

Hiltner (1959), a German worker, very successfully used mercuric chloride for the control of *Fusarium* disease of rye (*Calonectria graminicola*) as early as 1910. Mercury compounds have been in use for a long time for treating seeds, rhizomes, corms, bulbs etc., of vegetables and flowering plants for the control of seed-borne diseases. Both inorganic and organic type of fungicides has fungicidal and bactericidal activity. They are highly toxic towards animals and human beings and at times even phytotoxic. Because of their extreme toxicity the usage on foliage is limited. In very few instances some special type of organomercurial compounds are used as foliar spray on turf and apple trees. Mercury fungicides are banned in almost all countries including India because of their toxic nature and persistence to get into the food chain.



**i. Inorganic mercurials:** Mercuric chloride or calomei is the chemical largely used in earlier days for dipping the seed and other vegetatively propagated materials of vegetables and flower crops. In India it was first used by Burns in 1914 for steeping the potato tubers. It was used in a dilution of 1:1000 for potato tubers against *Rhizoctonia* sp. It is commonly used for surface sterilization of diseased plant materials used for the isolation of causal organism.

**ii. Organic mercurials:** According to Sharvelle (1960) the first organic mercury was suggested by Wesenberg of the I.G. Farben Industries in Germany in 1913 as a seed disinfectant. The first important commercial product was Uspulum, which contained 18.8% mercury in the form of chlorophenol – mercury.

Chemical compound	Commercial name
Aretan, Agallol, Wet ceresan	Methoxyethyl mercury chloride.
Agrosan GN	Tolyl mercuric acetate.
Ceresan Dry	Phenyl mercury acetate
Agrox	Phenyl mercury urea
Semesan	Hydroxy mercury chlorophenol
Panogen	Methylmercur dicyandiamide

These fungicides are mainly used for seed treatment by dry, wet or slurry method. Among these slurry method is the best because it reduces handling hazards. For dry seed treatment, 1% metallic mercury is applied at

0.25% concentration. For wet seed treatment, preparations containing 2.5 to 6.0 % mercury are applied at the rate of the 0.2 to 0.5 % concentration.

**Mechanism of action:** In general, The site of action of the fungicidal activity of mercury either as vapour or ion is at the sulphydryl (-SH) groups of the susceptible enzymes. In general, organomercurials are highly toxic than the inorganic ones because they are highly lipid soluble and can easily pass into the spore through the membrane. They have got good volatility and therefore they are good contact fungicides. On application to seeds, the organomercurials tend to move from places where it is an excess and deposit in places of less concentration. This mainly depends on the evaporation rate and diffusion rate of the fungicide. This kind of distribution and redistribution processes gets completed in about 2 after application in case of Panogen.

**Toxicity:** Increased doses over and above the safety level usually cause injury to seed and is exhibited as abnormal seed germination. The roots and hypocotyl regions will be hypertrophied and are commonly observed in abnormal germination of cereal seeds. The cells of such hypertrophied structures contain 'micro' and 'giant nuclei' and are the results of the incomplete meiosis.

## **2. Copper fungicides**

The copper compounds which were developed in nineteenth century had wide usage all over the world until organic fungicides came into use. Bordeaux mixture is the oldest copper fungicide. The discovery of Bordeaux mixture in 1882 by Millardet, Professor of Botany, University of Bordeaux, France laid the foundation stone for the development of fungicides. Downy mildew disease of grapevine appeared in France in 1878 when *Phylloxera* (insect) resistant grapevine stocks were imported from United States to replace the highly susceptible native varieties. He noticed that downy mildew was practically checked in vines along the roadsides, which had been applied with a solution of lime and copper sulphate to deprecate the pilferage of fruits by poachers. The fact that Bordeaux mixture as effective fungicide was announced by Millardet in 1885 which rapidly gained admirations from grapevine growers of France.

### **i. Bordeaux mixture**

Bordeaux mixture 1% is generally used for control of foliar diseases and some soil –borne diseases. It is used mainly for the control of downy mildew of grapevine, late blight of potato, koleroga of arecanut, foliar diseases like anthracnose, leaf spots, leaf blights, etc., Sometimes it is also effective against some bacterial diseases (citrus canker). It is used as soil drenching chemical for the control of nursery diseases like damping off caused by *Pythium* sp, *Phytophthora* sp., *Rhizoctonia* sp, and *Phytophthora* wilt of betelvine and basal stem rot of coconut. Especially for the control of coffee rust, Bordeaux mixture is used at 0.5% only.

### **Method of preparation**

Bordeaux mixture 1% is prepared with the following materials in the ratio of 1:1:100.

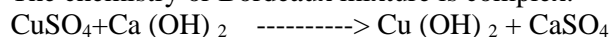
Copper sulphate (CuSO <sub>4</sub> )	-	1 kg
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Quick Lime [Ca (OH) <sub>2</sub> ] -	1 kg
Water	- 100 lit

Non-metallic vessels are used to prepare Bordeaux mixture. One kg of copper sulphate is taken, powdered and dissolved in 50 l. of water in an earthen / cement / porcelain/plastic vessel. Similarly 1kg of good quality quick lime is taken sprinkled with water to slaken it and dissolved in another 50 l. Of water in a separate vessel. Strain the lime solution to remove stones and other undissolved materials. Then the copper sulphate solution is slowly added to lime solution with constant stirring of the mixture. There is also a method, where simultaneous addition of the above two solutions is made in a third container and mixed well. The addition of lime is made to neutralize the copper sulphate solution which is acidic in nature. Copper sulphate solution when sprayed without neutralizing the pH will cause phytotoxic symptoms on the plants. The resultant solution (Bordeaux mixture) should be neutral and sky blue in colour. If the mixture is acidic, it shows the existence of free copper in the solution. Hence, it is essential to test the presence of free copper before the Bordeaux mixture is used on the crops.

### Chemistry of Bordeaux mixture

The chemistry of Bordeaux mixture is complex:



The ultimate mixture contains gelatinous precipitate of copper hydroxide and calcium sulphate. Cupric hydroxide is the active principle and is toxic to the fungi.

There are three important theories to explain the action of Bordeaux mixture.

- CuOH is brought into solution by atmospheric agencies more by CO<sub>2</sub> present in air.
- Leaves on which Bordeaux mixture sprayed exert some chemical action on the Bordeaux mixture and thus make CuOH suitable.
- The Bordeaux mixture itself is inert till the fungus falls on it and soon after the fungus comes in contact, it produces some chemical which makes CuOH soluble and copper enters the germ tube and kills the fungi.

### Methods of testing neutrality

The following methods are used to test the neutrality of the mixture.

**a. Field test:** Polished Iron knife or sickle is immersed in the prepared Bordeaux mixture for minutes. If reddish deposit is noticed on the polished surface of the knife/sickle it means that the mixture is in acidic pH. It indicates that the mixture has to be neutralized by addition of lime. In such condition add lime solution and test for neutrality by following the method.

### b. Laboratory tests

- Litmus paper: In the prepared Bordeaux mixture, dip a bit of blue litmus paper. Blue colour should not change, if it is neutral.
- pH paper: Dip a bit of the wide range pH paper and watch for the colour to the neutral pH in the paper.



- iii. Chemical test: Take five-ml of 10% potassium ferrocyanide in a test tube; add few drops of the prepared Bordeaux mixture to the above solution. If red precipitate is seen it shows that the prepared mixture is acidic. When the mixture is acidic add sufficient lime solution.

#### **Precautions**

- i. Good quality lime and copper sulphate should be used.
- ii. Metallic vessels should not be used for its preparation.
- iii. Always copper sulphate solution should be added to lime solution. If lime solution is added to copper sulphate solution there will be precipitate of free copper and the resultant mixture will be least toxic.
- iv. Bordeaux mixture should be prepared afresh every time and sprayed immediately after preparation. To keep it overnight add jaggery at 1g/lit.
- v. Some adhesives like casein, resin, Teepol, Triton-AE and vegetable oils can be added in regions of heavy rainfall.

#### **Merits of Bordeaux mixture**

- i. It can be prepared with locally available materials. It is an effective fungicide and highly adhesive.
- ii. Relatively cheaper fungicide.
- iii. It has got good tenacity.
- iv. It is non-poisonous and safe to handle. It is not phytotoxic to many plants.
- v. It controls both foliar diseases and soil borne diseases.
- vi. As it contains copper it cures copper deficiency in plants.
- vii. It has phytotonic effect and prevents defoliation.

#### **Demerits of Bordeaux mixture.**

- i. The process or preparation is laborious and cumbersome.
- ii. It has low keeping quality.
- iii. It is phytotoxic to apple, plums, peaches, pear, rose, sorghum, rice varieties like IR-8 and maize variety like Ganga hybrid, Makka No.3.
- iv. It leaves blemishes on the leaves after its spray which is unsightly in appearance in ornamental plants.
- v. It is corrosive to iron and zinc.
- vi. Since the coating of Bordeaux mixture on the leaf persists for sometimes temporary retardation of photosynthesis has been reported.

#### **ii. Bordeaux paste**

Bordeaux paste is a wound dresser and protects wounds and cut ends of the trees against the infection by fungal pathogens. It is generally used to swab the wounded portions in stem bleeding of coconut (*Ceratocystis paradoxa*), die-back and gummosis of citrus (*Colletotrichum gloeosporioides*) and *Dendrophthoe* affected mango and citrus trees. Bordeaux paste is prepared with the same materials as in Bordeaux mixture but here the ratio is 1:1:10. The method of preparation is same as in Bordeaux mixture but the resultant product is a paste. It is prepared with the following ingredients.

Copper sulphate- 1 kg

Quick lime	-	1 kg
Water	-	10 lit

### **iii. Burgundy mixture**

Burgundy mixture was developed by Mason in 1887 in Burgundy, France which had replaced the Bordeaux mixture. This mixture is prepared by mixing copper sulphate crystals and sodium carbonate crystals in water in the ratio of 1.8 kg: 1.8 kg: 225 l. The procedure for preparation of Burgundy mixture is same as that of Bordeaux mixture; here the lime is substituted with sodium carbonate. As there was scarcity of good quality of lime in those times in Europe. This formula was preferred to the Bordeaux mixture. The use of Burgundy mixture is much restricted. It is slightly less effective compared to Bordeaux mixture.

### **iv. Cheshunt compound**

This compound was formulated by Bewley in 1921. It is a compound prepared by mixing 2 parts of powdered copper sulphate and 11 parts of ammonium carbonate. Cheshunt compound is used as soil drench in nursery against damping off disease. These two chemicals are well powdered and thoroughly mixed. The resultant dry mixture is stored in an air tight container for 24 h. before using. If the dry mixture is exposed it gradually loses ammonia and becomes less effective. Thirty grams of this mixture is dissolved in little hot water and the solution is made up to 9 litres with cold water. This solution is used for spraying.

### **v. Chaubattia paste**

This is a wound dressing fungicide developed at Government Fruit Research Station, Chaubattia, Uttar Pradesh. This is prepared by mixing 800 g of copper carbonate and 800 g of red lead in one litre of raw linseed oil or lanolin. It is used against many diseases, (stem-brown, stem-black, stem canker, pink disease, of apple, pear and Collar rot of apple, apricot, peaches and plums and found to excel well among other fungicides especially in the Kumaon hills in Uttar Pradesh as it is not easily washed away by rain. It is applied to pruned parts for the control of the above diseases. A readymade paste is now sold commercially.

### **vi. Cuprous oxide**

Horsfall (1956) suggested the use of red oxide of copper as seed and foliage protectant. The efficacy of fungicide increases with fineness of particles. Cuprous oxide is usually red in colour but changes through orange to yellow in due course. The product is available in the form of dust and wettable powder. The commercial formulations are Copper Sandoz, Fungimar, Kirti copper and Perenox. The dust formulations contain 4 to 6% metallic copper while the wettable powders contain 50% copper. The former is used at the rate of 25 to 35kg of dust per ha while the latter is used at 0.25% concentration of spray fluid.

### **vii. Copper oxychloride**

The phytotoxic nature and tediousness in the preparation of Bordeaux mixture necessitated the discovery of a formulation which is less toxic and as

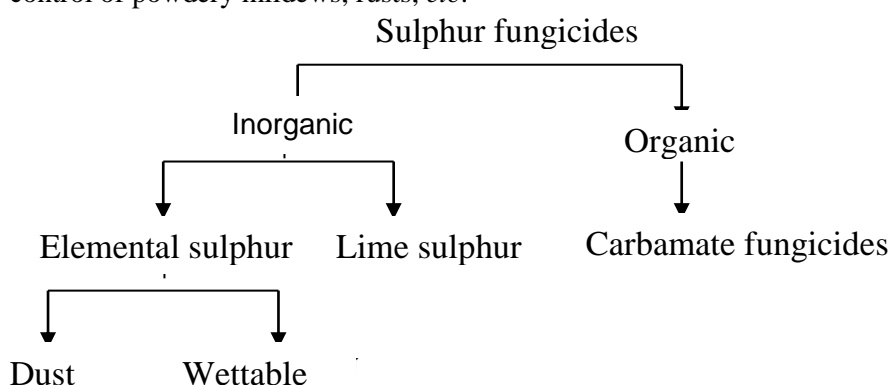
effective as the Bordeaux mixture. Copper oxychlorides ( $\text{CuCl}_2 \cdot 3 \text{Cu}(\text{OH})_2$ ) or the “fixed coppers” are the outcome now they are widely used against many fungal diseases. In the formulation of copper oxychlorides, the copper is fixed and thus it is less soluble in nature. This quality makes the fixed copper less phytotoxic. It is not as efficient as that of Bordeaux mixture since the latter is more tenacious in nature. It is available in the market as dust formulations and wettable powders. The former contains 4 to 12% of metallic copper while the latter contains 50% metallic copper. The commercial formulations are Blimix, Blitox, Coppersan, Blue copper-50, Cupromar, Fytolan, and Fytomix.

**Mechanism of action:** Copper kills spores of fungi by combining with the sulphhydryl group of certain amino acids and disrupts proteins and enzymes. Fungistatic action of copper arises through non-specific destruction of proteins and enzymes which is due to non-selective and non-specific affinity of the cupric ion for certain isogenic groups such as imidazole, carboxy, phosphate or sulphhydryl.

**Phytotoxicity:** Copper in Bordeaux mixture and other fungicides sometimes cause phytotoxicity on the crop plants. Bordeaux mixture causes russetting in apple fruits. Copper oxychloride becomes phytotoxic when sprayed on IR8 rice crop and Ganga Hybrid, Makka No.3 of maize. Copper fungicides at 0.2 percent were toxic to rapeseed and mustard.

### 3. Sulphur fungicides

Elemental sulphur has been in use from ancient times. Mention about its use has been made in the Bible. In 1821 Robertson experimentally proved for the first time about the effective control of peach mildew with the use of sulphur. Even now fungicides in this group are being successfully used against the control of powdery mildews, rusts, *etc.*



#### I. Inorganic

##### i. Elemental sulphur

Elemental sulphur in general is used in the form of finely divided powder as dust formulation. It is occurring in nature as deposits of pure crystals or along with gypsum and limestone underneath the earth. The efficacy of elemental sulphur increases with the fineness of the particles. The particles must pass through 200 or 300 mesh sieve which could allow 74 or 47  $\mu\text{m}$  particles respectively in diameter. It is imported every year for the formulation of

fungicides. Elemental sulphur is also available as wettable powder nowadays. Wettable sulphur form uniform suspension in water and are used as spays. It is made into a wettable form by grinding it with colloidal materials like casein, bentonite clay, etc. In India coarse elemental sulphur is micronized by grinding it in micronizer mill. They are used in the concentrations of 0.2% to 0.5% as foliar spraying against powdery mildews and rust diseases. It is sold in the names of Cosan, Solbar and Thiovit.

## **ii. Lime sulphur**

Lime sulphur came into use during the early part of the nineteenth century. It is used mainly as dormant sprays. It is prepared by boiling lime and sulphur together. The proportion of lime and sulphur varies and the following quantities are taken in general. Nine kg of rock lime and 6.75 kg of sulphur are boiled in 225 l of water. They are heated in an open pan for an hour. The boiled liquid is allowed to settle down and supernatant liquid is filtered off. The resultant liquid is called lime-sulphur or calcium polysulphide. It is chemically a mixture of calcium polysulphides and calcium thiosulphate. An effective lime sulphur can have 20% polysulphide content and the specific gravity of liquid not less than 1.28 at 60°F. Though this lime sulphur is being used for fruit trees for more than half a century, it is known to scorch some fruit trees and cause premature abscission of leaves and fruits. In spite of these ill-effects, lime sulphur is commonly used against apple scab, peach leaf curl and powdery mildews of some crop plants.

**Mechanism of action:** The mechanism of action of elemental sulphur is based on two theories viz, “oxidized sulphur theory” where the elemental sulphur is oxidized to form  $\text{SO}_2$ , which is fungicidal in action and “hydrogen sulphide ( $\text{H}_2\text{S}$ ) theory” where the elemental sulphur is reduced to form  $\text{H}_2\text{S}$ , which acts as the toxic substance on fungal spores. This was found out by Marsh (1929). The recent theory is the direct action theory by which the sulphur acts as a hydrogen acceptor in metabolic systems and it disturbs the normal hydrogenation and dehydrogenation process in the fungal cell. Sulphur fungicides emit sufficient vapour to prevent the germination and growth of fungal spores at a distance of several millimetres from deposits on leaves.

**Phytotoxicity:** Acute injury by sulphur is rare in temperate regions, but in warmer regions severe burning is sometimes caused on cucurbits when it is used against powdery mildew disease. In apple fruits it causes ‘Sulphur sun scald’ on the sun exposed sides. The sulphur placed on the stigma of apple blossoms inhibits pollen germination and leads to reduced fruit set. Lime sulphur applied on green leaves considerably reduces the photosynthesis by these leaves. The varieties such as Stirling Castle and Lane’s Prince Albert are sulphur sensitive. The mechanism of sulphur phytotoxicity may be due to reduction in  $\text{CO}_2$  assimilation.

## **II. ORGANIC**

Organic sulphur compounds are derived from dithiocarbamic acid and are widely used as spray fungicides; very few are used for seed and soil treatments. In 1931, Tisdale was the first to describe the possibility of the

fungicidal nature of carbamate, compounds but the commercial production was started after a decade. Dithiocarbamates fall under two main groups based on their mechanism of action. The first group, the dialkyl dithiocarbamates (ziram, ferbam and thiram) is derived from dialkyl amines. The second group, monoalkyl dithiocarbamates (nabam, zineb, vapam and maneb) is derived from monoalkyl amines or by the interaction of carbon disulphide and ethylenediamine. Common fungicides under this group are:

- i. Ziram (Zinc dimethyldithiocarbamate),
- ii. Ferbam (Ferric dimethyl dithiocarbamate)
- iii. Thiram (Tetramethyl thiuram disulphide)
- iv. Nabam (Disodium ethylenebisdithiocarbamate),
- v. Zineb (Zinc ethylene bisdithiocarbamate)
- vi. Maneb (Manganese ethylene bisdithiocarbamate) and
- vii. Vapam (Sodium methyl dithiocarbamate – SDMC).

## **DIALKYL DITHIOCARBAMATES**

### **i. Ziram**

Ziram (Zinc dimethyldithiocarbamate). It was developed in 1930 by E.I. du Pont de Nemours and Co. and Bayer A.G. It is commercially available as Cuman, Hexazir, Milbam, Zerlate and Ziram. The physical characters and compatibility of ziram is almost similar to thiram. It is a white powder with a molecular weight of 305.8. Ziram is compatible with most other pesticides except those containing either heavy metals like copper, iron, mercury or lime. Ziram is used mostly as a spray fungicide for controlling many fungal diseases of vegetables and ornamentals; Ziram is sprayed at 0.15 to 0.25 per cent concentration.

### **ii. Ferbam**

Ferbam (Ferric dimethyl dithiocarbamate) was developed in 1931. In the U.S.A. it was originally developed by E.I. du Pont de Nemours and Co. It is a black powder with a molecular weight of 416.5. Ferbam deteriorates under high heat and moisture. Like ziram it is also not compatible with copper, mercury or lime. It is marketed as Coromet, Ferbam, Ferberk, Fermate, Fermocide, Ferradow, Hexaferb and Karbam Black. In general, ferbam is used for spraying against number of diseases of temperate fruits, vegetables and ornamentals. This has been rarely used as seed dressing and soil drenching fungicide.

### **iii. Thiram**

Thiram (Tetramethyl thiuram disulphide). Thiram was first discovered as rubber accelerator. Later on, it was found to be fungicidal in action. E.I. du Pont de Nemours & Co. developed it in 1931. Thiram is prepared as wettable powder and dust formulations. Thiram is chiefly used as seed dressing chemical but also used as a foliar spray. It is available in the market as Arasan, Hexathir, Tersan, Thiram, Thiride and TMTD. As in the case of other dithiocarbamates thiram is also less toxic to animals and human beings. Thiram is a white powder with molecular weight of 240.4. It is insoluble in water but soluble in acetone and chloroform. It is compatible with most of the pesticides except those

containing copper and lime. Thiram is used as dry or wet seed treating chemical at the rate of 0.2 – 0.4% of the seed weight. Many soil-borne diseases caused by *Pythium* sp., *Rhizoctonia solani*, etc. are controlled by soil application at the rate of 15 to 25kg/ha. In a few instances it is being applied as foliar spray at a concentration of 0.2%. It is not advisable to spray the fruits since it taints the fruits and this cannot be used on the fruits intended for canning or deep-freezing.

**Mechanism of action:** Dialkyl dithiocarbamates are strong chelating agents that they acted by depriving the cell of needed metals. The free radicals are toxic with cellular thiols, which inturn result in the interference in the activities of sulphhydryl requiring enzymes. However, the toxicity of these compounds in the cell might involve one or more of the following.

- a) Chelation of required heavy metals.
- b) Attachment of a 1:1 complex of metal and dithiocarbamate ions of enzymes.
- c) Attachment of dithiocarbamate ions to metals bound to proteins.
- d) Reaction of free radical intermediates with cellular components.
- e) Lethal catalysis.

#### iv. Nabam

Nabam (Disodium ethylenebisdithiocarbamate) is the first dithiocarbamate produced which was later on replaced by zinc and manganese dithiocarbamates. It is unstable and too much soluble in water. It was discovered by W.F. Hester of the E.I. du Pont Co., U.S.A. in 1935. Dimond *et al.* reported its fungicidal properties in 1943. Heuberger and Manns in 1943 reported that nabam can be stabilized by the addition of zinc sulphate and lime at the rate of 1.135 kg or nabam, 454 g of zinc sulphate and 454 g of lime. It is sold under different trade names of Chembam, Dithane D14, Dithane A-40 and Parzate liquid. They are in the form of liquid. In the powder form it is less stable to heat, light and moisture. Nabam is used for the control of many foliage and flower diseases. While using in the field addition of zinc sulphate or zinc sulphate and lime is made for stable and effective action of nabam.

#### v. Zineb

Zineb (Zinc ethylene bisdithiocarbamate) is the popular fungicide used in large scale for control of many fungal diseases. It was developed by Rohm and Hass Inc. and E.I. du Pont and de Nemours & Co., in 1943. Zineb is yellowish white in colour with a molecular weight of 275.5. Its solubility in water is very little and is unstable in presence of heat, light and moisture. Some of the common trade names of this fungicide are Dithane Z-78, Hexathane, Lonocol, Parzate and Polyram. Zineb is compatible with most of the insecticides and fungicides but should not be mixed with any substance containing lime. Calcium will change the residual action of the zineb. Zineb is toxic to human beings and animals if consumed but on contact it causes irritation to mucous membranes. It gives very good control over late blight of potato. Apart from the fungicidal value zineb provides zinc to the plants and plants like tomato and paddy respond well towards this trace element when the soil is deficient. But it is phytotoxic to zinc sensitive plants. Sometimes it may cause defoliation in vines.

Zineb is very largely used as foliar spray against fungal leaf spot and rust diseases. But in few instances, it has been used as soil drench. Good results were obtained when zineb is applied along the furrow for the control of the post emergence damping off of cotton caused by *Rhizoctonia solani*. Rhizome rot of ginger is controlled by drenching the soil with 8 gallons of 0.15% zineb per plot of 8' x 5' before planting and three similar applications after planting at 3 weekly intervals. Zineb is nowadays mixed with copper oxychlorides in the ratio of 13: 33 and sold in the name of Miltox and Blitane. It is a recent advancement in the proprietary formulation.

#### **vi. Maneb and mancozeb**

Maneb (Manganese ethylene bisdithiocarbamate) is the next most important fungicide sold in the organic sulphur group of fungicides. It was developed in 1950 by E.I. du Pont de Nemours & Co., Rohm and Haas company, and Bayer A.G. of Germany. In India the commercial formulations include Dithane M-22, Manzate and MEB. Maneb is not available in pure form but is formulated by combining zinc ion (2%) and maneb (78%) and sold in the name of Dithane M. 45 or Indofil M45 (Mancozeb). In some countries maneb (53%) is mixed with nickel sulphate (19%) and sold as Dithane S-31. This controls cereal rusts effectively. Maneb is usually yellow in colour but it is also available as a grey coloured powder. The molecular weight is 265.3. It is compatible with almost all other insecticides and fungicides except fixed copper and Bordeaux mixture. It will be phytotoxic to tobacco seedlings, and some varieties of cucumbers and apple. It is unstable in heat, light and moisture. This is very widely used for vegetable crops especially for potato and tomato against blight disease. Very rarely it is used as foliar dust and for soil treatment.

#### **vii. Vapam or metham sodium**

Vapam (Sodium methyl dithiocarbamate, SDMC) was first introduced by Stauffer Chemical Co., of U.S.A in 1954. It has fungicidal, nematicidal and herbicidal properties. Some insecticidal property has also been reported. It is commercially available as Chem-Vape, Karbation, Vapam, Vitafume and VPM. It is a liquid used for treating the soil. When applied to soil. Vapam releases a fumigant called methyl isothiocyanate ( $\text{CH}_3\text{N} = \text{C} = \text{S}$ ). Soil temperature plays an important role in hastening the release of the fumigant. The higher the temperatures, the quicker the release. Usually Vapam is applied a week before planting or sowing. It is applied at the rate of 1.5 to 2.5 l per 10 sq. metre by using rose-can or other such devices. It effectively controls soil fungal pathogens like *Fusarium oxysporum*, *Pythium* sp., *Sclerotium rolfsii* and *Rhizoctonia* sp. Vapam also kills nematodes like root knot nematode (*Meloidogyne* spp.), *Tylenchulus* sp., and *Hoplolaimus* sp.

**Mechanism of action:** Introduction of hydrogen ion at the nitrogen atom in a dithiocarbamate structure considerably reduced the chemical stability of monoalkyl dithiocarbamates. Dithiocarbamates bearing free hydrogen at the nitrogen can split off hydrogen sulphide or the HS<sup>-</sup> ion with the formation of isothiocyanate groups, which show antifungal activities. Formation of volatile methylisothiocyanate from vapam also supported the isothiocyanate theory. In

general dithiocarbamates release thiocarbonyl ( $-N = C-S$ ) which inactivates  $-SH$  group.

#### 4. Quinones

Quinones are highly toxic to fungi. They are excellent seed treatment fungicides. In certain plants, quinones are naturally produced and confer protection against fungal diseases. Under this group only two fungicides viz., chloranil and dichlone are commercially developed. Ter Horsfall, 1956) first proposed investigation into the possibility of using tetrachloro - p-benzoquinone (chloranil) as a fungicide. Soon Cunningham and Sharvelle (1940) confirmed its fungicidal activity and Sharvelle *et al* (1942) recommended its use as a seed protectant on canning peas and lima beans. It however failed, on the whole, as a foliage fungicide. During subsequent research by Ter Horst and Felix (1943) another quinone namely viz., dichlone was developed, which was very much stable in sunlight and was accepted as a foliage fungicide, especially for apple scab. These fungicides are not commonly available at present for use in India.

##### i. Chloranil

Chloranil (2, 3, 5, 6 - tetrachloro - 1, 4 - benzo-quinone) is sold in the name of Spergon. It was released in 1940 in the U.S.A. as a seed treating chemical. It is stable in acidic pH, and decomposes in alkaline pH. It decomposes quickly in the presence of moisture and light and gives chloranilic acid. Chloranil can be used in combination with other seed treating pesticides and no mal-effect is noticed. As a seed protectant it is used at the rate of 4 to 8 oz/100 lb of seeds (114-228 g/45/kg.) In rare cases chloranil is used for downy mildew of cabbage, onion and tobacco.

##### ii. Dichlone

Dichlone is also used as seed treating chemical. It contains 2, 3-dichloro -1, 4 - naphthoquinone as its active ingredient. It is sold under the trade names of Phygon, Phygon XL, etc. Dichlone is stable in sunlight but unstable in alkali media. It is insoluble in water in the form of yellow crystals having no odour. It is used as a seed dresser at the rate of 1-oz per 100 lb seeds, It can also be used as spray fungicide and is used against apple scab (0.1%), peach leaf curl (0.2%), beans anthracnose (0.2 to 0.3%) tomato leaf spots (0.2%) and sugar beet seedling blight (1.0%).

**Mechanism of action:** The mechanism of fungitoxicity of quinones especially with chloranil and dichlone involves two ways:

- a. binding of the quinone nuclear to  $SH$  and  $NH_2$  groups in the fungus cell, and
- b. disturbance in the electron transport systems. In *Neurospora sitophila* it inhibited the activity of several enzymes.

#### 5. Heterocyclic nitrogenous compounds

This group of heterogeneous but some of the best fungicides, also known as dicarboximide fungicides, includes captan, folpet, captafol, vinclozolin, glyodin and anilazine. Captan, folpet, and captafol belong to old



class of dicarboximide and are now known as phthalamide fungicides. The new members of dicarboximide group are iprodione, procymidone, vinclozolin, etc. Captan, captafol and folpet are mostly used as foliar and fruit protectants and could be used for treating seed, soil or as a dip for planting materials are the common fungicides found in this group.

#### **i. Captan**

Captan (N-trichloromethyl-thio-4-cyclohexene – 1,2 – dicarboximide). acts mainly as a protectant. Kittleson (1952) prepared this compound and first reported its fungicidal activity. Hence, in the beginning it was called as Kittleson's killer. It is commercially sold under the names such as Captan 50W, Captan 75W, Esso Fungicide 406 Orthocide 406, Hexacap, Merpan, and Vancide 89. It is incompatible with alkaline materials since it gets decomposed at a higher pH. Hence it is incompatible with lime sulphur and Bordeaux mixture. Captan can be used as spray, dust and seed dressing fungicide. Foliar diseases of apple, banana, coffee, grapevine, mango etc. are controlled by spraying the crop with captan at concentration of 0.2 to 0.4%. Seed treatment against maize seedling blight, chilli damping off, etc., with captan at 0.25% is effective. It is less toxic to human beings and animals.

**Mechanism of action:** Sulphur or chloride of the compound may be responsible for killing the fungi. In general captan inhibits a number of enzymes and interferes with decarboxylation process and citrate synthesis. Captan competes with cocarboxylase (thiamine pyrophosphate) for sites on coenzyme-free carboxylase in the decarboxylation of pyruvate and thereby interferes with the process of decarboxylation. Captan acts by inhibiting number of enzymes in phosphorus metabolism, certain oxidases and dehydrogenases, carboxylase and co enzyme A. In certain experiments it was found that fungitoxic substance viz., the H<sub>2</sub>S formed after application of captan on *Saccharomyces pastorianus* by deriving the major part of S from captan applied and a little from the cell.

#### **ii. Captafol**

Captafol was introduced in 1961 by Chevron Chemical Co., the USA. It is closely related compound to captan. Chemically it is cis-N-(1, 1, 2, 2-tetrachloro ethyl thio) - 4-cyclohexene – 1, 2 dicarboximide showing good control over early blight of potato better than captan. It is available under different names such as Captaspor Difolatan, Foltaf, Difosan and Sanspor. Although it is mainly recommended for foliar sprays, it has been used for seed dressings and soil applications. This fungicide has high resistance to weathering and persists on the host surface for much longer period than other fungicides. Together with its low phytotoxicity this property enables use of three times higher dose as a single application treatment for control of apple scab. It is recommended against late blight of potato and tomato and several other foliar diseases. It is compatible with most other pesticides, but non-compatible with highly alkaline materials and oils.

#### **iii. Folpet**

Folpet is an analogue of captan it has the constitution of N – (trichloromethylthio) phthalimide. It was introduced by the Californian Spray

Chemical Corporation. It is sold more commonly as Phaltan. Folpet can as effectively control diseases as captan and sometimes more than that. The fungitoxicity is due to the presence of imide in them. The folpet imide is cent per cent more toxic than captan imide in relation to the spores of *Monilinia fructicola* and *Stemphylium sarcinaeforme*. The use of folpet is comparatively less and has been used against some powdery mildews and diseases of apple, rose, tobacco, etc.

#### **iv. Iprodione**

It is sold as Rovral, Chipco-26019 and Glycophene. It was discovered in 1970 by Rhone Powlenc of France. It is chemically 3-(3, 5- dichlorophenyl) – N- (1-methyl ethyl) – 2, 4 –dioso –1-imidazolidine carboxamide. It is a broad spectrum contact fungicide. It inhibits spore germination and mycelial growth but is only preventive in action. It can be curative in early stages of infection. Rovral is very effective against diseases caused by *Botrytis*, *Monilinia*, *Sclerotinia*, *Alternaria*, *Helminthosporium* and *Rhizoctonia*. It is generally used as a spray fungicide but can also be used for seed treatment and post-harvest dip of fruits.

#### **v. Vinclozolin**

It is sold as Ornalin, Ronilan or Vorlan and is used as foliar spray against *sclerotia* forming fungi belonging to *Ascomycotina* such as *Botrytis*, *Monilinia* and *Sclerotinia*.

#### **vi. Anilazine**

It is sold as Dyrene. It is chemically, 4, 6-dichloro-N-(2-chlorophenyl) - 1, 3, 5,-triazin – 2-amine. It is sold as 50 % wettable powder. It is used against the leaf spots of grasses due to *Drechslera* spp. and rots due to *Fusarium* and *Rhizoctonia* spp. It is also effective against *Botrytis*, *Septoria*, *Colletotrichum* spp. as a protectant when applied at 170-250 g a.i. /100 l and is obtained as 50% W.P.

#### **vii. Glyodin**

In 1946 Wellan and McCallan discovered the fungicidal properties of the derivatives of glyoxalidine which led to the introduction of glyodin. chemically, glyodin is 2-heptodecyl-2-imidazoline acetate or 2-heptadecyl imidazoline glyoxide. Glyodin is sold under the trade names Crag Fruit Fungicide 341, Glyodin and Glyoxalidine. Glyodex is a combined formulation of 50% glyodin and 16% dodine. It is available as 30% liquid in 70% isopropanol and 70% WP. Glyodin loses fungitoxicity when slurried with hot water. It has a narrow spectrum of activity and is used as foliar spray for the control of apple scab and cherry leaf spot. It is not compatible with oils or emulsifiable concentrates. It competes with histidine and purines for enzymic binding sites. It may also act through metal chelation. It is fungistatic in action. It inhibits respiration by interfering with the availability of respiration substrates than by effect on enzymes.

## 6. Aromatic compounds

### i. Dinocap

Dinocap is the important member under this group. It is a mixture of 2, 4- dinitro –6-octyl phenyl crotonate and 2, 6-dinitro-4-octyphenyl crotonate. It was developed in 1946 by the Rohm and Haas Co., of the U.S.A. It is a dark brown liquid, insoluble in water but soluble in most organic solvents. It is sold under the names of Karathane, Arathane, Capryl, Mildex, DNOPC, Mildont and Crotothane. In India, it is available both in W.P. (25%) and liquid (48%) formulations. It is a good acaricide and contact fungicide and it controls powdery mildew of fruits and ornamentals effectively. This is successfully and safely used on sulphur sensitive plants like cucurbits and apple varieties against powdery mildew diseases. It is sprayed at a concentration of 0.05 to 0.1%.

### ii. Halogenated phenols

Halogenated phenols often serve as good fungicides but their use is much limited since they are highly phytotoxic. Pentachlorophenol is commonly used for wood preservation but hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB) and tetrachloronitrobenzene are used against some plant diseases. Hexachlorobenzene is used as a seed dresser against bunt disease of wheat.

#### a. Pentachloronitrobenzene (PCNB)

Pentachloronitrobenzene or Quintozene was first synthesized in Germany in 1930. Now it is sold in the market as Brassicol, PCNB, Quintozene, Terraclor and Tritisan. It is a seed dresser and soil drenching chemical. It is used as a good substitute for organomercurial seed treating chemicals. As a soil drench it controls many important soil inhabiting fungi like *Rhizoctonia* sp., *Sclerotium* sp. and *Sclerotinia* sp. PCNB prevents sporulation of fungi and inhibits the growth. PCNB has got nematicidal properties also. A great reduction of a virus diseases in strawberries transmitted by the nematode *Longidorus elongatus* was noticed when PCNB is applied at the rate of 1360 kg/ha in soils infested heavily by the nematodes, by way of reducing the population. It was also found that when PCNB was applied to soil, most of the actinomycetes and other fungi were suppressed, but some fungi like *Fusarium* sp., which are not affected by this fungicide are growing well as the other competitive organisms have been killed.

**Mechanism of action:** It is suggested that PCNB is antimitotic, which acts as inhibitor of inositol, as essential growth factor for many fungi. It is highly persistent in moist soil, but loses its character under submerged condition. PCNB acts primarily by disrupting the semipermeability of the cell membrane and causes lysis of cells.

### iii. Fenaminosulf or diazoben

It is sold as Dexon. It is sold as 5% granule and 70% WP. It is chemically, Sodium p-dimethyl amino benzene –diazo sulphonate. It is used against damping off and root-rot caused by species of *Pythium*, *Aphanomyces* and *Phytophthora*. It is fungistatic in action against *Phytophthora cinnamomi*. In

sensitive fungi it inhibits respiration. In the mitochondria of *Pythium* it inhibits oxidation of nicotinamide adenine dinucleotide. (NADH). It is carcinogenic and hence, its use was discontinued.

**iv. Chlorothalonil:** Chlorothalonil (tetrachloro-isophthalonitrile) is a contact fungicide. It is sold as Bravo, Daconil, and Termil is a popular fungicide effective against many diseases. As spray materials Daconil and Bravo have been used against leaf spots, late and early blights, downy mildews, rusts, anthracnoses and scab. In many areas Daconil is preferred over mancozeb for control of late blight of potato. Termil is a tablet formulation of chlorothalonil which is used in green houses for control of *Botrytis* on ornamentals and for several molds and blights of tomato. The mechanism of fungicidal action of *Chlorothalonil* is attributed to reaction with the thiol groups of certain enzymes in the susceptible fungus.

**v. Dichloran or DCNA:** Dichloran (2, 6-dichloro-4, introaniline) was developed by Upjohn Co. of the U.S.A. It is available as 50% WP or 75% WP. It is sold as Botran or DCNA and is used against fruit and vegetable diseases as spray and soil treatment material. It is effective against sclerotia producing fungi and is also used as post-harvest dip or spray for fruits, vegetables and flowers. It is compatible with WP formulations of most of the fungicides and insecticides, but with EC formulations of organic phosphorus compounds it may cause plant injury. It shows low phytotoxicity. It should not be used on germinating seeds on annual seedlings. DCNA generally fails to inhibit spore germination but suppresses mycelial growth. DCNA is a non-specific toxicant disorganizes cell division and growth. DCNA is the specific inhibitor of protein synthesis.

## 7. Non-aromatic organic compounds

### i. Dodine

By 1959 dodine was released for commercial use in the U.S.A. It is a derivative of guanidine. It is chemically known as n-dodecyl guanidine acetate. It is a salt of strong base and weak acid and is a surface active compound. It is sold under the commercial names of Cyprex, Guanidol, Melprex and Syllit. Dodine is used against the control of apple scab and several other diseases caused by fungi belonging to Ascomycotina and Deuteromycotina. Despite its solubility, the tenacity of the fungicide is high and shows an eradicant action against *Venturia* sp. It penetrates the host tissues and exhibit local systemic eradicant properties. Though it is non-injurious on foliage of apple, there are reports to say that spraying with dodine causes delay in ripening of fruits and often russetting. It is compatible with most pesticides but is incompatible with strongly alkaline compounds like Bordeaux mixture or lime and oils. Malathion may reduce its eradicant action. It is not compatible with streptomycin.

**Mechanism of action:** The spores of certain fungi absorb dodine in abundance. It interferes with the Permeability of cellular membranes of the yeast cells and causes heavy loss of the 'P<sup>32</sup>' compounds and amino acids in the cells. Besides this action, dodine inhibits the activities of intracellular enzymes.

## 8. Organo-tin compounds

Inorganic compounds of tin do not have any fungicidal value. Van der Kerk and Luijten, in 1954 published that many organotin derivatives having a general formula of  $R_3 Sn X$  are powerful fungicides. In this general formula, 'R' is normally a hydrocarbon (Alky<sup>1</sup>, Aralky<sup>1</sup> or Aryl) group which is attached to the metal atom (tin) by means of a carbon atom. 'X' may be a halogen, hydroxyl, oxygen or an organic or inorganic acid radical which is not attached to metal via, carbon atom. Majority of the organic tin fungicides are phytotoxic. The following 3 organic tin compounds are having fungicidal properties:

- i. Triphenyl tin acetate (TPTA)
- ii. Triphenyl tin hydroxide (TPTH)
- iii. Triphenyl tin chloride (TPTC)

These tin compounds have anti-bacterial activity also. TPTC has some systemic activity. TPTH has a anti-feeding properties on many insects and it is effective against rust mites.

### i. Triphenyl tin acetate (TPTA) or fentin acetate

It is sold as Brestan 40% and 60% WP. It is a non-systemic fungicide. It is effective against diseases caused by *Cercospora*, *Alternaria*, *Phytophthora*, *Pyricularia*, *Ramularia* and *Septoria*. As protectant fungicides it is used against *Cercospora* leaf spot of sugarbeet, carrot and celery, *Septoria* leaf spot of celery, downy mildew of beet, and early blight and leaf spots of potato. It controls brown spot of rice, groundnut early and late leaf spots and rust. The normal rate of application is 0.1%. It is about 10 to 20 times as effective as copper fungicides. It has good sticking properties and thus provides long-term protection. It is compatible with the wettable powder formulations of common pesticides. Even when it is mixed with alkaline salts or compounds of high pH values like Bordeaux mixture and lime-sulphur, it is hydrolyzed to TPTH which also has the same fungicidal efficacy as TPTA. However, it is not compatible with oils or oil containing formulations. It is phytotoxic to various vines, hops, fruits, ornamentals and green house plants. It is phytotoxic to four weeks old wheat seedling when used at 0.05% spray. It inhibits spore germination as well as kills the germinated spores.

### ii. Triphenyl tin hydroxide (TPTH) or fentin hydroxide

It was developed by Philips – Duphar laboratories, Holland under the trade name Du-Ter. The other commercial names are Farmatin and Tubotin. It is safer than other Organotin fungicides. It controls diseases caused by *Cercospora*, *Helminthosporium*, *Pythium*, *Phytophthora*, *Rhizoctonia* and *Septoria*. It is used to control the diseases in potatoes, sugarbeet, celery, groundnut, onions, carrot, tomato, vegetables, fruits and ornamental crops. It effectively controls *Cercospora arachidicola*, *Cercospora personata*, *Puccinia*, *Alternaria*, *Phytophthora*, *Pyricularia* at 0.2% concentration. It is compatible with Wettable powder formulations of other pesticides but is incompatible with emulsifiable formulations and oils. Phytotoxicity at higher rates of application has been reported in potato and wheat. Generally, TPTH sprayed crops remain greener for a longer period. A waiting period of 3-4 weeks between last spray on the harvesting is recommended.

### iii. Triphenyl tin chloride (TPTC) or fentin chloride

It is sold as Brestanol 45% WP and Tinmate. Its activity is similar to TPTA or TPTH. It is effective against *Cercospora* leaf spot of sugarbeet, rice blast, aerial blight of soybean (*Rhizoctonia solani*), rust of soybean (*Phakopsora pachyrhizi*) and brown stripe downy mildew of maize (*Sclerophthora rayssiae* var. *zeae*). It causes phytotoxicity to wheat seedlings.

## 9. Organophosphates

### i. Edifenphos

The chemical name of edifenphos is O-ethyl-S,S, diphenyl dithiophosphate. Hinosan, is available as 50% emulsifiable concentrate. It is a clear liquid of yellow to light brown colour with a characteristic thiophenol odour. It proves to be a specific fungicide against rice blast fungus, *Pyricularia oryzae* and acts as a curative and protective fungicide. Apart from its specific action against blast fungus, it also effectively acts against sheath rot and sheath blight of rice. This fungicide is chiefly used as spray at a concentration of 0.1%-0.2% for the control of rice diseases. It is compatible with methyl demeton, Lebaycid and methyl parathion. The pH has a pronounced effect on its stability. It is less stable at higher pH than neutral pH.

**Mechanism of action:** It inhibits the chitin synthesis in sensitive fungal cells as iprobenphos, a systemic fungicide.

## 10. Nickel compounds

Nickel chloride (used at 170 g/67 of water) is effective in the control of tea blister-blight in Sri Lanka. A mixture of 210 g of copper oxychloride + 210 g of nickel chloride per ha sprayed at 5 days interval from Jun to Sept. and 11 days intervals in Oct-Nov gives economic control. Nickel sulphate or nickel chloride as foliar spray (0.3%) induced resistance against bacterial leaf blight disease of rice and it controlled this effectively.

## 11. Miscellaneous fungicides

### i. Chinomethionate or oxythioquinox

It is manufactured by Farbenfabriken Bayer A.G. It is chemically 6-methyl- quinoxaline – 2,3-dithiol – cyclic carbonate. The commercial name of this fungicide is morestan. It is available as 25% W.P. It has excellent protective and government eradicant action against powdery mildews of fruits, cucurbits and ornamental plants. It is slightly more effective than Karathane against powdery mildews and mites. Generally 0.03 to 0.05 % ( for vegetables 0.025 to 0.05%) at 7-9 days or 10-14 days interval is recommended. It has very good acaricidal action. It gives control of eggs, active larval stages and of adult spider mites. It also has effects against pear psylla (*Psylla piricola* ), whitefly, and aphids. It is not toxic to honey bees. This fungicide causes russetting of fruits in Golden delicious variety of apple. High dosage under bright sunlight in grapevine causes leaf brown, russetting and etching.

## ii. Mineral oil

Light viscosity oils atomized into very fine droplets of 50-100 µm in dia provide very good control over Sigatoka leaf spot of banana (*Mycosphaerella musicola*). It apparently stops the growth of the established pathogen but do not inhibit the production or germination of spores. The nature of its control over this pathogen is obscure. Oils usually cause phytotoxicity slightly when a quantity of 5.11 to 7.76kg /ha is applied. It is believed that it physically restricts the growth of the fungus by obstructing gas exchange and by blocking stomatal opening. High sugar pathogens (sugar loving pathogens) such as powdery mildews and rusts are found to be susceptible to oils. Phytotoxicity of mineral oils has been reported in citrus, banana and a few other crops due to inefficient spraying leading to accumulation in concentrated areas where by necrotic flecks arise.

## iii. Salicylanilide (salicylamide)

It is chemically 2-hydroxy-N-phenylbenzamide. It is a broad spectrum non-systemic fungicide mainly used in glass houses. It is toxic to Oomycetes and Ascomycetes. If 0-hydroxyl group is replaced by a chlorine or bromine it loses its toxicity to above fungi and becomes specifically toxic to Basidiomycetes.

## 12. Systemic fungicides

The introduction of systemic fungicides in 1966 is a major landmark in the history of management of plant diseases. The successful use of synthetic systemic compounds of non-microbial origin, oxathiin was first demonstrated by von Schelming and Kulka in 1966. This discovery of oxathiin fungicides was soon followed by confirmation of systemic activity of pyrimidines (Elias *et.al.* 1968) and benzimidazoles (Delp and Klopping, 1968). A systemic fungicide is defined as a systemic, fungitoxic compound that controls a fungal pathogen remote from the point of application, and that can be detected or identified. These compounds are absorbed by the plants and get translocated with in it thus provides protection and eradication of already established infection.

It resembles the antibiotics, but the antibiotics are of microbial origin and differ in their translocability from the systemic fungicides. An ideal systemic fungicide should have the following characteristics:

1. The substance may either be toxic to the pathogen concerned or be converted in the host plant to become a fungitoxicant.
2. The substance (or a derivative formed in the plant) may alter the metabolism of the host so that biochemical or physiological resistance to pathogen may be induced or enhanced.
3. It should not adversely affect the host plant and reduce the quantity and quality of the crop.
4. In systemicity the substance must be absorbed sufficiently and translocated from the point of application to the site of the pathogen and should have a considerable degree of stability within the plant.
5. If it is applied to an edible portion of the plant the mammalian toxicity must be at very low level to avoid residue problems at the consumer stage.
6. It should be a water soluble substance and persistent inside to give protection sufficiently for a long time.

7. They should be cheap, stable under normal conditions, agreeable to handle and simple to apply.

During the last 35 years there has been a major breakthrough in the field of systemic fungicides. There are a number of commercially viable, systemic fungicides in the market. On the basis of chemical nature and structural relationship these fungicides are classified as detailed below.

- i. Acetamides: *e.g.*, cymoxanil
- ii. Acylalanines: *e.g.*, Metalaxyl, Furalaxyl, Benalaxyl
- iii. Aliphatics: *e.g.*, prothiocarb, Propamocarb
- iv. Benzimidazoles: *e.g.*, Benomyl, Carbendazim, Thiabendazole, Fuberidazole, Cypendazole.
- v. Oxathiins or Carboximides: *e.g.*, Carboxin Oxycarboxin, Fenfuran,
- vi. Benodanil, Cyclafuramid, Mebenil, Pyracarbolid.
- vii. Dicarboximides: *e.g.*, Procymidone
- viii. Imidazoles: *e.g.*, Imazalil, Fenafanil
- ix. Morpholines: *e.g.*, Dodemorph, Tridemorph
- x. Organophosphates: *e.g.*, Triaminphos Iprobenphos, Pyrazophos
- xi. Alkyl phosphonates: *e.g.*, Phosetyl - Al
- xii. Piperazine: *e.g.*, Triforine
- xiii. Pyrimidines and purines: *e.g.*, Dimethirimol, Ethirimol, Triarimol, Bupirimate, Pyroxychlor
- xiv. Thiophanates: *e.g.*, Thiophanate, thiophanate – methyl
- xv. Triazoles: *e.g.*, R.H-24, Tricyclozole, Fluotrimazole, Triadimefon, Triadimenol, Biloxizole
- xvi. Phenol derivatives: *e.g.*, Chloroneb
- xvii. Miscellaneous: *e.g.*, Ethazole, Buthiobate, Furmecyclox, Isoprothiolane

#### **i. Acetamides**

Acetamides (acetic acid amide) are the amido derivatives of acetic acid.

**a. Cymoxanil:** Cymoxanil (DPX – 3217) was developed by E.I. du Pont Nemours & Co., Inc., U.S.A. It is commercially sold as Curzate (DPC 3217) and Remiltine. It is chemically Cyano-N- (ethylaminocarbonyl) –2- (methoxyimino) acetamide. It is available as 50% and 80% WP for spraying. It controls downy mildew of grapevine, late blight of potato. In soil it has low mobility and short persistence. hence it is not suitable as a soil fungicide. Mode of action is not known. Another compound, N-cyanoethyl chloracetamide sold as Udonkor was found to be effective against powdery mildew fungi.

#### **ii. Acylalanines**

Acylalanines belong to phenylamide group. In 1973, Acylalanines were found out by CIBA-Geigy, Switzerland to have systemic and curative functions against most of the pathogenic Oomycetes. Metalaxyl, furalaxyl and benalaxyl are the three important acylalanine fungicides.



**a. Metalaxyl:** It was discovered in 1973 and developed under the code number CGA-48988 by CIBA-Geigy, Switzerland. Metalaxyl is the common Name for methyl –dI-N- (2, 6-dimethyl phenyl) – N- (2' methoxy acetyl) alaninate. It is commercially sold as Ridomil 25% WP, Ridomil IG, 2G and 5G, Apron 35 SD, Apron 25%, WP and Subdue 2E. It is formulated as wettable powder (WP), emulsifiable concentrate (EC) seed dressing (SD) and granules (G). It is highly effective against *Pythium*, *Phytophthora* and many downy mildew fungi. It is sold under the name Ridomil for foliar application and as Apron for seed treatment. Another formulation Subdue is used for ornamentals and turf grasses. It is widely used as a soil or seed treatment material for control of seed rot and damping off caused by *Pythium* *Phytophthora*, as soil treatment for control of *Phytophthora* diseases of citrus and other perennial and annual crops and downy mildews of maize, pearl millet and sorghum. It is also used as post harvest fruit dip to prevent brown rot of citrus. It has curative effect and can eradicate infection from treated plants. It is available in combination with mancozeb, Ridomil MZ-72 WP which contains 8% metalaxyl +64% mancozeb. This combination having systemic and contact action and effective against damping-off, root-rots, stem-rots and downy mildew of grapevine and millets. Metalaxyl is quite water soluble and readily translocated from roots to aerial parts but its lateral translocation is slight. It is compatible with most insecticides, acaricides and fungicides in common use. It interferes with fungal colonization, affects nucleic acid synthesis and may stimulate phytoalexins, thus promoting resistance.

**b. Furalaxyl:** Furalaxyl has been discovered and developed by CIBA-Geigy, Switzerland. It is marketed as Fongarid, 50WP and Fonganil. Chemically, it is methyl-N-(2,6-dimethyl – phenyl). –N- furoyl – (2) alaninate. It is a soil fungicide with curative and systemic properties. It is available as 25% and 50% WP. It is suitable for control of soil-borne diseases caused by *Phytophthora* and *Pythium* spp. on ornamentals and downy mildew of lettuce.

**c. Benalaxyl:** It is chemically, N-2,6 –dimethyl phenyl –N-(phenylacetyl) – methyl ester. It is commercially available as Galben 25% WP and 5% G. It is effective against blue mould of tobacco, late blight of potato and tomato and downy mildew of grapevine, hops and lettuce.

### iii. Aliphatics

**a. Prothiocarb:** Prothiocarb was introduced by Schering AG, Plant Protection Division, Berlin and the trade names are Previcur Dynone and SN-41703. Chemically it is S- ethyl-N- (3-dimethylamino propyl) – thiocarbamate hydrochloride. It is specifically active against soil-borne Oomycetes like *Pythium* and *Phytophthora* causing stem and root diseases. As soil application (5.6 kg a.i. per ha) alone or when combined with 0.3% a.i. foliar sprays, controls the above diseases.

**b. Propamocarb:** It is chemically propyl – (3-dimethylamino) – propyl carbamate monohydrochloride (PDAC). It was developed by NOR-AM Agricultural products, Inc., USA. It is commercially sold as Banol, Prevex, Previcure-N, Filex, Dynone-N and SN-66752. It is effective against soil-borne

Oomycetes, mainly *Phytophthora* spp. It controls Phycomycetous diseases by soil or foliar applications. Like prothiocarb, it also needs to be used in relatively high concentration.

#### iv. Benzimidazoles

This group includes benomyl, carbendazim, thiabendazole and cypendazole. Most benzimidazoles are converted on the plant surface to methyl benzimidazole carbamate (MBC) and this compound interferes with nuclear division of sensitive fungi. Benzimidazoles are in general inhibitors of DNA synthesis (benomyl) and carbendazim) and respiration (TBZ and Fuberidazole). They have broader spectrum of activity against many fungi in Ascomycotina and Basidiomycotina. However, they are not effective against bacteria and fungi belonging to Oomycetes.

**a. Benomyl:** It was developed by the E.I. du Pont de Nemours and Co., Inc., USA., and sold as Benlate 50 WP. Chemically it is, Methyl –N- (1-butylcarbamoyl) –2- benzimidazole carbamate. It is an excellent systemic fungicide which has eradicant and protectant activities. It has ovicidal action in mites. It is effective against powdery mildews of cucurbits, cereals and legumes, rice blast, apple scab, black spot of rose, diseases caused by *Botrytis*, *Cercospora*, *Colletotrichum*, *Cephalosporium*, *Fusarium*, *Gloeosporium*, *Monilinia*, *Verticillium* etc. It is highly effective against and suppresses the infection by *Rhizoctonia*, *Thielaviopsis* and *Cephalosporium*. Benomyl has no effect against Oomycetes and some dark coloured fungi such as *Alternaria* and *Helminthosporium*. It is used as foliar, seed and soil treatment fungicides. This fungicide usually requires a surfactant to avoid intense agitation for dispersion. It is usually used with ‘Surfactant-F’ for proper dispersion. Resistance against benomyl has been reported in a large number of fungi.

**b. Carbendazim:** It is chemically Methyl –2- benzimidazole carbamate. It is sold as Bavistin 50WP, MBC, Derosol 60%WP B.Sten 50, Zoom, Tagstin, Agrozim and Jkenstin. It is both prophylactic and curative. It has fungitoxic properties as benomyl but it is more stable. It is also used as seed treatment at 1-2 gms/kg of seeds to control seed-borne diseases of cereals and root-rot diseases of different crops. As a foliar spray at 0.05-0.1%, it effectively controls leaf spots by different fungi, anthracnose, powdery mildews and rust diseases. It is also used as soil-drench against wilt diseases especially banana wilt. It is also used for seedling dip and for post harvest treatment of fruits. Like benomyl, it also interferes with the DNA synthesis.

**c. Thiabendazole:** It is chemically 2 – (4'-thiazolyl) benzimidazole, It is sold as Thiabendazole has got a systemic fungitoxic action and shows an upward movement from root to other aerial portions. It is particularly very effective against blue and green mold of citrus fruits. Thiabendazole W-7, Mertect, 60%WP, Tecto and Storite. It is a broad spectrum fungicide effective against fungi like *Botrytis*, *Ceratocystis*, *Cercospora*, *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Septoria*, and *Verticillium*. It is also effective for the control of post-harvest diseases of fruits and vegetables. It is used as a pre-harvest spray or post-harvest dip for controlling fruit rots during storage,

shipping and marketing. It is not active against *Mucor fumosus*, *Oospora citriauranti*, *Phoma betae*, *Phytophthora* spp., *Pythium ultimum* and *Rhizopus* spp. It is non-phytotoxic at the recommended rates.

**d. Fuberidazole:** It was developed by Bayer-AG of West-Germany. It is chemically 2,(2-furyl) – benzimidazole. It is sold as Voronit. It is used for treatment of seeds against *Fusarium nivale* on rye and *F.culmorum* on peas. As a seed treatment fungicide it reduced infections of *Puccinia triticina* and *Erysiphe graminis* for several weeks in wheat and barley.

**e. Cypendazole:** Cypendazole was introduced by Bayer – AG, West Germany under the Trade Code number chemagro Bay Dam 18654. Chemically, it is methyl 1-(5-Cyanopentyl carbamoyl) benzimidazole –2-yl carbamate. It has protectant, eradicant and systemic activity. The compound is broad spectrum and is active against apple scab, *Botrytis* spp. Powdery mildews, *Cercospora* spp. storage rots, vascular and seedling diseases.

#### **v. Oxathiins or carboxamides**

It is also known as carboxylic acid anilides or carboxanilides or anilides. The members of this group are substituted derivatives of salicyl amides. Carboxin, oxycarboxin, furamamide, fenfuran, benodanil, pyracarbolid are included in this group. Fungitoxicity of these fungicides are in general due to inhibition of mitochondrial respiration at or close to the site of succinate oxidation. They are found to be effective chemotherapeutants for many smuts and rust diseases. Oxycarboxin is very effective than carboxin where long lasting chemotherapeutic activity is required e.g., in the control of cereal and bean-rust diseases. But, carboxin gives better protection against rust because of its greater stability in plants. The systemic fungicidal activity of oxathiin derivatives Carboxin and oxycarboxin was first reported by Von Schmeling and Kulka, in 1966. Oxathiins were the first developed systemic fungicides in plant disease management.

**a. Carboxin:** Carboxin was discovered and developed by Uniroyal International Division of Uniroyal Inc., U.S.A. It is chemically known as 5, 6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide or DMOC. It is sold as Vitavax 10%D, Vitavax 75%WP, Vitavax 34% liq. and Vitaflow. Fungi belonging to Basidiomycotina are particularly sensitive to DMOC and also *Verticillium albo-atrum* and *Monilia cinerea* f.sp. *americana* have been found to be sensitive to DMOC. This systemic fungicide is used for seed treatment of cereals against bunts and smuts especially loose smut of wheat (250 g /100 kg of seeds). When used with copper oxyquinolate the synergistic effect controls *Septoria nodorum*. *Helminthosporium gramineum* and *Fusarium nivale* which are not affected by carboxin alone. It also controls *Rhizoctonia* disease of cotton and sugarbeet. This fungicide interferes with synthesis of protein, RNA and DNA in rapidly metabolizing cells. It also inhibits mitochondrial respiration.

**b. Oxycarboxin:** - Oxycarboxin was discovered and developed by Uniroyal chemical, U.S.A under the code number Uniroyal F-461. It is chemically 5, 6-dihydro-2-methyl-1, 4-oxathiin-3-carboxianilid-4, 4-dioxide or DCMOD. Trade names are Plantvax 5G, Plantvax 5% liq., Plantvax 1.5 EC, 10% dust and 75

WP. It is reported toxic to (in addition to Basidiomycetes) *Helminthosporium sativum*, *Curvularia*, *Aspergillus*, *Cladosporium*, *Botrytis*, *Monilinia* and *Cunninghamella* also. This systemic fungicide is used for the control of rust diseases of cereals, pulses, ornamentals, vegetables and coffee. Complete control of plant disease such as rust and smut was achieved when oxycarboxin seed treatment and foliar application or soil and foliar applications were combined.

**c. Pyracarbolid:** Pyracarbolid was developed by Hoechst Aktiengesellschaft, West Germany under the code name HOE -13764. It is chemically 2-methyl-5, 6-dihydro-4H-pyran-3-carboxylic acid anilide. It is sold as Sicarol 50 WP. It controls rusts, smuts of many crops and *Rhizoctonia solani* and blister blight of tea. It is slightly more effective than carboxin.

**d. Fenfuran:** It is a new selective cereal seed dressing fungicide. It was first developed by Shell Research Ltd. It is sold as Panoram 25, 40 and 75% Dust and Panoram 35% liquid. Its chemical name is 2-methyl-furan-3-carboxylic acid anilide. It is active against smuts and bunts and controls bunt of wheat, barley smut (*Ustilago hordei*), loose smut of barley and wheat, smut of oat (*U.avenae*) and flag smut of wheat (*Urocystis tritici*). It is generally used at 1 to 2g/kg of seed. As liquid formulation its use is 2 to 3 ml/kg of seed.

**e. Benodanil :** It was developed by BASF-AG of West Germany. It is sold as Calirus. It is available as 50% wettable power and as 20% emulsifiable concentrate. It is effective against rust disease in cereals like yellow rust of wheat and barley, brown rust of wheat and barley, black rust of wheat, carnation rust, fig rust and chrysanthemum rust. It is used at the rate of 1.5 to 2.0 kg/ha or 1.5 to 3.0 /ha of wettable powder and EC formulation respectively.

**f. Mebenil:-** It was developed by BASF-AG of West Germany. Chemically it is O- methyl-benzoic acid anilide- (2-toluanilide). It can be used as soil, seed and foliar fungicide for the control of rust, smut and root-rot-fungi. It effectively controls yellow rust of wheat and barley and brown rust of barley as foliar spray. Seed treatment of potato is effective against infection by *Rhizoctonia* sp. As seed treatment at 1kg/100kg of seed controlled onion smut.

**g. Cyclafuramid:** Cyclafuramid was developed under the code number BAS 3270F and 3271F of BASF-AG of West Germany. Chemically it is, N-cyclohexyl -2,5 -di-methylfuramide. It is effective against smuts, bunts and rusts of cereals, coffee rust, blister blight of tea, smut and red rot of sugarcane, *Fusarium* wilt of tomato, *Rhizoctonia* sp. on potato, groundnut and rice as well as *Armillaria* sp. on rubber. The general application rate as spray is of 0.25 to 1.0 kg a.i., per hectare as seed or sett dip is 0.25 to 0.75 per cent a.i., suspension for 5 to 15 minutes and as soil spray or drench is 2.5 to 5.0 kg a.i. per hectare. A waiting period of 14 days is suggested for cereals, potatoes, groundnuts, sugarcane, tea and coffee.

#### vi. Dicarboximides

**a. Procymidone:** Procymidone is developed under the code number S7131 by Sumitomo Chemical Co., Ltd., Japan. Chemically it is N-(3'5' - dichlorophenyl) -1,2-dimethylcyclo -propanedicarboximide. It is sold as Sumilex and Sumisclex, as 50% WP and 25% flowable. It is highly fungitoxic to Ascomycetes and a few Deuteromycetes. It is selectively toxic to *Sclerotinia* spp., *Botrytis* spp. and *Monilinia* spp. It is used to control the diseases caused by species of *Sclerotinia* *Botrytis*, *Helminthosporium* and *Monilinia* on cereals, beans, vegetables, fruits etc., It effectively controls *Botrytis cinerea* which caused grey mould of eggplant, cucumber, tomato, strawberry, onion, ornamentals and grapevine and stem-rot of beans, lettuce, paprika and brown rot of peach caused by *Sclerotinia* sp.

#### vii. Imidazoles

**a. Imazalil:** Imazalil is developed under the code number R-23979 by Janseen Pharmaceutical of Beers, Belgium. Chemically it is 1-(2-(2, 4-dichloro-phenyl) -2- (2-propenyl-oxy) ethyl -H- imidazole. It is especially effective against fungi which have developed resistance against benzimidazoles such as *Penicillium italicum* and *P. digitatum*. on citrus fruits causing fruits rots. It is sold as Funga-flor, Bromazil, Deccozil and Nuzone. It is available as EC formulation having 20, 50 and 80% W/V; as water soluble powder 75% w/w, liquid 5-8% w/v, oily liquid 50% w/v and 50% granule. It possesses protective, curative and antisporeulant properties.

**b. Fenapanil :** Fenapanil was developed by Rohm and Haas Co., USA. Chemically it is butyl -a- phenyl-1- H-imidazole -1- propanenitrile. It is a broad spectrum systemic, protectant and curative imidazole fungicide. It is sold as Sisthane 25% EC. It is effective against a large number of Ascomycotina, Basidiomycotina and Fungi Imperfecti. It is highly toxic to spot blotch of barley (*Bipolaris sorokiniana*) when sprayed at 0.05%. As seed treatment fungicide it controlled brown spot of rice, common bunt and loose smut of wheat, loose and covered smut of barley and barley powdery mildew. It inhibits sterol biosynthesis. It also showed abnormal germ tube development.

#### viii. Morpholines

**a. Tridemorph:** It was developed under the code number BAS - 22005F by BASF-AG West Germany. It is marketed as Calixin 75EC Bardew and Beacon. Chemically, it is N-tridecyl -2, 6-dimethyl morpholine. It is an eradicant fungicide with systemic action. It has been used against powdery mildew of cereals (wheat), vegetables (pea, cucurbits) and ornamentals. The fungicide is also reported to be effective against the stripe or yellow rust of wheat and barley, rusts of pulses groundnut and coffee, Sigatoka leaf spot of banana (*Mycosphaerella musicola*), pink disease of rubber (*Corticium salmonicolor*) and blister blight of tea, The uptake and translocation is acropetal. It is phytotoxic at 0.05% to brinjal plants.

**b. Dodemorph:** Dodemorph was developed under code number BAS 238F by BASF-AG, West Germany. It is sold as Meltatox 40% EC. Chemically it is 4-

cyclododecyl 2, 6 – dimethylmorpholin. It is effective against powdery mildews of ornamental crops including roses at 0.1%.

#### **ix. Organophosphates**

**a. Triamiphos:** It was the first practically applied systemic fungicide. It was introduced by Philips-Duphar, Holland. It is commercially sold as Wepsyn. Chemically it is, p-5 –amino – (bis – (dimethylamino)-phosphoryl) –3- phenyl – 1, 2, 4 – triazole. It effectively controls powdery mildews of ornamentals including powdery mildew of roses. But it was phytotoxic to apple. It is presumed that it modifies the host plant metabolism and makes them to resist the pathogen. It has insecticidal and acaricidal properties also. It has limited utility because of high phytotoxicity.

**b. Iprobenphos (IBP):** It was developed by Kumiai Chemical Industry Co.Ltd., Japan. It is sold as Kitazin 48%EC, Kitazin 17G and Kitazin 2%D. Chemically it is, S-benzyl –o, o-di-isopropyl phosphorothioate. It is used to control rice blast and rice sheath blight. It has insecticidal property (rice leaf hopper, *Nephotettix* spp.) and plant hoppers (*Sagata* sp.) also.

**c. Pyrazophos:** It was discovered and developed and introduced by Hoechst, Frankfurt. It is sold as Afugan, HOE 2873 30EC, Missile EC and Curamil WP. Chemically it is, O,O-diethyl –O- (5-methyl –6- ethoxycarbonyl –pyrazolo) (1.5a) – pyrimidine –2-yl) thionophosphate. It has systemic, preventive and curative action. It stimulates growth of ornamentals and strawberries. It is used to control powdery mildews of cereals, vegetables, (cucumbers), fruits and ornamentals. It is used at 0.03 or 0.04%.

#### **x. Alkyl phosphonates**

**a. Fosetyl-Al or Fosetyl-Al or Aluminium-Tris :** It was developed by Rhone-Poulenc. France and released in 1977. It is commercially sold as Aliette, EPAL and EXP 1659. It is available as 80% WP. Chemically it is Aluminium tris' –o- ethylphosphonate. It is the only commercially available alkyl phosphonate compound. It is a specific fungicide for Oomycetes especially damping off, late blight of potato, diseases caused by *Phytophthora* in Citrus, pepper, rubber and grapevine downy mildew. It is recommended in combination with protectant fungicides like folpet or mancozeb. Mikal, a mixture of fosetyl-Al (50%) with folpet (25%) and Rhodex, a mixture of fosetyl-Al (44%) with mancozeb (26%) are the combination formulations available for use.

#### **xi. Piperazines**

**a. Triforine:** In 1967 W.Ost of C.H. Boehringer Sohn, Germany synthesized a new systemic fungicide coded as CELA.W 524. Chemically it is 1, 4-bis (2, 2, 2 – trichloro –1- formamidoethyl) - piperazine. It is sold as Sapol – EG and Fungitex. It is effective against powdery mildews, rusts and apple scab. It is also active against storage diseases of fruits.

#### **xii. Pyrimidines and pyridines**

**a. Dimethirimol:** It was introduced in 1968 by ICI Plant Protection Division, LONDON. It is commercially sold as Milcurb. Chemically it is, 5 - butyl – 2 -

dimethylamino – 4 - hydroxy – 6 - methyl pyrimidine. It is very effective against powdery mildews of chrysanthemum and cucurbits. It is not effective against grapevine powdery mildew. It is highly active against *Sphaerotheca fuliginea* on cucumber and *Erysiphe cichoracearum*. It is less active against *Erysiphe graminis*, *Podosphaera leucotricha* and *Sphaerotheca pannosa*. It is not effective against *Uncinula necator*. The protected plants are more vigorous.

**b. Ethirimol:** It was introduced in 1968 by ICI Plant Protection Division, London. It is sold as Milgo E, Milcurb super and Milstem. Milliatem 80 WDP. Chemically it is, 5 - butyl - 2 - ethylamino - 4 - hydroxy – 6 - methyl pyrimidine. It is effective against powdery mildews of cereals (wheat and barley), cucumber and ornamentals. Ethirimol is considered more effective as soil or seed treatment fungicide than as foliar spray material.

**c. Triarimol:** It was discovered and developed under the code number EL-273 the Eli Lilly company, USA. Chemically it is,  $\alpha$ -2, 4 -dichlorophenyl - $\alpha$ -phenyl –5- pyrimidine –methanol. It is a broad spectrum fungicide and highly effective against apple and pear scab, powdery mildews of fruits, cereals and roses and yellow rust of wheat.

**d. Bupirimate:** It was introduced in 1975 by Imperial Chemical Industries, England. It is sold as Nimrod. Chemically it is, 5-butyl –2- ethylamino –6- methyl –pyrimidine –4- yl –dimethyl sulphamate. It is available either as an emulsifiable concentrate (250 g a.i./l) or as dispersible powder containing 250 g a.i./kg. It was commercially introduced in 1975. It is used for the control of powdery mildews of fruit crops (apple, peach, apricot, mango, strawberry) and ornamentals (rose and chrysanthemum) and vegetables (cucurbits, sugarbeet, pea and pepper). It has both eradicant protectant properties.

**e. Pyroxychlor:** It was discovered under the code number DOWCO – 269) by Johnston and Tomita (1969) and developed by Dow Chemical Co., U.S.A. It is sold as Nurelle. Chemically it is 2-chloro –5- methoxy –4- trichloro methyl phsidin. It is available as Emulsifiable. It is effective against pythiaceacious fungi. It is effective as seed treatment for the control of *Pythium ultimum* and for Tobacco black shank, *Phytophthora parasitica* var. *nicotianae* as soil drench.

**f. Fenarimol:** Chemically it is,  $\alpha$  - (2-chlorophenyl) - $\alpha$ - (4-chlorophenyl) –5- pyrimidinemethanol. It is commercially known as Rubigan, Bloc and EL-222. Formulations available are 50% WP. EC. and 1 lb/gal aqueous suspension. It is effective against powdery mildew of grapevine, apple and rose and rust and scab of apple. As seed treatment 2 g/kg of seed, it is phytotoxic to wheat. It is supposed to act through inhibition of the ergosterol biosynthesis in the sensitive fungi.

**g. Nuarimol:** Chemically it is  $\alpha$ -(2-chlorophenyl) - $\alpha$ - (4-fluorophenyl) –5- pyrimidine methanol. It was developed under the code number EL-228 by Eli Lilly and Co. It is sold as Trimidal. It is available as 0.75 lb/gal E.C., 1 lb/gal aqueous suspension and 5% solution formulations. It is used as protectant,

curative and eradicator. As seed treatment it controls seedling infection in barley by *Cochliobolus sativus*. It also controls take-all disease of wheat.

### **xiii. Thiophanates**

**a. Thiophanate:** It was developed and marketed by Nippon Soda Co., Ltd. Japan. It is chemically, 1, 2 – bis (ethoxy carbonyl –2- thioureido) benzene. It is sold in the names of Topsin 50 WP, Cercobin 50 WP and Enovit. It is used as seed treatment, soil drenching and foliar sprays. It is effective against scab of apple and pear, powdery mildews and diseases caused by *Botrytis*, *Cladosporium*, *Plasmidiophora* and *Sclerotinia* on various crops.

**b. Thiophanate – methyl:** It is also called as mildothane, trade names are Topsin-M 70 WP Cercobin – M - 70 WP and Enovit-methyl. It was developed by Nippon Soda Co., Ltd., Japan. Chemically it is 1-2 bis – (3-methoxy carbonyl –2- thioureido) benzene. It is effective against scab of apple and pear, Sigatoka leaf spot and anthracnose of banana, powdery mildew of apple, pear, grapevine and cucurbits, blast of rice and diseases caused by *Cercospora* and *Botrytis* of various crops. As soil treatment fungicide it is effective against *Verticillium* wilt of tomato and *Macrophomina* root rot in soybean.

### **xiv. Triazoles**

**a. Bitertanol (Biloxazol):** It was discovered and developed by Bayer-AG, West Germany. It is sold as Baycor and Sibutol. Chemically it is, p- (1, 1- biphenyl –4- yloxy) – $\alpha$ - 1, 1-dimethylethyl) –1- H-1, 2, 4 triazole –1- ethanol. It is formulated as 25% and 50% WP, EC having 200 g a.i. /l and as seed dressing. Sibutol is a seed dressing formulation for cereals. It is a broad spectrum fungicide. It is effective against powdery mildews and rusts of various crops, apple scab, *Monilinia* on fruit crops, late leaf spot of groundnut and Sigatoka leaf spot of banana. It is not effective against Oomycetes, *Botrytis*, *Fusarium*, *Penicillium* and *Rhizoctonia* and *Septoria*.

**b. Diclobutrazol:** It was introduced by Jealott's Hill Research Station, Plant Protection Division of ICI, U.K. It is sold as Vigil. Chemically it is, (2F, 3R) and (2S-3S) –1(2, 4, dichlorophenyl)-4-4 –dimethyl –2- (1, 2, 4 –triazol –1 yl) pentan –3-ol. It is formulated as W/V suspension concentrate on non-cereal crops and as aqueous suspension 12.5% W/V for use on cereal crops. It controls rusts and powdery diseases as foliar spray or by soil application.

**c. Etaconazole:** It was developed under code number CGA-64251 by Ciba-Geigy Ltd., Switzerland. Its chemical name is: 1 – (2-(2', 4' – dichlorophenyl) – 4- ethyl –1, 3 – dioxolan –2- yl) methyl –1H –1, 2, 4 –triazole. It is available as 10% WP and 1.25% DS. It is a broad spectrum fungicide with protective and curative activities. It specifically controls powdery mildew, rust, scab of apple and pear and diseases caused by *Sclerotinia*. As seed treatment fungicide it is effective against fungi like *Helminthosporium*, *Tilletia* and *Ustilago*. It inhibits the ergosterol biosynthesis.

**d. Flutriazol:** It was discovered by ICI Plant Protection Division and was tested under code number PP-450. Chemically it is, RS-2,4<sup>1</sup> – difluoro – $\alpha$ - (1H, -1, 2,4 -



triazole –1-yl-methyl) benzhydryl alcohol. It is available under the trade name Impact, (PP-450). It is formulated as suspension concentrate (SC) having 125 g a.i. /l. It is compatible with most insecticides, herbicides plant growth regulators and fertilizers. It controls powdery mildews, brown and yellow rust of wheat and barley as a foliar fungicide. It inhibits ergosterol biosynthesis in sensitive fungi.

**e. Flusilazol:** This fungicide is being developed under the code number DPX – H6573 by E.I. du Pont de Nemours & Co.Inc., U.S.A. It is also known under the trade name Nustar. It is chemically, 1-(Bis-(4-fluorophenyl) (methylisly) methyl –1H, 1, 2, 4 –triazole. It has preventive, curative and systemic activity, It controls early and late leaf spots of groundnut, leaf and glume blotch of wheat (*Septoria* sp.) cereal powdery mildew, scab and powdery mildew of apple, wheat foot-rot, (*Pseudocercospora herpotrichiodes*), cedar apple rust (*Gymnosporangium juniperi-virginianae*), brown rot (*Monilinia fructicola*), cherry leaf spot (*Coccomyces hiemalis* and orange rust of raspberry, coffee rust, black spot of rose, powdery mildew of bhendi, (*Sphaerotheca fuliginea*) powdery mildew of cucumber and *Mycosphaerella fragariae* on strawberry.

**f. Myclobutanil:** It was developed under the code number RH-3866 by Rohm and Haas company, USA. Chemically it is  $\alpha$ -butyl - $\alpha$ - (4-chlorophenyl) –1- H-1, 2, 4 –triazole –1- propnenitrile. It is available as Systhanse. It is a systemic fungicide possessing protective and curative properties. It is highly effective against apple scab, cedar apple rust and powdery mildew (*Podosphaera Leucotricha*). It inhibits ergosterol biosynthesis.

**g. Tricyclazole:** It was developed by Research and Development division of Eli-Lilly & Co., U.S.A. with the code number EL-91. It is marked as Beam and Bim. It is available as wettable powder (20% WP, 75% WP) dust 1% D, and granule 4% G. It is a specific fungicide which is highly effective against blast of rice. This chemical is effective for the blast incidence at panicle stage also.

**h. Triadimefon:** It was discovered and developed by Bayer, West Germany. It is sold as Bayleton and Amiral. Chemically it is, 1-(4-chlorophenoxy) –3, 3-dimethyl –1-1 (H-1, 2, 4 –triazole –1-yl) – butan –2-one. It is formulated as wettable powder 5% or 20%, emulsifiable concentrate having 100g a.i./l.suspension concentrate, paste and 1% dust. It is very effective against powdery mildews and rusts of different crops, (*Erysiphe*, *Sphaerotheca*, *Puccinia*, *Uromyces Phakopsora*, *Hemileia* and *Gymnosporangium*).

**i. Triadimenol:** It was discovered and developed by Bayer, West Germany. It is sold as Baytan and Bayfiden. Chemically it is, 1-(4- chlorophenoxy) –3, 3-dimethyl –1- (1, 2, 4 –triazole –1- yl) -butan 2-ol). It is available as seed dressing, wettable powder, emulsifiable concentrate, emulsion water and dry powder formulations. It is a broad spectrum fungicide effective against rusts, smuts, bunts, powdery mildews and some members of Fungi Imperfecti. (*Helminthosporium graminea*, *H. sativum*, *H. avenae*, *Bipolaris sorokiniana*, *Rhizoctonia solani*). It causes temporary stunting in wheat, barley and soybean.

**j. Triazbutyl:** It (RH-124) was developed and discovered by Rohm and Haas Co., USA. It is sold as Indar. Chemically it is 4-n-butyl 1, 2, 4 – triazole, (BT). R.H –124 is available as 80% active water soluble liquid concentrate for foliar sprays and 25% active wettable powders for seed treatment. The fungicide can be used as foliar spray, seed treatment and soil application. It is selectively effective against leaf rust of wheat caused by *Puccinia recondita* f.sp. *tritici* and not to rust diseases caused by other species of *Puccinia* or *Uromyces*.

#### **xv. Phenol derivatives**

**a. Chloroneb:** It was developed by E.I. du Pont de Nemours and Co., USA. It is sold as Demosan 65 WP, Tersan SP and Turf fungicide. Chemically it is, 1, 4-dichloro – 2,5 –dimethoxy benzene. It is used as supplemental seed treatment or as an in-furrow soil treatment at planting time for the control of seedling diseases of beans, cotton, soybean and sugarbeet. It is effective against damping off and seedling blight caused by *Pythium*, *Rhizoctonia* and *Sclerotium*. It is slightly effective to *Fusarium*.

#### **xvi. Miscellaneous**

**a. Buthiobate:** It was developed by Sumitomo Chemical Co., Ltd. Japan. It is sold as Den Mert. Chemically it is, S-n-butyl –s-tert –butyl (benzyl –N-3 pyridyldithio carbonimidate. It is effective against powdery mildew diseases of barley and cucumber.

**b. Cyproconazole:** It is sold as SAN 619. It is very effective against rust and powdery mildew diseases. It also controls groundnut early and late leaf spots.

**c. Difenconazole :** It is sold as Sare 25 EC. It is effective against rust, powdery mildew and leaf spot diseases of many crops.

**d. Ethazole:** It was developed under the code number OM-2424 by Ohi Mathieson Chemical Corporation, U.S.A. Chemically it is 5-ethoxy –3-trichloromethyl 1, 2, 4 – thiadiazole (ETMT). Trade names of this fungicide are Terracoat Aaterra, Koban, Pansol EG, Pansol 4% DP and Turban WP, Terrazole 30% WP, Terrazole 95% WP, Terrazole 25% EC. Terrazole was introduced in 1966 as protective fungicide as seed and soil treatment. It is effective against seed and seedling diseases of bean, cotton, cucumber, potato, sorghum, soybean, and tomato. It is effective against diseases caused by *Pythium* and *Phytophthora* and *Rhizoctonia* and vegetables, fruits and ornamentals.

**e. Furmecyclox:** It was developed as BAS 39501 by BASF, Germany. Chemically it is, N-cyclohexyl –N- methoxy –2, 5- dimethyl –3 furan carboximide. It is a systemic fungicide for cereal seed treatment and is effective against *Ustilago* and *Tilletia caries* and *Pyrenophora graminea*.

**f. Hexaconazole:** It was developed under code number PP-523 by ICI, England. Anvil 5C and Contaf –5 EC are the trade names. It is chemically, RS-2- (2, 4-dichlorophenyl) –1-(1H –1, 2, 4, triazol –1 yl) hexan-2-ol. It has both protectant and eradican properties. It is effective against powdery mildews, scab and rust of apples. It also controls groundnut early and late leaf spots and powdery

mildew of vegetables, peach brown rot and Sigatoka leaf spot of banana. It inhibits ergosterol biosynthesis in target fungi.

**g. Isoprothiolane** ; It was developed by Nihon Nohyaku Co., Ltd. Japan. It is sold as Fuziwan. Chemically it is diisopropyl –1,3, dithiolan –2-ylidenemalonate. It is available as 40% EC, 2.5% dust and 12% granule formulations. It is a specific fungicide against rice blast. It also controls stem rot of rice.

**h. Penconazole:** Chemically it is, 1-(2-(2, 4, -dichlorophenyl) –n- pentyl) –1H-1, 2, 4 – triazole. It is sold as Award, CGA – 71818, Topas, Topaz and Topaze. It is available as emulsifiable concentrate 100% EC. It is a systemic fungicide for protective, curative, and eradicated use against powdery mildews, scab of pome fruit and other pathogenic Ascomycetes, Basidiomycetes and Deuteromycetes and for use in grapes, deciduous fruits, cucurbits and other vegetables.

**i. Probenazole:** Orizemate is the commercial name of this fungicide which is effective against rice blast.

**j. Propiconazole:** It was developed under the code number CGA – 64250 by Ciba – Geigy Ltd., Switzerland. The trade names are Tilt and Desmel. Tilt 3.6E (3.6 lb/gal) in the U.S.A and Tilt 250E (250g. /l) are some of the formulations. Chemically it is, 1-(2-(2, 4- dichlorophenyl) –4- propyl –1, 3- dioxolan –2yl) methyl –1H –1, 2, 4 –triazole. It is a broad spectrum foliar fungicide with eradicant and systemic activity. It is effective against diseases caused by fungi in Ascomycotina, Basidiomycotina and Deuteromycotina. It is effective against Sigatoka leaf spot of banana, *Alternaria* blight of cotton, early and late leaf spot of groundnut, powdery mildew of grapevine, head smut of maize, sheath blight of rice, pineapple disease of sugarcane and leaf rust, *Septoria* glume blotch and Karnal bunt of wheat.

**k. Pyroquilon:** It is chemically 1,2,5,6 – tetrahydro –4- pyrolo (3,2-1-i,j) quinoline 4-one. Trade names are Fungorene 50 WP and Fungorene 5G. It effectively controls rice blast.

**l. Tebuconazole :** It is commercially sold as Folicur 25EC and also Raxil 2DS. It is used as a seed treatment fungicide for the control of smuts. It is also effective against powdery mildew and rust diseases.

### 13. Antibiotics

Antibiotic is defined as a chemical substance produced by one microorganism which in low concentration can inhibit or even kill other microorganisms. They are specific in their action against plant pathogens like fungi, bacteria and phytoplasmas. Most of the antibiotics are the products of actinomycetes. Few antibiotics are obtained from fungi and bacteria. Penicillin was discovered by Alexander Fleming, the Nobel laureate in 1928. Ever since penicillin was rediscovered in 1939 – 40 by Florey and his associates, considerable interest has developed in the study of various microorganisms for

production of antibiotics. During the past six decades many of the known and newer antibiotics have been tested for their use in plant disease management. More than 350 antibiotics have been found out in the control of plant diseases. But majority of them are of no practical value because of their instability and due to one or other undesirable properties. It is now established that different fungal and bacterial plant diseases can be controlled by few antibiotics. Gliotoxin is the first antibiotic to be used for plant disease control. It was isolated and purified even before the discovery of penicillin. Chloramphenicol is the first antibiotic to be synthesized on a commercial scale. The chemical formulae of most of the antibiotics are complex and they are not related to each other. Antibiotics used in plant disease management are generally absorbed and translocated systemically by the plants. Antibiotics may control plant diseases by acting on the pathogen or on the host or after undergoing transformation within the host. This capacity depends on the type of antibiotic and the plant species involved. Antibiotics act both as eradicant and as protectant.

Antibiotics like penicillin, streptomycin and tetracyclines are antibacterial while antibiotics like aureofungin, griseofulvin, cycloheximide, etc., are antifungal.

**Advantages:** The advantages of using the antibiotics in plant disease control are as follows:

- i. Relatively less phytotoxic
- ii. Specificity of their action on plant pathogens – fungi or bacteria or both, Phytoplasmas etc.,
- iii. Easy decomposition.
- iv. Activity in low concentration
- v. Easy and non-hazardous manufacture
- vi. No operation hazards
- vii. Systemic in action. They are absorbed by roots or leaves and trans located to varying plant parts.

**Disadvantages:** Most of the antibiotics are unstable. Phytotoxicity is one of the major disadvantages in antibiotics. They are usually used in very small quantities. If their dose is increased beyond permissible limits plants are damaged. This is the reason why the highly fungitoxic antibiotics like griseofulvin and cycloheximide could not become popular in plant disease control. Another disadvantage with antibiotics is their narrow spectrum of antipathogenic action. The antibacterial antibiotics are mostly ineffective against fungi while the antifungal antibiotics have no antibacterial activity and are effective only against specific fungal plant pathogens. Resistance to several antibiotics has been reported in fungi. Strains of *Erwinia amylovora*, the fire blight (of apple) bacterium, that were resistant to the systemic antibiotic, streptomycin had been known for several years.

The antibiotics are broadly grouped into two viz. antibacterial and antifungal.

#### **i. Antibacterial antibiotics**

**a. Streptomycin:** Streptomycin is an antibacterial antibiotic produced by the actinomycete, *Streptomyces griseus*. Chemical name of streptomycin is 2, 4 – diguanidino – 3, 5, - trihydroxycyclohexyl –5- deoxy – 2 – 0 – (2-deoxy – 2 methylamino – x – glucopyranosyl) – 3 – formyl pentofuranoside. Streptomycin is strongly basic in character and is marketed either as sulphate or hydrochloride. Streptomycin or streptomycin sulphate is sold as Agrimycin – 100, Agristrep, Embamycin, Orthostreptomycin, Paushamycin, Plantomycin, Phytomycin, Phytostrip, Streptomycin sulphate, Streptocycline, Agrimycin – 100 contains 1 % streptomycin sulphate + 1.5 % terramycin (oxytetracycline). Phytomycin contains 20 % streptomycin. Agristrep contains 3 % streptomycin sulphate. Streptocycline and Paushamycin contains 9 parts of streptomycin and 1 part of tetracycline hydrochloride. Streptomycin is effective against both Gram positive and Gram negative bacterial plant pathogens, but they do not show any fungitoxicity against true fungi.

Streptomycin acts against a broad range of bacterial pathogens causing spots, blights, wilts, rots etc. This antibiotic is mostly used as foliar spray at concentrations of 100-500 ppm. Important diseases controlled are fire blight of apple and pear (*Erwinia amylovora*), Citrus canker (*Xanthomonas axonopodis* pv. citri), cotton bacterial blight (*X. axonopodis* pv. malvacearum), bacterial leaf spot of tomato and pepper. (*X. pv. vesicatoria*), brown rot of potato and tomato (*Burkholderia solanacearum*), wild fire of tobacco (*Pseudomonas syringae* pv. tabaci) and soft rot of vegetables (*Erwinia carotovora* pv. carotovora).

Streptomycin as soil drench is used to control foot rot of geranium caused by *Xanthomonas* sp. It is also used as a dip for potato seed pieces against various bacterial rots and as a disinfectant in bacterial pathogens of cereals, beans, cotton, crucifers and other vegetables. Moreover streptomycin is also effective against some diseases caused by Oomycetous fungi, especially foot-rot and leaf rot of betelvine caused by *Phytophthora parasitica* var. piperina and hops downy mildew caused by *Pseudoperonospora humuli*. Streptomycin act as uncoupling agent and inhibit electron transport. It also inhibits protein synthesis. Streptomycin and Agrimycin are compatible with DDT, fixed copper fungicides, wettable sulphur and zineb and not with BHC, chlordane and glyodin. Glycerine, copper oxychloride, etc., are often added to streptomycin to improve its efficacy.

**b. Tetracyclines:** Tetracyclines are produced by many species of the actinomycetes, *Streptomyces*. Among tetracycline antibiotics chlortetracycline was the first isolated antibiotic. They are all broad spectrum antibiotics, being effective against a large number of Gram positive and Gram negative bacteria, rickettsiae and the larger viruses. Oxytetracycline isolated from *Streptomyces rimosus* is most common. Terramycin or Oxymicin (oxytetracycline), Achromycin and Tetracycline (tetracycline) and Aureomycin (chlorotetracycline) are commonly used for plant diseases control. All these antibiotics are bacteriostatic, bactericidal and phytoplasma static. These are very effective against seed-borne bacteria. This group of antibiotic is very effective in managing phytoplasmal diseases on a wide range of crops. These are mostly used as combination products with streptomycin sulphate in controlling a wide range of bacterial diseases including bacterial blight of pome fruits.

Oxytetracyclines are effectively used as soil drench or as root dip for controlling crown gall diseases in rosaceous plants caused by *Agrobacterium tumefaciens*. Tetracyclines injected into the trees infected with phytoplasmas stop the development of disease incidence and induce remission of symptoms, *i.e.*, the symptoms of the disease disappear and trees resume growth as long as some tetracycline is present in the trees. Remission of symptoms has been reported in phytoplasmal diseases like sandal spike and brinjal little leaf. In general tetracycline group of antibiotics are known to inhibit protein synthesis, amino acids and ribosomal proteins.

## ii. Antifungal antibiotics

**a. Aureofungin:** It is a polyene (heptaene) antibiotic produced in submerged culture of *Streptovorticillium cinnamomeum* var. *terricola*. It was discovered by Thirumalachar *et al.* (1964-66). Aureofungin-sol. is now commercially manufactured and sold by Hindustan Antibiotics Ltd., Pimpri, Pune India. The manufacturer recommended use of 6 g copper sulphate for every 6 g (active ingredient of aureofungin-sol for better disease control. Aureofungin belongs to a new aromatic sub-group among the heptaenes. The aromatic moiety is N-methyl, -aminoacetophenone and mycosamine. It is absorbed and translocated to other parts of the plants when applied as foliar spray or given to roots as drench. It is sold as Aureofungin-Sol which contains 33.3% aureofungin and 66.7% solubilizing agents and normally sprayed at 50-100 ppm. The diseases controlled by Aureofungin-Sol are citrus gummosis of citrus (*Phytophthora* spp), powdery mildew of apple (*Podosphaera leucotricha*), apple scab (*Venturia inaequalis*), groundnut early and late leaf spots, downy mildew, powdery mildew and anthracnose of grapevine and potato early and late blights. Soaking seeds in 20 ppm aureofungin + 20 ppm copper sulphate solution controls brown spot disease in the rice nursery. Aureofungin is effective against variety of fungi. It is not soluble in water but is soluble in the presence of alkali or soap solution at 1g in 10ml of soap solution. Spraying aureofungin at 7.5 g/ha controls blast of ragi caused by *Pyricularia oryzae*. Post harvest diseases of fruits and vegetables like *Penicillium* rot of apples and citrus. Diplodia rot of mango, (dip treatment of 100 ppm) *Alternaria* rot of tomato (dip treatment of 500 ppm) and *Pythium* rot of cucurbits are also controlled by aureofungin. Soaking rice seeds affected by *Helminthosporium oryzae* in 20 ppm solution mixed with 20 ppm copper sulphate for one h gave good control of the diseases. As a root feeding, 1.3 g of Aureofungin-Sol+0.5 of copper sulphate in 100ml of water effectively reduced basal stem rot (Thanjavur wilt) of coconut caused by *Ganoderma lucidum*. The shelf life of Aureofungin-Sol is about two years. Aureofungin has no activity against bacteria including *Rhizobium* spp. Aureofungin causes disruption of cell wall of the fungi. It also changes the host physiology and makes it unfavourable to the pathogen. As seed treatment it effectively checks brown spot and blast diseases in rice.

**b. Griseofulvin:** Griseofulvin was discovered first by Oxford *et al.* (1939) as a metabolic product of *Penicillium griseofulvum*. *Penicillium* viz., *P.janczewski*, *Penicillium nigricans*, *P.patulum*, *P.raciborskii* and *P.urticae* and are now found to produce griseofulvin. Griseofulvin is chemically known as 7-chloro -4, 6-dimethoxycoumaran - 3 one - 2 - spiro - 1' - (2' - methoxycyclohex) -2' - en

-4'-one. It is a condensed aromatic compound. It is a neutral antibiotic. Griseofulvin has no activity against bacteria and yeasts. Its use against plant disease is limited. It is commercially available as Fulvicin, Griseofulvin, and Grisovin. It is highly toxic to diseases caused by the fungus, *Botrytis*, powdery mildew of beans (*Erysiphe polygoni*) and roses (*Sphaerotheca pannosa*), downy mildew of cucumber (*Pseudoperonospora cubensis*) and powdery mildew of chrysanthemum (*Oidium chrysanthemi*). It is also used as foliar spray to control *Alternaria solani* in tomato and *Ascochyta pisi* on peas. *Sclerotinia fructigena* in apple (1000 ppm), *Botrytis cinerea* in lettuce at concentration between 100 and 1000 ppm and tulip fire (*Botrytis tulipae*), Brain *et al.* (1946) were the first to report that griseofulvin caused abnormal development of fungal hyphae. Griseofulvin inhibits chitin synthesis and protein synthesis in fungi.

**c. Cycloheximide:** Cycloheximide, a glutarimide fungicide was introduced as a fungicide in 1949. Cycloheximide was discovered and developed by Upjohn Research Laboratories in the U.S.A. It is chemically known as  $\beta$ - (2, (3, 5 - dimethyl -2- oxycyclohexyl) -2- hydroxyethyl glutarimide. It is obtained as a by-product in streptomycin manufacture. It is produced by *Streptomyces griseus* and *S. novae*. It is toxic to fungi, yeasts, algae and protozoa. It is of comparatively low mammalian toxicity, though the toxicity level is much higher than streptomycin or griseofulvin. It is commercially manufactured and is used in plant disease control since 1952 in the U.S.A. It is commercially available as Actidione, Actidione -PM, Actidione RZ and Actispray. Cycloheximide has been used successfully used as a protectant, eradicant and systemic fungicide. It is systemic in nature and is easily taken by roots and leaves are rendered highly antifungal. It is active against a wide range of fungi and yeasts. Its use is limited because it is extremely phytotoxic. It is effective as foliar spray against rust diseases, powdery mildew of beans (*Erysiphe polygoni*), powdery mildews of grapevine (*Uncinula necator*) and cherry (*Podosphaera* sp.), bunt of wheat (*Tilletia* sp.), covered smut of oats (*Ustilago hordei*), brown rot of peach (*Sclerotinia fructicola*) and post harvest rots of strawberry (*Rhizopus* spp. and *Botrytis* spp.) Cycloheximide is also used in sweet wine preservation. Cycloheximide is mixed with PCNB in the ratio of 15:75 and is sold as 'Actidion RZ'. Cycloheximide can be mixed with DDT, malathion, ferbam, glyodin, captan, thiram and organomercurials and not with chlordane or pesticides containing lime or alkaline in reaction. It is of comparatively low mammalian toxicity, though the toxicity level is much higher than streptomycin or griseofulvin. Cycloheximide inhibits protein and nucleic acid synthesis and incorporation of some amino acids in fungi. It is used at 10-100 ppm depending on the disease and the host plant.

**d. Blastidicin:** It is a product of *Streptomyces griseochromogenes*. It is a broad spectrum antibiotic inhibiting fungi, some Gram positive and Gram negative bacteria including *Pseudomonas*. It is specifically used as protectant fungicide against blast of rice (*Pyricularia oryzae*). It is commercially sold as Bla-S. Blastidicin-S inhibits protein synthesis, ribosomal proteins and amino acids.

**e. Antimycin:** Antimycin is a macrolactone type antibiotic and is produced by *Streptomyces griseus* and *S. pitasawaensis*. Its use is limited because of

phytotoxicity. It is effectively used against early blight of tomato, rice blast and seeding blight of oats (*Helminthosporium* sp.) and apple scab.

**f. Kasugamycin:** It was obtained from *Streptomyces pasugaensis* in 1965. It is commercially available as Kasumin. It is a water soluble base. It is more effective as an eradicant than as a protectant. It is also very specific antibiotic against rice blast disease. Besides rice blast it is also effective against *Pseudomonas* spp. It is effective in low pH and at 50-100 mcg/ml of its active ingredient. Kasugamycin interferes with the nucleic acid metabolism and protein synthesis of fungi.

**g. Thiolutin:** It is a water soluble pyrrothine type antibiotic. It is produced by *Streptomyces albus*. It effectively controls late blight of potato.

**h. Endomycin:** It is a product of *Streptomyces endus* and effectively used against leaf rust of wheat and fruit rot of strawberry (*Botrytis cinerea*). Endomycin is non-toxic to higher plants but it has been found to be useful to control only few plant diseases.

**i. Bulbiformin:** It is an antifungal polypeptide antibiotic. It is produced by a bacterium, *Bacillus subtilis* and is very effectively used against wilt diseases like pigeonpea wilt (*Fusarium udum*).

**j. Nystatin:** It is produced by *Streptomyces noursei*. It is commercially marketed as Mycostatin and Fungicidin. It is successfully used against anthracnose diseases of banana and beans. It also controls downy mildew of cucurbits. As a post harvest dip, it effectively reduces brown rot of peach and anthracnose of banana in store houses. It is used as seed treatment against stripe disease of barley. It is also employed in combating meat moulds.

**k. Eurocidin:** It is a pentaene antibiotic. It is produced by *Streptomyces anandii*. It effectively controls diseases caused by species of *Colletotrichum* and *Helminthosporium*.



# 14

## Formulations

Fungicides are seldom used at full strength. They are formulated in various ways to make them easily available. Proper formulation is therefore necessary for the successful employment of fungicide. Fungicides formulated commercially are of two types viz., Liquid formulations and solid formulations.

### **i. Liquid formulations**

- a. Emulsifiable concentrates
- b. Solutions
- c. Flowables
- d. Aerosols
- e. Emulsions
- f. Suspensions or slurries
- g. Liquified gas

### **ii. Solid formulations**

- a. Dusts
- b. Granules
- c. Wettable powders and water dispersible powders
- d. Soluble powders

### **i. Liquid formulations**

**a. Emulsifiable concentrate (EC):** The great majority of sprays are applied as water-based emulsions using emulsifiable concentrates. This common and versatile formulation contains the toxicant, a solvent for the toxicant and an emulsifying agent. Incorporation of a small amount of an emulsifier (surface-active material) into the mixture ensures emulsion desired stability and wetting and spreading characteristics. When mixed with water, the concentrate forms an emulsion of oil in water type. When sprayed, the solvent evaporates quickly leaving a deposit of toxicant from which the water also evaporates.

**b. Solutions:** True solutions are formulations in which active ingredient or a combination of active ingredients and a solvent is dissolved in water. The common organic solvents used in pesticide formulations are amyl acetate, carbon tetrachloride, cyclohexanane, dibutyl phthalate, ethylene dichloride, kerosene, monochlorobenzene, petroleum naphtha or Stoddard's solvent, pine oil, technical di- and tri-methyl naphthalenes and xylene. Solvency, toxicity to plants and animals, fire hazard, compatibility, odour and cost are important factors to be taken into consideration in choosing a solvent. Solutions are liquid concentrates that may be used directly or after dilution. Those used directly from the container are low concentrates. Most low concentrates are solutions in highly refined oils. Solutions have the advantage of requiring no agitation

after formulation is added in water. Special kind of high-concentrate solution is the ultra low volume (ULV) concentrate. ULV formulations are applied without dilution with special aerial or ground equipments to produce an extremely fine spray.

**c. Flowables (F):** In some instances pesticidal compounds can be made only as solids or semisolids. They are wet-milled with a clay diluent and water to give a pudding-like consistency. This formulations is mixed with water for spraying. Flowables should be constantly agitated to prevent the pesticide from coming out of suspension and settling to the bottom of the spray tank.

**d. Aerosols (A):** Toxicants used in aerosols are dissolved in volatile petroleum solvents. eg., Methyl chloride or Freon (Dichlorodifluoromethane) is normally in gaseous form but can be liquified under pressure at ordinary temperature. On release of pressure the liquid containing the toxicant vapourizes and leaves a residue over the entire surface. Aerosols have a low percentage of active ingredient and therefore expensive. They are used mostly in glass houses.

**e. Emulsions:** Emulsions are prepared when the active ingredient is not soluble in water or other cheap solvent. It is prepared by dissolving the active ingredient in small quantity of solvent and emulsifying it in water to the required degree of concentration. The emulsifying agent is not dissolved in the solvent. The colloidal concentrate is prepared mechanically and diluted with water for spraying. Emulsions become unstable when stored for long periods and are more expensive than water dispersible powders for equivalent quantities of the active ingredients.

**f. Suspensions or slurries:** In suspensions or slurries dry form of active ingredient is mixed with a liquid. Suspension or slurry has a high percentage of active ingredients as wettable powders. They are mixed with water for final use. They require agitation. Fungicidal slurries are used to treat seeds in seed processing units.

**g. Liquified Gases (LG):** Fumigants when placed under pressure turn into a liquid. Liquified gases are stored in metal bottles under pressure and are released into the soil by injection.

## **ii. Dry formulations**

**a. Dusts:** Dusts are the oldest and simplest solid formulations of fungicides. They are prepared by milling fungicidal compound into a fine powder. The powder is diluted with diluents like attapulgite, kaolin, talc, pyrophyllite, diatomaceous earth, bentonite, calcium silicate, hydrated silica, calcium carbonate, magnesium carbonate, gypsum and lime. Compatibility, particle size, abrasiveness, absorbability, specific gravity

and cost are some important basic points to be considered in the selection of dusts. They are light and are easily carried by wind to a considerable distance. Dust formulations usually contain 1 to 10 % active ingredient. They are easy to apply in small areas. They are applied in the early morning when the plants are wet with dew. They are applied directly as dry form on the target areas in foliage. Dusts are cheaper and easier, least effective and least economic. The efficacy of dust formulation depends on the degree of intimate mixing between the active ingredient and the diluent. For dusts, particles of 30 µm to 50 µm are preferred.

**b. Granules:** Granular formulations are prepared by applying liquid fungicide to coarse particles of a porous material. These materials may be formed from corn cobs, walnut shells, clay or other minerals. The fungicide is absorbed into the granule, coats the outside, or does both. The amount of active ingredient in granular formulation ranges from 2 to 10%. Because of the size of the granular particles it is safer (not inhaled) to apply than dusts or emulsifiable concentrates. Due to greater weight of the granular particles, there is very little drift which prevent undesirable contamination and undue loss of fungicide. The toxic material in the granular formulation is released over a longer period than does a spray deposits. The granules vary from 0.25 to 2.38 mm in dia. Granular application may cause scorching sometimes if the chemical is concentrated in a small volume of carrier. It has limited application to soil. Proper coverage may be problem with this formulation. The efficacy of the granular formulation applied to soil is dependent on many factors such as dosage, type of formulation, soil type, moisture conditions, method of application, rate of release of the toxicant, type of crop/ plant, rate of respiration and growth stage of the crop/plant and weather conditions.

**c. Wettable powder (WP):** Wettable powders look like dusts while they are in the containers. They are formulated to be mixed with water and sprayed on surfaces. When the wettable powder is used agitation is generally done in the spray tank to keep uniform dispersion. A surfactant added to the dust allows wetting during the mixing processes. When water is mixed a particle suspension results. Wettable powders are much more concentrated than dusts and contain 15 to 95 % active ingredient. Like the Flowables, frequent agitation is required to keep the fungicide in uniform suspension. Wettable powders usually cause less phytotoxicity than emulsifiable concentrates. They are more abrasive to spray pumps and nozzles. Wettable powders should never be used without dilutions. The active ingredient is incorporated, usually at the rate of 30 to 80% with a finely ground inert dust (filler) such as kaolin, a wetting agent and a suspending agent. The commonly used suspending agents are sodium lignin sulphonate (sulphite lye), methyl celluloses, polyvinyl acetate and

aluminium silicate. In addition, spreader- sticker is sometimes desirable, especially on plants with glossy or waxy leaves.

**d. Water dispersible powder (WDP):** WDP is easily wetted and they disperse well in water. A wetting agent is usually present in most water dispersible powder formulations but addition of a spreader-sticker is desirable when it is sprayed on plants with glossy or waxy leaves. Agitation is not generally required in water dispersible powder since it remains well dispersed for a long time. A highly developed type of water dispersible powder is called colloidal powder. A colloidal powder is a substance which is so finely divided that the individual particles will never sediment out. eg., Some copper fungicides.

**e. Soluble powders (SP):** Soluble powder dissolves in water and forms a true solution. Some agitation is needed to get soluble powders into solution. But after they are dissolved no agitation is needed. It always requires dilution.

### **Synergists and auxiliaries or adjuants**

Synergists are added to increase the toxicity of fungicides. The fungicides are commonly applied by dusting or by spraying. In dust formulations diluents are added to obtain proper coverage. In spraying, toxicant is made into a suspension in water. In order to increase the efficiency of the sprays, substances like wetting agents, dispersing agents, spreaders, stickers *etc.* are added during formulation. These materials are called as auxiliaries or adjuants. The various auxiliaries or adjuants used commonly are

- |                       |                     |
|-----------------------|---------------------|
| a. Solvents           | f. Spreading agents |
| b. Diluents           | g. Stickers         |
| c. Dispersing agents  | h. Safeners         |
| d. Emulsifying agents | i. Deodorants       |
| e. Wetting agents     |                     |

**Synergists:** Synergists are chemicals which are not toxic to pathogens by themselves, when it is added to fungicide it increases the toxicity. They are often added in the ratio of 8 (synergist):1 (fungicide) to 10:1. eg., piperonyl butoxide, sulfoxide and MGK 264. Many of these are used to prepare sprays.

**Adjuants:** Chemicals called auxiliaries or adjuants are added. They increase the efficacy of water mixed sprays. They are added mostly during the formulation of fungicides. They improve adhesion, mixing, surface tension or smell or serve to carry the fungicide.

**a. Solvents:** Many of the organic compounds used for fungicides are insoluble in water. Before they can be turned into spray concentrates or aerosols, they must be dissolved. Solvents are used to dissolve them. In choosing the solvents, solvency, phytotoxicity, animal toxicity, odour and cost are the factors to be considered. eg., Carbon tetrachloride, kerosene and xylene.

**b. Diluents:** Diluents are combined with concentrated fungicides as carriers. The addition of diluent is necessary to obtain proper coverage of the treated area. Diluent can be either liquid or solid. Liquid diluents are usually water or refined oils. When water is used it is essential to add wetting and dispersing agents for proper suspension of the fungicide. Emulsifying agents are required where oil solutions are used with water. Solid diluents are used to formulate pesticide dusts or granules. Here coarse or finely ground particles are used as carriers. eg., Organic flours like soybean flour and minerals like bentonite clay, talc and volcanic ash.

**c. Dispersing agents:** Dispersing agents or deflocculating agents are substances which keep fine particles away from each other to prevent deflocculation. When added to formulations they ensure uniform suspension and retard sedimentation of particles in spray suspension. They are added to get uniform concentrations of spray all over the treated area. e.g., Gelatin, plant gums, milk products, sodium carboxy and methyl celluloses.

**d. Emulsifying agents or Emulsifiers:** They are used for making stock emulsions. Oils and water immiscible liquids on agitation with water break into small droplets on standing rapidly coalesce to form a separate layer. This can be prevented by addition of emulsifier which helps to stabilize the emulsion. Many surface active substances (wetting and spreading agents) like soap function as emulsifier. They help in uniform mixing of substances in water suspensions. The separation between oil and water is due to difference in their specific gravity. The emulsifier resists the tendency of oil droplets to coalesce when mixed with water by providing a film between water and oil droplets which is held by both. This is obtained by the processing of oil, water and emulsifier mixtures in an emulsifying mill.

Emulsifier reduces the surface tension of the spray allowing it to spread and wet the treated surface and thus ensuring a better contact of the spray. This factor largely determines the spray performance. The size of the droplets is governed to a great extent by the kind and amount of emulsifier in the mixture. Alkaline soaps, organic amines, sulfates of long chain alcohols, sulfonated aliphatic esters and amides, mixed aliphatic – aromatic sulfonates, non-toxic types (ethers, alcohols and esters of

polyhydric alcohols and long chain fatty acids) and natural materials such as gums, proteins, carbohydrates, lipids, alginates and saponins are normally used as emulsifying agents.

**e. Wetting agents:** Wetting agents are materials which facilitate contact between spray and sprayed surface. They reduce the surface tension of the spray liquids and improve the surface of contact and help to spread. Wetting agents when added in fungicidal preparations help in easy deposition on sprayed surface. eg., polyethylene oxide condensate, esters of fatty acids and flour. The commercial preparations like Teepol, Tergitol and Triton-AE are good examples of wetting agents.

**f. Spreading agents:** Like wetting agents, spreading agents or spreaders also improve the contact between spray materials and plant surface. They help in the good coverage of the fungicide. Wetting precedes spreading. e.g., Soap, flour, sulphated amines, saponins, mineral oils, glyceride oil, terpene oil, resins and petroleum sulphonic acids.

**g. Stickers:** Stickers or adhesives are added to the spray liquids to improve the tenacity of fungicidal preparations. They retain the active ingredients of a fungicide on a plant surface for a longer period. e.g., Polyvinyl acetate, polybutanes, fish oil, linseed oil, milk casein, gelatin, dextrans, polyethylene polysulphide, starch, gum arabic, hydrocarbon oils and bentonite clays. Milk casein and gelatin also act as good spreading and wetting agents besides acting as stickers.

**h. Safeners:** A chemical which reduces the phytotoxicity of another chemical is called safener. Copper sulphate when sprayed alone on the foliage causes phytotoxicity. Lime is added to copper sulphate in the preparation of Bordeaux mixture as a safener. Glyceride oils are also used as safeners.

**i. Deodorants:** Deodorants are materials added to fungicides to mask unpleasant smell. They are particularly added in formulations used for some home gardens. e.g., cedar oil, pine oil, various flower scents. Deodorants are incorporated at 0.1 to 1.0 per cent concentration.

# 15

## Mode of Action and Fungicide Resistance

### MODE OF ACTION OF FUNGICIDES

Majority of fungicides kill or inhibit the fungi responsive to them through direct effect on fungal cells or spores (conidia) after entering them. Solubilization of fungicides on the host surface is facilitated by free water, CO<sub>2</sub> and ammonia in rain water or dew, guttation fluids and other exudates from the plants, spore exudates and the ability of spores to accumulate fungicides from very dilute solutions. The killing of fungal cells by fungicides may be brought about by

- i. injurious effects on cell walls and on cell division.
- ii. effect on the permeability of cell membrane,
- iii. effect on enzyme system of the fungal cells,
- iv. chelation and precipitation of chemicals and
- v. by antimetabolism.

The protectant fungicides (copper, mercury, sulphur, etc., and organic compounds of mercury and sulphur) have fairly unspecific modes of action. They are applied more or less in insoluble form on the surface of the plant where they kill germinating spores but cannot penetrate the plant. Within the fungus, they exhibit fairly unspecific chemical reactions with sulphhydryl, amino, hydroxylic or carboxylic groups in proteins and nucleic acids or their precursors. Sulphur interferes with electron transport along the cytochromes of fungi and is then reduced to hydrogen sulphide which is toxic to most cellular proteins. Copper ion is toxic to all cells because it reacts with sulphhydryl group of certain amino acids and causes denaturation of proteins and enzymes.

Many organic fungicides also inactivate fungal proteins and enzymes in the same manner. Dithiocarbamates and ethazol, when taken up by fungal cells, release thiocarbonyl which inactivates sulphhydryl (-SH) groups. The chlorinated aromatic and heterocyclic compounds such as PCNB, chlorothalonil, chloroneb, captan and vinclozolin inactivate enzymes in the fungal cells.

The systemic fungicides and antibiotics are absorbed by the host, are translocated internally through the plants and are effective against the pathogen at the site of infection both before and after infection. These systemic fungicides are much more specific and affect only one or two

functions in the pathogen rather than a variety of them as in the case of protectant wide spectrum fungicides. Oxathiins inhibit only the enzyme, succinic dehydrogenase which is essential for mitochondrial respiration. Benzimidazoles interfere with nuclear division by binding to protein subunits of the spindle microtubules. The organophosphorus fungicides iprobenphos and edifenphos primarily act by inhibiting chitin synthesis in the pathogen. Fungi that have no chitinous walls are not responsive to these fungicides. Metalaxyl and related compounds affect ribosomal RNA of the fungi and interfere with protein synthesis. Triazole fungicides have become well known as sterol inhibiting fungicides because they inhibit ergosterol biosynthesis. Ergosterol is a cellular compound that plays a crucial role in the structure and function of the membranes of many fungi. These fungicides penetrate the leaf cuticle and therefore have curative post infection applications.

Very little has been known about the possible role of fungicides in stimulating resistance responses to the host. The fungicide, fosetyl-A1 is supposed to reduce infection by increasing the resistance of the host to the pathogen. This is probably done by altering the constitution of the host cell walls, by limiting the availability of essential coenzymes in the host or by altering the rate or direction of metabolism in the host, which they may be in a better position to defend itself. The secondary effects including the induction of mild shift in resistance expression, promotion of mycorrhizal growth and enhancement lytic capabilities of the antagonistic microflora in the rhizosphere may substantially contribute to host resistance in specific diseases. These observations suggest that indirect effects of fungicides also play some role in disease suppression. However the major role of fungicides is direct effect on the pathogens.

## **DEVELOPMENT OF FUNGICIDE RESISTANCE**

Among the several constraints in the extensive use of fungicides, resistance developed by pathogenic fungi to fungicides, resurgence of pathogens and residues retained by the plants are emerging as major problems. Fungicide resistance can be defined as the ability of organisms to tolerate higher dosage of chemicals. Resistance was first observed in 1969 in cucumber downy mildew fungus, *Sphaerotheca fuliginea* when treated with benomyl fungicide. Fungicide resurgence has been observed in the form of increase in other diseases or non-target pathogens. Fungicide residue is defined as persistence of a chemical over a long period after application of chemical.

Resistant strains are frequently cross resistant to structurally related chemicals or to chemicals with identical mode of action. Usually the pathogen/strains develop resistance to systemic fungicides than non-systemic fungicides because



1. Systemic fungicides act on a specific site in fungi whereas non-systemic fungicides possess nonselective toxicity and multisite action
2. The organism can acquire resistance to systemic fungicides easily by mutation of a single gene and not to non-systemics which involve polygenic mutation.
3. Successive use of one systemic fungicide or of systemic fungicides belonging to a single cross resistance group can promote the development of resistance of the fungal cell wall as well as their growth or reproduction rate.

Some of the fungi listed as having developed resistance to systemic fungicides are *Phytophthora*, *Pseudoperonospora cubensis*, *Plasmopara*, *Sphaerotheca*, *Venturia inaequalis*, *Fusarium*, *Cercospora*, *Colletotrichum*, *Verticillium* and *Ustilago*.

**Mechanisms of development of fungicide resistance:** The mechanisms by which a pathogen can develop resistance to a toxic chemical are

- i. decreased permeability of pathogen cell membranes to the chemical e.g. *Cladosporium cucumerinum* to 6-azouracil,
- ii. detoxification of the chemical through modification of its structure or through binding it to a cell constituent, e.g. *Botrytis cinerea* to PCNB
- iii. decreased conversion of the chemical to real toxic compound ,e.g. *Cladosporium cucumerinum* to 6-azouracil
- iv. decreased affinity at the reaction site in the cell, e.g. *Sphaerotheca fuliginea* to benomyl .
- v. bypassing of a blocked reaction or function through a shift in metabolism e.g. *Ustilago maydis* to Antimycin - A and
- vi. compensation for the effect of inhibition by producing more of the inhibited product,

Table. List of resistant strains of pathogenic fungi to fungicides

Fungicide	Fungal pathogen	Crop
<b>Benzimidazoles</b>		
Benomyl	<i>Uncinula necator</i>	Grapevine
	<i>Erysiphe cichoracearum</i>	Egg plant
	<i>E.graminis</i>	Blue grass
	<i>Corynespora melongenae</i>	Egg plant
	<i>Botrytis cinerea</i>	Apple, Beans,
	<i>Penicillium expansum</i>	Grapes
	<i>P.italicum</i> , <i>P.digitatum</i>	Apple
	<i>Glomerella cingulata</i>	Citrus

	<i>Venturia pirina</i>	tea
	<i>Venturia inaequalis</i>	Pear
	<i>Sphaerotheca pannosa</i>	Apple
	<i>Cercospora beticola</i>	Cucumber
	<i>Botrytis</i> sp.	Sugarbeet
	<i>B. tulipae</i>	Vegetables
		Tulip
Carbendazim	<i>Pseudocercospora herpotrichides</i>	Wheat
<b>Acylalanines</b>		
Metalaxyl	<i>Pseudoperonospora</i> sp.	Cucumber
	<i>Phytophthora infestans</i>	Potato
	<i>Plasmopara viticola</i>	Grapevine
	<i>Bremia lactucae</i>	Lettuce
	<i>Phytophthora citricola</i>	Citrus
<b>Dicarboximides</b>		
(Dichlozoline, iprodione, procymidone, vinclozolin)	<i>Botrytis cinerea</i>	on fruits
<b>Triazoles</b>		
(Tridemeton triadimenol and fenpropimorp)	<i>Ustilago avenae</i>	
	<i>Erysiphe graminis f.sp. hordei</i>	Barley
<b>Hydroxy pyridines</b>		
Thiophanate methyl	<i>Botrytis cinerea</i>	Apple, beans and grapes
	<i>Erysiphe cichoracearum</i>	Egg plant
	<i>E.graminis</i>	Citrus
	<i>Penicillium digitatum, P. italicum</i>	Cucumber
	<i>Rhizoctonia solani</i>	
	<i>Sphaerotheca fuliginea</i>	
	<i>Venturia inaequalis</i>	Apple
	<i>Colletotrichum musae</i>	Banana
	<i>Cochliobolus miyabeanus</i>	Rice
<b>Organophosphates</b>		
Edifenphos	<i>Pyricularia oryzae</i>	Rice
Pyrazophos	<i>P.oryzae</i>	Rice

Iprobenphos	<i>P.oryzae</i>	Rice
<b>Oxathiins</b>		
Oxycarboxin	<i>Puccinia horiana</i>	Chrysanthemum
<b>Organicfungicides</b>		
Dodine	<i>Venturia inaequalis</i>	Apple
<b>Pyrimidines</b>		
Dimethirimol	<i>Sphaerotheca fuliginea</i>	
Ethirimol	<i>Erysiphe graminis</i>	
<b>Antibiotics</b>		
Streptomycin	<i>Erwinia amylovora</i>	Apple, pear
Pimaricin	<i>Cladosporium cucumerinum</i>	Rice
	<i>F.oxysporum</i>	
	f.sp. <i>narcisi</i>	Pear
Kasugamycin	<i>Pyricularia oryzae</i>	
	<i>Alternaria kikuchiana</i>	
Polyoxins	<i>A.maci</i>	Apple
Antimycin A	<i>Ustilago maydis</i>	Maize
<b>Dithiocarbamates</b>		
Mancozeb	<i>Bipolaris oryzae</i>	Rice
	<i>Helminthosporium maydis</i>	Maize
	<i>Rhizoctonia bataticola</i>	Many crops
<b>Aromatic compounds</b>		
Dinocap	<i>Sphaerotheca fuliginea</i>	Cucumber
PCNB	<i>Rhizoctonia</i> spp.	Many crops
	<i>Botrytis cinerea</i>	

There are ways and means to preserve the efficacy of systemic fungicides and prevent appearance of resistant strains of the pathogens. Most important is the modification in the method of their use. They are

- i. Use of mixtures of specific systemic and wide spectrum protectant fungicides. e.g., mix with benomyl either captan, dichloran or iprodione for the control of diseases caused by *Botrytis* or *Sclerotinia* strains or mix with metalaxyl either maneb or zineb for control of downy mildews.
- ii. Alternate sprays of systemic with protectant fungicides.
- iii. Spray during half season with systemic and other half with protectant fungicides.
- iv. Avoid cultivation of very susceptible cultivars of host to reduce need for fungicide application.

# 16

## Compatibility and Phytotoxicity

### Compatibility

When two or more chemicals (for example fungicides and insecticides) are mixed to make up a successful spray or dust mixture, they are said to be compatible. In general the active ingredients in a successful combination do not react with each other to destroy effectiveness of either compound, or the chemical reaction is so slow that effectiveness of the combination is not greatly by changed before use. Spray or dust mixtures that cause severe injury on plants are said to be incompatible even though the combination may destroy the pests and diseases.

Very often it is necessary to apply fungicide along with pesticides (insecticides, acaricides etc.) on a particular crop to control the diseases and pest complex. The necessary of mixing fungicide with insecticide or acaricide may also be due to the appearance of pests and diseases together or are may quickly follow the other. Sometimes there is little time available for separate sprays because of short intervals between rains particularly in the monsoon season.

Mixing them together before application of each save the labour, time and expense since the number of applications are reduced. Sometimes mixing two agrochemicals (fungicides with fungicide/ with others) result in increase of the fungitoxicity of certain fungicide. This kind of synergism has been observed with fungicides. The best examples are copper oxychloride with zineb, ziram, captan and wettable sulphur; sulphur with zineb, ziram and copper oxychloride; zineb with captan, folpet and copper oxychloride and captan with copper oxychloride. Synergistic effect has been observed based on laboratory observations and therefore test verifications under field conditions are important . Mixture of fungicides shows synergistic effect only when they are mixed in certain ratios and not otherwise.

When two chemicals are brought together in a single spray mixture, due to reaction, a compound differing from either parent may be formed. A knowledge of the effects of such compounds on the plants when applied is essential to avoid phytotoxicity. The incompatibility in such cases may be

**1. Chemical incompatibility:** Different compounds are formed due to reaction of various material.

**2. Phytotoxic incompatibility :** The component parts though by themselves are not injurious to the plants and do not show any chemical reaction when mixed, the mixture causes injury to plant.

**3. Physical incompatibility :** In which the chemicals used change their physical form to one that is unstable and hazardous for application.

Compatibilities between various agrochemicals are given in the chart.

**Warning :** Do not mix fungicides in wettable powder form with liquid concentrates of insecticides.

Do not mix two concentrates together when mixing a multiple spray , prepare one first to the correct dilution and then add the second concentrate.

Table : Compatibility chart of plant protection chemicals

	Wettable Sulphur	Captan	Zineb, Thiram, Ziram, Maneb	Copper Oxychloride	Bordeaux Mixture	Carbaryl	Dimethoate	Phosphamidon	Malathion	Methyl Demeton	Methyl Parathion
Methyl Parathion	++	++ <sup>5</sup>	++	++	- <sup>4</sup>	++	++	++	++	++	
Methyl Demeton	++	++	++	++	- <sup>4</sup>	++	++	++	++		++
Malathion	++	++ <sup>5</sup>	++	++	-	++ <sup>6</sup>	++	++		++	++
Phosphamidon	++ <sup>5</sup>	++	++	++	- <sup>4</sup>	++	++		++	++	++
Dimethoate	++	++	++	++	-	++ <sup>6</sup>		++	++	++	++
Carbaryl	++	++	++	++	-		++ <sup>6</sup>	++	++ <sup>6</sup>	++	++
Bordeaux Mixture	++	+1	+ <sup>2</sup>	++		-	-	- <sup>4</sup>	-	= <sup>4</sup>	- <sup>4</sup>
Copper Oxychloride	++	+1	+ <sup>3</sup>		++	++	++	++	++	++	++
Zineb, Thiram, Ziram, Maneb	++	++		+ <sup>2</sup>	+ <sup>2</sup>	++	++	++	++	++	++
Captan	++	-	++	+ <sup>1</sup>	-	++	-	++	++	++ <sup>5</sup>	+ <sup>5</sup>
Wettable Sulphur		++	++	++	++	++	++	+ <sup>5</sup>	++	++	++

++ = Safe

+ = Caution

- = Incompatible

1. Not usually mixed together or compatibility not known.
2. When mixed with water decomposes after standing. With a ziram mixture, adding 500 gm skim milk to 500 litres of spray may prevent decomposition.
3. Not recommended, except as directed by manufacturer. Presence of calcium compound may change residual fungicidal nature of dithiocarbamates to eradicant type without residual action.
4. Bordeaux mixture may be used with guthion or parathion on grapes with caution No.2.
5. Use wettable powder forms only.
6. Combinations of carbaryl and malathion may cause injury to cotton .

### **Phytotoxicity**

# 17

## Evaluation of Fungicides

Fungicides are evaluated to know the efficacy of fungicides to control the pathogen and disease, for comparing the efficacy of different fungicides.

The following are the commonly used methods for testing the efficacy of the fungicides.

- i. Slide germination method
- ii. Test tube dilution method
- iii. Poisoned food technique
- iv. Inhibition zone technique.

### **i. Slide germination method**

Fungicides to be evaluated are prepared in different concentrations namely 100ppm, 500ppm and 1000ppm. Spore suspension of the test fungus is also prepared (50,000 spores of conidia/ml). Two drops of fungicidal solution and two drops of spore suspension are pipetted into the cavity of the cavity slide. Separate slides with a different concentration of fungicide with spore suspension are prepared separately. They are mixed. The slides are kept in moist chamber and incubated at 24-25°C. Required replications are made to minimize the error. After 6, 12 and 24 hrs of incubation, each slide is observed under microscope for the number of spores / conidia germinated. Sterile distilled water (instead of fungicidal solution) serves as control. The total number of spores in each slide and number of spores germinated are counted and the germination percentage is calculated. Dosage response curve / toxicity curve is prepared. The per cent inhibition of spore germination is plotted on 'Y' axis against the concentration on the 'X' axis. The unit for comparison is LD<sub>50</sub> value, the dose that inhibits germination of 50 per cent of the spores.

### **ii. Test tube dilution method**

It is also a slide germination technique. Two ml of each concentration of the test fungicide is taken in a test tube. The germination stimulant like sucrose orange juice is added to the spore suspension in equal volume. 0.5 ml of spore suspension is added to the fungicidal solution in the test tube, mixed well. Two drops from each test tube is taken and placed on a glass slide and incubated. A control is maintained using sterile distilled water. Germination count is made and the dosage response curve is fitted. LD<sub>50</sub> value is found out from the graph.

### iii. Poisoned food technique

In this technique the medium containing the nutrients is poisoned with the fungicide and the test fungus is allowed to grow. In this technique either solid medium or liquid medium may be used.

**a. With solid (agar) medium:** Potato dextrose agar (PDA) is prepared as given below and obtained by using usual sterilizing procedures.

Potato	-	250 g
Dextrose	-	20 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	7.0

PDA medium is prepared in flasks and sterilized. Different concentrations of fungicide are prepared by mixing the required quantity of fungicide with the agar medium (100, 250, 500 and 1000 ppm). The fungicide is thoroughly mixed by stirring. The medium is poured into sterilized petriplates and allowed to solidify. A disc of 9mm dia of test fungus grown on a solid medium (for a period of 7 days) is cut with the help of a sterilized cork borer and is placed aseptically in the centre of the petriplates containing the poisoned medium. Suitable checks (control) are kept where the fungal culture disc is grown under the same conditions on PDA without the test fungicide. The petriplates are incubated at room temperature for 7 days. The colony diameter in each concentration is compared with the check and the minimum inhibitory concentration of the fungicide is determined.

**b. With liquid medium:** Any fungus which can grow on a liquid medium can be used as a test fungus. Generally Richard's medium (without agar) is prepared in conical flasks, sterilized and used. Different concentrations of the fungicide are prepared by adding the required quantities to the medium. A disc of 9mm dia of fungal growth (7 days old) on solid medium is cut with a sterile cork borer and transferred to the medium @ one fungal disc / flask of each concentration of the fungicides. The fungal discs grown in Richard's medium without the fungicide serve as control. The mycelial mat is removed after 7 days of incubation by filtration. It is placed in a hot air oven and dried and the dried weight is determined. The minimum inhibitory concentrations of the fungicide are worked out.

### iv. Inhibition zone technique

It is also known as paper disc plate method. Spore suspension of the test fungus is prepared with sterile distilled water from 7 day old culture on a solid medium. Different concentrations of the fungicide



namely 100,250, 500 and 1000 ppm are prepared. Twenty ml of the PDA medium is poured into a Petri dish and 3ml of the spore suspension ( $1 \times 10^6$  spores/ml) is added and rotated clockwise and anticlockwise direction for thorough mixing. Petri dish is allowed to solidify. Then Petri dishes are frozen to condense water. Different concentrations of the fungicide are prepared and filter paper of 10-12 mm dia is dipped in the fungicide solution. The filter paper is then placed at the centre of the seeded medium. The petriplates are incubated at 28-30°C for 24-48 hours. Inhibition zone of the fungal growth (diameter) around the treated disc is measured in centimetres. The paper discs dipped in the sterile water serve as control. This method is also used for evaluating the efficacy of different antibiotics against fungi and bacteria. If it is against bacteria nutrient agar medium is used in the testing method.

### **Evaluation of soil fungicides**

Air-dried soil is sterilized in an autoclave for a period of 1 hr at  $1.15 \text{ kg/cm}^2$ . The sterilized soil is placed in a glass jar / beaker to a height of 2.5 cm. A fungal disc of 10mm dia from the outer margin of the PDA culture is cut with sterile cork borer, removed and placed on the top of the soil. It is covered with another 2.5cm layer of sterilized soil. 5 ml of the fungicidal solution of a known concentration is taken in a pipette and added on the soil surface. The glass jar/beaker is incubated at 25°C for 24 hrs. Fungal disc placed in the sterilized soil at a depth of 2.5 cm in a column of 5.0 cm. Soils without adding the fungicidal solution serves as control. After the incubation period the disc is removed by sieving the soil. The disc is washed with sterile water to remove the adhering soil. The discs of mycelium is picked out with sterile forceps and placed on the Petri dish containing PDA medium. The growth of the fungus is observed and is compared with the growth from the disc removed from the sterilized soil without the fungicide. The efficacy of fungicides at different concentrations is worked out.

### **Evaluation of systemic fungicides**

The systemic fungicides are evaluated to find out the penetration and translocation in the plant system to the locus of infection. Translocation of fungicides in plants may be,

- i. upward translocation (apoplastic movement) or
- ii. downward translocation (symplastic movement) or
- iii. Amphimobile translocation

Apoplastic fungicide is transported in the direction of the transpiration stream with long distance transport occurring in the xylem (upward translocation). Symplastic fungicide is transported in the direction assimilate movement with long distance transport occurring in the phloem (downward translocation). Some fungicides are transported by the above two ways and are termed as amphimobile.

**i. Test for upward translocation (apoplastic movement)**

Different concentrations of a systemic fungicide are prepared in sterile water in a conical flask / test tube. The seedlings are pulled out carefully and the roots are washed free of soil using sterile water and immersed in the known concentration of the fungicidal solution kept in conical flask / test tube for a period of 2 hours. The flask / test tube is covered with a black paper to prevent the roots becoming green. The top portion of the seedlings are cut into bits and placed in the seeded agar medium. The upward translocation of the fungicide is assayed following previously described inhibition zone technique. The bits from the top portion of the seedling dipped in the sterile water serve as control.

**ii. Test for downward translocation (Symplastic movement)**

Different concentrations of the systemic fungicide solutions are prepared using sterile water. Seedlings are raised in the pots. Seedlings with 4-5 leaves stage are sprayed with the test solutions separately. The seedlings are incubated at room temperature for 24 hrs. The plant parts (root, stem and leaf bits) washed in sterile water and placed on seeded agar medium. Inhibition zone method is followed in testing the efficacy. At the end of the incubation period the inhibition zone in each type of plant parts (root, stem and leaf) is recorded. The seedlings sprayed with sterile water serve as control. The symplastic movement is shown by the formation of inhibition zone around the root bit in the agar medium.

**iii. Amphimobile translocation**

The amphimobile nature of the systemic fungicide is explained by the upward movement of the chemical when the seedlings are dipped in the fungicidal solution and by the downward movement of the chemical when the seedlings are sprayed on the aerial parts. Inhibitory zones are observed in the aerial parts and in the roots respectively.

# 18

## Methods of Application

Proper selection of a fungicide and its application at the correct dose and at the proper time are essential for the effective management of plant disease. In the application method the fungicide is delivered to the target (plant parts) where the active ingredient acts. This is mostly achieved generally by spraying, dusting, granule application. Seed or soil is also treated with the chemicals. In addition, some trees and shrubs can be protected by injection of fungicide liquid into the trunk or by brushing wounds with fungicide paste or slurry. Fungicides are applied through roots or applied in the corm or pseudostem. In the case of sprays, mists, aerosols and fogs the fungicide is in the droplets of water or another fluids. In the case of dusts and granules, the fungicides is straightly mixed with an inert carrier, impregnated into it or coated on the particles which are applied mechanically.

The object of spraying or dusting is to cover the entire susceptible surface of host with a thin covering of a suitable concentration of the fungicide before the pathogen has come in contact with the host. The fungicidal application varies according to the nature of the host part diseased and nature of survival and spread of the pathogen.

### **1. Foliar application**

#### **i. Spraying**

This is the universal method to apply fungicides on the foliage. Spraying of fungicides is done on leaves, stems, inflorescence and fruits. Spraying gives better coverage on the plant surface. Loss due to drift and pollution risk is very less. It has long residual effect. Spraying can be done at relatively high wind velocities. Spraying leads to less hazards to the operators than dusting. Wettable powders and emulsifiable concentrates are commonly used for preparing spray solutions. The most common diluent or carrier is water. The dispersion of the spray is usually achieved by its passage under pressure through nozzle of the sprayer. The amount of spray solution required for a hectare of land depends on the nature of crops to be treated. For trees and shrubs, more amount of spray solution is required. Depending on the volume of fluid used for coverage, the sprays are categorized into high volume, medium volume, very low volume and ultra low volume (ULV).

Type of spray	Quantity of spray solution required (litre/ha)	
	Ground crops	Orchard crops/Trees
High volume	700	1200
Medium volume	400 to 700	800 to 1200
Low Volume	225 to 400	350 to 800
Very low volume	60 to 225	250 to 350
Ultra low volume	20	50

### Preparation of spray solution

Usually wettable powders or emulsifiable concentrates are diluted with water and the resultant solution is used for spraying. A known quantity of the fungicidal formulation is taken in a container to which a small quantity of water is added and mixed. Then the required quantity of water is added to make the required concentration and stirred well. It is taken in the sprayer and applied either in the morning or in the evening. For crops which have glossy leaf surface (banana) or hairy leaf surface (sugarcane, rice, etc) spreading agents like soap (khadi soap at 1g/litre of spray solution) or Teepol (1ml / litre ) is added in the spray solution. The required concentration of the fungicide should be prepared. If it is less than the required concentration the targeted disease(s) is not effectively controlled and if it is more, the chemical may cause phytotoxicity to the crop. Generally the following concentrations are used for the management of plant diseases.

Fungicide	Concentration recommended	Chemical required per one litre of water
Carbendazim	0.05%	0.5g
	0.1%	1.0g
Mancozeb	0.2%	2.0g
Copper oxychloride	0.25%	2.5g
Wettable sulphur	0.3%	3.0g

### Requirement of chemical

For spraying one acre of field crop generally 200 litres of spray solution (500 litres/ha) is required. The requirement of chemical for this area varies with the concentration of the solution and number of sprayings recommended. For e.g for two spraying in 2.0 ha of crop area with carbendazim 0.1% the following is the calculations for the fungicide requirement.

e.g. carbendazim 1g/litre of water	0.1%
500g/500 litre of water/ha.	0.1%
For 2.0 ha. = 2 x 500g / 2 x 500 l of water	0.1%
= 1000/1000l of water	

For 2 sprays in 2.0 ha.                    =            2 x 1000g/2x1000 l of water  
   =            2000g/2000 l of water  
   2.0 kg of carbendazim is required.

## **ii. Dusting**

Dusting requires less labour for the operation. Dusting covers more area in a day than with spraying. Dusting equipments are lighter in weight and less risk of corrosion. Dusts are applied to all aerial parts of a plant as an alternative to spraying. Dry powders are used to cover the host surface. Generally dusting is practiced when the plant surface is wet with dew or rain drops. Dusting is made during early hours in a day. Dust is applied at the rate of 25 kg/ha e.g. Sulphur dust to control powdery mildew diseases of different crops in rainfed areas. The equipments employed for the dusting operation are bellows duster, rotary duster, motorized knapsack duster and aircraft.

## **2. Seed treatment**

The seed treatment with fungicides is essential since the pathogens are carried on or in the seed. Seed treatment protects the germinating seeds and growing seedlings from soil-borne pathogens. Seed treatment is an effective and economic method of disease control. Seed treatment is therapeutic when it kills pathogens that infect embryos, cotyledons or endosperms under the seed coat, eradicated when it kills pathogens that contaminate seed surfaces and protective when it prevents penetration of soil-borne pathogens into the seedlings. Seed treatment methods are broadly divided into three categories.

- i. Mechanical methods
- ii. Chemical methods
- iii. Physical methods.

### **i. Mechanical methods**

Some pathogens attack the seeds and reduce the size, change the shape and reduce the weight of seeds. Hence it is possible to detect the infected seeds and separate them from the healthy ones. In the case of ergot disease of pearl millet, sorghum and rye the sclerotia are usually larger in size and lighter in weight than healthy grains. So by sieving or floatation, the infected grains may easily be separated. Such mechanical separation eliminates the infected materials from the seeds. This method is also useful to separate “tundu” diseased seeds of wheat.

**Method of removal of ergots in pearl millet seeds:** Dissolve 2 kg of common salt (Sodium chloride) in 10 of water (20% solution) and take it in a vessel. Drop the seeds into the salt solution and stir well. Remove the ergot affected seeds and sclerotia which floats on the water surface.

Wash the seeds in fresh water 2 or 3 times to remove the salt on the seeds. Seeds are dried in the shade and used for sowing.

## **ii. Chemical methods**

Using fungicides on seed is one of the most efficient and economical method. Seed treatment chemicals may be seed disinfectant or seed protectant.

**Seed disinfectant:** Seed disinfectant, disinfects the seed but may not remain active for a longer period after the seed has been sown.

**Seed protectant:** Seed protectant, disinfects the seed surface and stick to the seed surfaces for sometimes after the seed has been sown, and thus giving temporary protection to the young seedlings against soil-borne fungi. Systemic fungicides protect deep seated infections in the seed.

The seed dressing chemicals may be applied by,

- a. Dry treatment
- b. Wet treatment
- c. Slurry

### **a. Dry seed treatment**

In this method, the fungicide adheres in a fine form on the surface of the seeds. A calculated quantity of fungicide is applied and mixed with seed using machinery specially designed for the purpose. The fungicides may be treated with seeds of small lots using simple rotary seed dresser (Seed treating drum) or of large seed lots at seed processing plants using grain treating machines. Normally in field level, dry seed treatment is carried out in rotary seed treating drums which ensures proper coating of the chemical on the surface of seeds. Dry seed dressing method is also followed in pulses, cotton and oilseeds with the antagonistic fungi like *Trichoderma viride* at the rate of 4g/kg of seed.

**Dry seed treatment in rice:** Mix a required amount of fungicide with the required quantity of seeds in a seed treating drum or polythene-lined gunny bag to provide uniform coating of the fungicide over the seeds. Treat the seeds 24 hr prior to soaking for sprouting. Thiram or captan or carboxin or Tricyclazole or carbendazim is treated at 2g /kg of seed.

### **b. Wet seed treatment**

This method involves preparing fungicide suspension in water and dipping the seeds or seedlings or propagative materials in it for a specified time.

**Seed dip/Seedling dip:** Seed soaking is done to certain crop seeds. Here the fungicide adheres as a thin film over the seed surface which gives protection against invasion by soil-borne pathogens.

**Seed dipping in rice:** Fungicidal solution is prepared by mixing any of the recommended fungicides viz., carbendazim or pyroquilon or tricyclazole at 2g/litre of water and seeds are soaked in the solution for 2 hrs. Drain the solution and keep the seeds for sprouting.

**Seed dipping in wheat:** Prepare 0.2% solution of carboxin (2g/litre of water) and soak the seeds for 6 hrs. Drain the solution and dry the seeds and sow. It eliminates the loose smut pathogen, *Ustilago nuda tritici*.

**Seed dip/root dip :** The seedlings of fruits and vegetables are normally dipped in 0.25% copper oxychloride (2.5 g /litre of water) or 0.1% carbendazim solution (1g/litre of water) for 5 minutes to protect against seedling blights and rots.

**Rhizome dip:** The rhizomes of cardamom, ginger and turmeric are treated with 0.1% Emisan solution for 20 minutes to protect them from the attack by the pathogens present in the soil.

**Sett dip/sucker dip:** The setts of sugarcane and tapioca and suckers of pineapple are dipped in 0.1% Emisan solution or 0.1% carbendazim solution for 30 min.

**c. Slurry treatment (Seed pelleting)** In this method, chemical is applied in the form of a thin paste (active material is dissolved in small quantity of water) to the seed. The required quantity of the fungicide slurry is mixed with the specified quantity of the seed so that during the process of treatment slurry get deposited on the surface of seeds in the form of a thin paste which later dries up. Seed processing units have usually slurry treaters, which mix the fungicide slurry with specified quantity of seeds before the seed lot is bagged.

**Seed pelleting in ragi:** Mix 2.5g of carbendazim in 40 ml of water and add 0.5g of gum to the fungicidal solution. Add 2 kg of seeds to this solution and mix thoroughly to ensure an uniform coating of the fungicide over the seed. Dry the seeds under shade and sow after 24 h after treatment.

**Acid-delinting in cotton:** This treatment helps to kill the seed-borne fungi and bacteria in cotton. The seeds are treated with concentrated sulphuric acid @ 100 ml/kg of seed for 2-3 min. The seeds are then washed 2 or 3 times thoroughly with cold water and shade dried. After drying, they are again treated with captan or thiram @ 4g/kg of seed before sowing.

### **iii. Physical methods**

The physical agents like hot water or hot air or steam is used to eliminate the seed-borne infection. They are employed successfully in controlling internally seed-borne disease like loose smut of wheat and systemically infected diseases caused by virus and phytoplasmas.

**a. Hot water treatment:** The seeds are soaked in cold water at 20 to 30°C for 5 hr to induce the dormant mycelium to grow. Then the seeds are immersed in hot water at 50-54°C for 10 min to kill the mycelium. It is very effectively used to eliminate loose smut of wheat. The setts of sugarcane can be treated at 50°C for 2 hr to eliminate grassy shoot pathogen (Phytoplasma). The main drawback in the hot water treatment is that the seeds may be killed or lose its germinability, if the period of treatment exceeds the specified time.

**b. Hot air treatment (HAT) :** Sugarcane setts are treated with hot air at 50°C for 2 hr to eliminate sugarcane mosaic virus.

**c. Aerated steam therapy (AST):** Sugarcane setts are exposed to aerated steam at 50°C for 3 hr to eliminate sugarcane mosaic virus.

**d. Moist hot air treatment (MHAT):** It is used in sugarcane to eliminate grassy shoot phytoplasma. Initially the setts are exposed to hot air at 54°C for hrs, then exposed to aerated steam at 50°C for 8 h and finally to moist hot air at 54°C for 2 hr.

**e. Solar heat treatment (SHT) :** As the thermal death point of the fungus and the embryo are very close, the extensive care should be taken to avoid killing of the embryo. Luthra in 1953 in India devised a method to eliminate the deep seated infection of *Ustilago nuda tritici*. This method is popularly known as solar heat or solar energy treatment.

**Method:** The seeds are soaked in cold water for 4 hr in the forenoon on a bright summer day followed by spreading and drying the seeds in hot sun for 4 hr in the afternoon. Then the seeds are again treated with carboxin or carbendazim at 2g/kg and stored. This method is highly useful for treating large quantities of the wheat seed lots.

### 3. Soil treatment / soil application

In soil dead organic matter supports active or dormant stages of pathogens. soil treatment is largely curative in nature as it mainly aims at killing the pathogens in soil and making the soil safe for the growth of the plant.

#### i. Physical methods

**a. Soil solarization:** It is generally used for controlling soil-borne pathogens like *Pythium*, *Verticillium*, *Rhizoctonia* and *Fusarium* sp. in small areas like nurseries. Irrigate the nursery bed to moisten the soil to a depth of 10 cm. Cover the bed after 2 days with thin transparent polyethylene sheets for 4-6 weeks and then irrigate beds once in a week. The purpose of irrigation is to increase the thermal sensitivity of resting structures of fungi and to improve heat conduction.

**b. Steam sterilization:** It is mostly practiced under glass house and green house conditions. Steam is passed through perforated pipes at a depth of 15 cms to sterilize the upper layers of soil.



**c. Hot air sterilization:** Hot air is passed through pipelines to sterilize the soil in the nursery areas.

**d. Hot water treatment:** It is mainly done in pot culture studies to kill the fungi and nematodes. The pots containing soil are immersed in boiling water at 98°C for 5 minutes or drenching boiling water @ 20 m<sup>2</sup>.

## **ii. Chemical methods**

Chemical treatment of the soil is comparatively simple, especially when the soil is fallow as the chemical is volatile and disappears quickly either by volatilization or decomposition. Soil treating chemicals should be non-injurious to the plants in the soil adjacent to the area where treatment has been carried out.

Methods of soil treatment are

- a. Soil drenching
- b. Broadcasting
- c. Furrow application
- d. Fumigation and
- e. Chemigation

**a. Soil drenching:** It is followed for controlling damping off and root rot infections at the ground level. Required quantity of fungicide suspension is applied per unit area so that the fungicide reaches to a depth of at least 10-15 cm. It is applied with rose can or through sprayer after removing nozzle.

**b. Broadcasting:** It is followed in granular fungicides wherein the pellets are broadcasted near the plant.

**c. Furrow application:** It is done for the control of diseases where the direct application of the fungicides on the plant surface leads to phytotoxicity. For the control of powdery mildew of tobacco, sulphur dust is applied in the furrows and not on the foliage.

**d. Fumigation:** Volatile (fumigants) toxicants such as methyl bromide, chloropicrin, formaldehyde and vapam are the best chemical sterilant for soil to kill fungi and nematodes. Soil fumigants penetrate the soil efficiently. Fumigations are normally done in nursery areas and in glass houses. The fumigant is applied to the soil and covered by thin polythene sheets for 5 to 7 days and removed. Eg. formaldehyde is applied at 400 ml/sq.m. The treated soil is irrigated and used for sowing / transplanting 2 weeks after treatment. Vapam is normally sprinkled on the soil surface and covered. Volatile liquid fumigants are also injected to a depth of 15 cm using soil injector guns.

**e. Chemigation:** The fungicides are directly mixed in the irrigation water. It is normally practiced when sprinkler or drip irrigation system is followed in a field or garden.

#### 4. Post harvest application

Fruits and vegetables are largely damaged after harvest mostly by fungi and bacteria. The spoilage by these pathogens can be reduced by refrigeration. But it is an expensive method of management and will be useful only in certain fungal diseases. Fungi like *Penicillium* and *Botrytis* are not affected by refrigeration. Hence use of chemical method is found to be economical and effective. Fumigation, dipping them in solutions and wrapping of the harvested products with fungicide impregnated waxed papers are the important methods adopted in control of post harvest diseases. Fumigation is followed in limited cases. Further fumigants with the desirable qualities like quick volatility, highly effective against micro organisms, non-phytotoxic and non-tainting in nature are very few. Commercial fumigant commonly used for this purpose is sulphur dioxide. Tetrachloroethylene, dichloromethane, ammonia, nitrogen trichloride are also used as fumigants. Dipping the fruits in fungicidal solution is better than in suspension. In suspension the fungicide deposits on the surface of fruits. Borax (sodium tetraborate) solution was specifically used for treating oranges at 6 to 8% concentration. But borax had some undesirable qualities like crystallization after dipping and accumulation of boron in the peel even after washing. Later, in its place sodium -D-phenylphenate solution with 1% hexamine was suggested for oranges. It is also used for apples also. A dip in Dichloran (2,6-dichloro 4 nitro aniline) 0.1-0.2% aqueous solution for 1 or 2 min is effective against *Rhizopus* rot of peaches. Wrapping citrus fruits with waxed paper impregnated with diphenyl or phenylbenzene or lining the packing container with the paper are followed in fruit industry to avoid *Penicillium* spp. infection. The wrapped fruits leave a slight tint on the fruits. But it disappears when the treated fruits are exposed to air for few days. Of late post harvest treatment of fruits and vegetables with systemic fungicides like benomyl or thiabendazole is being practiced.

#### 5. Special methods

##### i. Corm injection

Corm injection with the fungicide carbendazim is followed to control the dreadful disease of banana namely, Panama wilt (*Fusarium oxysporum* f.sp. *cubense*). Carbendazim is applied either in the form of 2% solution or in the form of capsule.

**Capsule application method:** Carbendazim 50% WP 50 to 60 mg is taken in a gelatinaceous capsule. The soil above the corm in the banana tree is removed to expose the corm. A capsule applicator made of iron is employed to make a hole to apply the carbendazim capsule. Capsule applicator is made with 7mm thickness iron rod. The length of the rod is 45 cm. One end of the rod is sharpened. A iron plate is welded 7 cm above the pointed end for the purpose of inserting the rod at a constant

depth of the corm. At the top of the iron rod a wooden handle is fixed. The applicator is inserted upto the iron plate into the exposed corm at an angle of 45° to make a hole. Carbendazim capsule is pushed to the hole and covered with clay soil. The corm is again covered with the removed soil.

**Application of carbendazim 2% solution:** Two per cent solution of carbendazim 50% WP is prepared by dissolving 20 g of carbendazim in one litre of water. A whole in the banana corm is made by following the method in the above capsule application. Three ml of carbendazim 2% solution is injected into the hole through syringe and the hole is plugged with clay soil.

## **ii. Root feeding**

Root feeding with the antibiotic, Aureofungin-Sol + copper sulphate is employed in the control of basal stem rot (Tanjore wilt or *Ganoderma* wilt) of coconut caused by *Ganoderma lucidum*. Tridemorph (Calixin) can also be used at 2ml/100ml water. When tridemorph is used need not be added. It is an improved and effective method compared to trunk injection, which is an old and dangerous method. which may cause even copper sulphate death of the tree?

**Root feeding in method:** One pencil thickness, active coconut root is selected and exposed outside from the soil by removing a layer of soil and is cut at a distance of 60 cm from the tree. A slanting cut is made at the tip to have more surface area for absorption. Root feeding chemical is prepared as follows:

Aureofungin-Sol	- 1.3 g
Copper sulphate	- 0.5 g
Water	- 100 ml

Copper sulphate is powdered well and it is dissolved in 100ml of water along with the Aureofungin-Sol purchased in the market. The solution is taken in a polythene bag. The tip of the root is inserted into the polythene bag containing 100ml of the fungicidal solution. Later the mouth of the bag is tied tightly with the root keeping the cut end touching the bottom portion of the bag. The solution is normally absorbed within 24 hrs. If it is not absorbed in 24hrs, another healthy root should be selected as done before and the root feeding is given. It is followed thrice in a year for every four months for effective control of the disease.

# 19

## Fungicide Application Equipments

### 1. Sprayers

The important methods of application of fungicide in the field are spraying and dusting. These methods are in vogue for a longer time. Selection of right kind of fungicide application equipments (sprayers and dusters) is an important factor for the success of disease management.

#### Parts of a sprayer

The important parts of a sprayer are:

i. Tank, ii. Agitator, iii. Filter, iv. Pump, v. Nozzles, vi. Pressure chamber, vii. Pressure gauge, viii. Valve, ix. Hose, x. Spray lances, xi. Spray cut-off devices, xii. Boom and xiii. Power source.

**i. Tank:** The spray fluid may be contained in a tank that is built in the sprayer as in the case of any knapsack sprayer or in a separate container as in the case of pedal pump. In manually operated sprayers the tank should be so designed that the operator does not feel any discomfort. Tanks are usually made of galvanized iron or steel coated with anti-corrosion material particularly on the inner side. Tanks from cold rolled brass sheets or copper lined tanks are used to spray of copper fungicides. Tanks with resin bound glass fibres or lined with inert plastic materials are available in the market. It is desirable to have rounded corners and bottom. Its capacity may range from less than one litre as in the case of pneumatic hand sprayers to 10-25 l in knapsack sprayer or mist blower and up to 2700 l in large power driven sprayers. Volume of the spray to be used/volume of the tank will give an approximate idea of the number of fillings needed. The liquid in the tank should be sufficient for operation for at least 15-20 min. spraying. The tank should be provided with a good agitator, a strainer in the filler hole and a drain plug at the bottom for proper mixing, easy pouring of spray fluid and cleaning after use.

**ii. Agitator:** If the spray fluid is a solution of the toxicant it does not settle and hence does not require being agitated. But emulsions slowly settle. But suspensions of wettable powders quickly settle and hence such fluids require being agitated to prevent uneven spray strengths. Most sprayers with built-in tanks have paddle-like agitators that rotate in the tank and keep the material uniformly dispersed. In the case of sprayers with no such built-in tanks the return flow of excess spray fluid from the

pump helps to agitate the fluid in the separate container. Agitators are usually provided in the power sprayers by a return flow pipe as well as separate mechanical agitators.

**iii. Filter / Strainer:** Filters are provided in the sprayers to strain off dirt and coarse particles from entering the nozzle and blocking the flow of spray fluid. Even partial block of nozzles will disturb the spray pattern and distribution. In addition to the coarse particles filter at the entrance to the tank, filters are provided in the line between the tank and the pump and the boom or spray lance and also in individual nozzles.

**iv. Pump:** This is the most expensive and vital component of the sprayer. It is needed for atomization of the liquid through air. While selecting a pump for a sprayer, two factors viz., nature of the spray liquid-its formulation as concentrate, viscosity, corrosiveness and delivery-the time and the pressure at which the liquid has to be delivered ultimately through nozzle are to be considered.

Depending on the mechanism of action the source of power the pumps used in the sprayer are broadly grouped into following categories viz., Pneumatic pumps, positive displacement pumps, Plunger pump or piston pump, Rotary pump, Diaphragm pump and Centrifugal or impeller pump.

**a. Pneumatic pumps or Air pumps :** -It is used mostly in hand compression or pneumatic sprayers. It is used to force air into the airtight tank up to a certain pressure. The compressed air forces the liquid or release of valve, exerts pressure on the liquid and forces it through the nozzle. It is really a force pump.

**b. Positive displacement pumps:** Positive displacement pumps are those which take in a definite volume of liquid from inlet and without possibility of escape transfer it to the outlet.

**c. Plunger pumps or piston pump:** It is used in power sprayer and can generate high pressure up to  $70 \text{ kg/cm}^2$ . It consists of a piston which operates inside a cylinder. In the suction stroke it sucks in the liquid through an inlet valve. This valve closes on the pressure stroke and forces the liquid through the outlet valve into the delivery circuit. Two or three cylinders are commonly used and an air vessel is introduced to even out the pressure. These pumps are stoutly made and consist of a number of component parts. Pistons are fitted with plunger caps(washers) which are of moulded rubber and other materials and they must fit in with or be seated against, the inside of the cylinder. The inside of the cylinder is usually lined with enamel. When muddy water with fine sand and clay is used then there is a rapid wear of the plunger cap with possible corrosion and unevenness of the lining. The capacity of the pump depends on the number of cylinders, their diameter, number of strokes per unit time and length of the stroke. The rate of movement of water is slow and hence these types of pumps are unsuitable for viscous liquids. They are suitable only for high volume spraying.

**d. Rotary pumps:** They have the advantage that they can be directly coupled with the driving shaft of the power source without involvement of the crank shaft needed in a reciprocal pump. Common types used are gear types and roller vane type.

**Gear types:** Here the pumps are fitted with a continuous delivery system which is maintained by movement of a pair of gears running together in mesh in a casing. The liquid enters between the teeth as it comes out of the mesh and is carried round between casing and teeth to be discharged at a later point before the teeth enmesh once more. The pump is made of brass, bronze or high carbon steel and can be operated smoothly only when clean water is available. The use of formulations of wettable powder will cause heavy wear in such pumps. Hence such pumps are not recommended for use with wettable powder. They are used for low volume spraying as the pressure generated is low,  $4.2 \text{ kg/cm}^2$ .

**Roller vane pump** is operated by a single rotor which is eccentrically mounted inside a casing. The casing is divided into a section of vanes into which rollers are fitted and thrown against the casing by centrifugal force. The liquid enters through the inlet part (opening for intake of liquid) and is trapped between the rollers and then ejected through the outlet subjected to pressure being trapped between the rollers. These pumps are simple, but more expensive than gear pumps, but they develop higher pressure than gear pumps ( $8.4 \text{ kg/cm}^2$ ). Most medium output sprayers are fitted with pumps of this type.

**e. Diaphragm pumps:** These operate on the same principle as plunger pumps. The pressure is generated on the rear of the diaphragm by a reciprocating plate which acts like a plunger. The diaphragm is moved up and down a short distance by means of a rod. With the downward movement of the diaphragm a vacuum is created above it and the liquid enters through an inlet valve. The diaphragm is then pushed up and the liquid is ejected under pressure through the outlet valve. Liquid is sealed from moving parts, hence there is little damage due to abrasion, and wear and tear is much less. But these pumps are limited in capacity conditioned by strength and movement of diaphragm. High pressure as obtained in plunger pump cannot be generated in these sprayers fitted with these types of pumps.

**f. Centrifugal or impeller type of pumps:** These take in the liquid at its axis and throw it to the periphery by centrifugal force, where it is delivered. The liquid moves out quickly because of high speed. Due to the absence of reciprocating action liquid discharge is even. Pressure generated reaches up to  $7 \text{ kg/cm}^2$ . Hence these pumps are not suitable for high pressure sprayers. They are more expensive than gear and vane types but easy to maintain.

**v. Nozzles:** A nozzle is strictly the end of a pipe through which liquid can emerge as jet. It is an important part of a sprayer and specially designed aperture to breakup the liquid coming out of the spray tank into

fine droplets. The nozzle helps to control the rate and pattern of distribution. These factors depend on

- a. the nozzle design or type
- b. its operating pressure
- c. the size of the opening
- d. its discharge angle
- e. its distance from the target

These are different types of nozzles devised to suit different types of work like hydraulic energy, gaseous energy, centrifugal energy and thermal energy. But in general there are six basic nozzle types.

Solid stream nozzle

Flat fan nozzle

Hollow cone nozzle

Solid cone nozzle

Atomizing nozzle

Broadcast nozzle

**Solid stream nozzle:** This type of nozzle is used in hand guns to spray a distant target and for crack and crevices treatment in buildings. It is also used in a nozzle body to apply pesticides in a narrow band or inject into the soil.

**Flat fan nozzle:** There are three types of flat fan nozzles: regular, even flat and flooding nozzles. The **regular flat fan** nozzle makes a narrow oval pattern with lighter edges. It is used for broadcast spraying. This pattern is designed to be used on a boom, and to be overlapped 30-50 per cent for even distribution. The **even flat fan nozzle** makes a uniform pattern across its width. It is used for band spraying and for treating walls and other surfaces. The **flooding nozzle** makes a wide-angle flat spray pattern. It works at lower pressures than the other flat fan nozzles. Its pattern is fairly uniform across its width. It is used for broadcast spraying.

**Hollow cone nozzle:** There are two types of hollow cone nozzles: the core and disc and the whirl chamber. The pattern of spray is circular with tapered edges, and little or no spray in the centre. It is used for spraying foliage.

**Solid cone nozzle:** This type of nozzle produces a solid circular pattern. The spray is well-distributed throughout the pattern. It is used for spraying foliage.

**Atomizing nozzle:** This type of nozzle makes a fine mist from liquid pesticides. It is used indoors for special situations.

**Broadcast nozzle:** This type of nozzle forms a wide flat fan pattern. It is used on bloomless sprayers and to extend the effective swath width when attached to the end of a boom.

### **General guidelines for selecting type of nozzle**

Spraying jobs can be done by more than one nozzle type or pattern.

For weed control – regular flat fan, flooding fan, even flat fan, hollow cone.

For disease control – hollow cone, solid cone

For insect control – regular flat fan, hollow cone, solid cone.

To minimize drift – flooding fan, whirl chamber, hollow cone.

Nozzles are available in various materials as given below :

- Brass – inexpensive, wears quickly from abrasion, probably the best material for limited use.
- Stainless steel – will not corrode, resists abrasion, especially if it is hardened.
- Plastic – resists corrosion and abrasion, swells when exposed to some solvents.
- Aluminium – resists some corrosive materials, easily corroded by some fertilizers.
- Tungsten carbide and carbide and ceramic – highly resistant to abrasion and corrosion but expensive.

**vi. Pressure chamber:** The pressure chamber is provided in sprayers operated with hydraulic pumps. It prevents fluctuations in the pressure and, hence affects uniformity in spraying. However, in larger pumps, pulsations in the discharge are overcome by incorporating into them two or more cylinders with separate pistons which work alternately.

**vii. Pressure gauge:** It is an important instrument on a spraying machine. It is fixed on the discharge line. It helps to assess if other parts of the sprayer are functioning correctly and for adjusting the pressure required for the job. The pressure gauge should be so located that it can be seen easily by the operator. Further, it should be connected to the pipeline as near the nozzles as possible.

**viii. Valves:** The valves constitute an important part of a sprayer because they govern the direction of the flow of the spray fluid. They are fitted into the pipe system, so that they allow the liquid to pass in the direction of the nozzles. They are of two main types: ball valves and spring-loaded valves.

**a. Ball valve:** It is more commonly employed in the sprayers. It consists of a ball of metal that fits perfectly on a circular seat when the ball is pressed against the seat, it produces a watertight seal and prevents the return flow of the liquid.

**b. Spring-loaded valve:** It is employed for regulating the flow of the liquid as well as of the air. It may be designed in many ways, but basically it consists of an aperture which is blocked. The piece of the valve producing the blockage is held in position with a spring. This spring can be set in such a way that it will allow the blockage to be raised



slightly when given pressure is applied to it. Thus, below a given pressure, the spray fluid or air is not allowed to pass through the aperture. However, when this pressure is reached, the spray fluid or air passes freely. Valves based on this principle are also used as relief valves. The relief valve is a safety device provided on the pressure or discharge side of the pump, especially in the case of powerful compression sprayers and power-operated hydraulic sprayers.

The pressure at which the safety valve should swing into action can be adjusted by changing the tension on the spring of the valve through the manipulation of the control screw. Tension on the spring increases by its clockwise movement and decreases by driving it anti-clock wise. As the adjustment is made, the effect is seen on the pressure gauge. Once the valve has been set, the excess pressure is relieved by the liquid or the compressed air forcing the valve off its normal position so as to clear the blockage of the aperture by the valve. In the case of hydraulic sprayers, the issuing liquid returns to the tank.

**ix. Hose:** The hose is attached to the sprayer on the one end and the spray lance on the other, using hose couplings and clamps. It should be light, non-absorbent, oil-resistant, durable and flexible. Its bursting pressure should be over three times greater than the spraying pressure. It should not impart friction to the free-flow of liquid through it, because it may result in loss of pressure, though the length and diameter of the hose may also influence such loss. Hoses of synthetic rubber are heavier, less flexible and liable to crack at hose couplings. The other materials used for making hoses are natural rubber, cotton, fabrics, plastic and nylon. The plastic and nylon hoses are more common because they are light and cheap. Nylon-braided plastic hoses are also available.

**x. Spray lances:** The nozzle of a sprayer is usually attached to a brass rod (extension rod, spray lance) of variable designs. The length of the rod varies from 35 to 90 cm. Normally, the spray lance is of seamless construction and can be easily detached. The wall of the tubing used to make the lance should not be less than 0.6 mm in thickness and the internal diameter of the lance should not be less than 6 mm. Usually, the lance has a 120° bend to form a goose neck. It is desirable to have the goose neck detachable, since it makes the sprayer more versatile. Nozzles may be fixed to the spray lance by a screw-thread mechanism. In some cases, the nozzle may form a part of the lance and may also have arrangements for manipulating the plunger rod of the lance, which moves at its distal end the vortex plate of the nozzle up and down. This arrangement enables a quick adjustment of the spray pattern. Such special lances are also known as impeller guns or spray guns. These guns are used for spraying trees, fence rows and buildings.

**xi. Spray cut off devices:** Cut-off devices are provided to shut off the flow of the liquid. They are either spring activated(trigger control) or are operated by a knob.

**xii. Boom:** Sometimes a number of nozzles can be arranged in a horizontal tube called the **boom** or **spray bar**. It is normally coupled with power sprayers. Booms are usually used for treating row crops. To a boom, nozzles may be arranged either singly or in pairs. A number of nozzle placement combinations are available for specific jobs.

**xiii. Power source:** The 2-stroke or 4-stroke internal combustion engines are most commonly used in the power-operated sprayers. Electric motors may sometimes be used to provide power for stationary or semi-mobile sprayers. The source of motive power in the case of tractor-operated sprayers may be the power take-off from the engine of the tractor, provided the tractor has sufficient power for moving itself and the sprayer. Battery-operated light-weight portable sprayers are also available.

### **Types of sprayers**

The spraying machines may be either manually operated or power operated. In either category there is sprayers working with hydraulic pressure or with air (or pneumatic) pressure. In sprayers working with hydraulic pressure, pressure is developed by the direct action of the pump on the spray fluid. This pressure forces the liquid through the nozzle. In sprayers working with air compression system, pressure is developed on the air contained in the spray tank.

**i. Manually operated hydraulic sprayers:** In this category of sprayers, hydraulic pressure is thrust upon the liquid by the hand operated pumps. As a result, the liquid is forced through the nozzle in the form of a spray of droplets, which are mostly 300-400  $\mu$  in dia. Sprayers of this type are high volume, high pressure and suitable or complete coverage of both ground and field crops. Different types of hydraulic energy sprayers commonly used are as follows:

**a. Hand syringe or garden syringe:** It is a single acting pump working on the principle of a bicycle pump. It consists of a cylinder or pump barrel and a plunger or piston. Spray fluid has to be contained in a separate tank. The fluid is drawn either through the nozzle aperture itself or through a separate aperture, provided with ball valve, near the nozzle. The liquid is drawn on the return stroke of the plunger and ejected during the compression stroke. After each ejection, the spray fluid has to be drawn in. The spray is made of large droplets and is just like drenching. It is useful for small scale spraying in kitchen garden and pot plants. It is simple and they may last for years, provided they are well maintained. It is very tiresome to operate this syringe for a longer period. It is difficult to control the rate of application by this sprayer.

**b. Bucket sprayer or stirrup pump:** It may consist either of a double acting pump with two cylinders or a single acting pump with one cylinder. The other parts of the sprayer are the plunger assembly, foot valve

assembly, hose, lance, nozzle, a stirrup and an adjustable foot valve assembly. Plunger assembly has plunger shaft, a handle and a travel-limitation-device. The pump has to be put in a bucket or any container having the spray fluid. In the single acting pump the spray discharge is discontinuous since the fluid is ejected only during the downward compression stroke, while in the double acting pump the discharge is continuous as the fluid is discharged during both suction and pressure strokes. However, in both the cases a continuous pumping is necessary. This type of sprayer is useful for spraying small areas. It is very tiresome to work with it and rate of application cannot be controlled.

**c. Knapsack sprayers:** The type commonly available in India is the lever-operated plunger or diaphragm type. It has a flat bean-shaped tank. The frame of the sprayer is so shaped as to conveniently fit on the back of the operator. The capacity of the container is from 10-14 litres. It is generally made of galvanized iron, brass or stainless steel. It is provided with a double action lever operated pump which may be either inside or outside the sprayer. The operator with his one hand, usually left hand, operates the lever which is extended along the left hand side of the operator. Spray liquid is delivered through the delivery system, consisting of lance and nozzle, which is connected with the pump by a flexible hose. Spraying is done by right hand. Coarse nozzles are normally used to undertake spraying of any type of material. It is preferred for spraying rice crop, low crops, vegetables, tea, coffee and nurseries. Different types of nozzles and tail boom may be fitted to suit desired conditions.

**d. Rocker/Rocking sprayer:** It consists of a pump assembly, a rocking-lever, pressure chamber, suction hose with a strainer, delivery hose, cut off valve and spray lance with nozzle. By rocking movement of the lever, pressure can be built up in the pressure chamber and this helps to force the liquid through the nozzle. There is no built in tank. It can be used for spraying crops like sugarcane, banana, citrus, mango, arecanut, coconut etc., Therefore, a separate spray tank is necessary. A high pressure of 14-18 kg / cm<sup>2</sup> can be built up in the tank. In some, it may be as much as 36 kg.cm<sup>2</sup>. It can, therefore, be used for spraying tall field crops and trees up to 5-m high. Long hose connections up to 30 m are made to one or two outlets. Uniform spraying can be done if sufficient pressure is maintained in the pressure chamber. It needs two persons to operate the sprayer, one for operating the pumping system and another for the application of spray liquid.

**e. Foot sprayer / Pedal pump sprayer:** It consists of a plunger assembly, a stand, a suction hose, a delivery hose, an extension rod with a spray nozzle. etc. One end of the suction hose is fitted with a strainer and the other with a flexible coupling. Similarly, the delivery hose has one end fitted with a cut-off valve and the other with a flexible coupling. It is operated by foot and the principle is the same as in the case of the rocking

sprayer. The pump is fixed on an iron stand, and a pedal attached to the plunger rod operates the sprayer by its upward and downward movements. It does not have a built-in tank. It is used for field crop and fruit trees up to 4 m. in height. It may or may not be mounted on a trolley. Constant pedaling is required for continuous spraying. It develops a pressure of 17-21 kg/cm<sup>2</sup>. It is easy to operate and can be used for spraying tall crops as well as fruit trees up to 4-m high. By using a bamboo support for the spray lance or an extra-long spray lance made of aluminium pipe, this sprayer can be used to spray trees up to 6-m high. According to the requirements, one or two hoses are coupled with the sprayer. One sprayer with a single nozzle can easily treat about 1 ha of a medium-sized crop in a day.

**ii. Manually operated pneumatic sprayers (Compression sprayers) :**

In these sprayers air pressure is employed for forcing the liquid through the nozzle for atomization. The containers of these sprayers should not be filled completely with the spray fluid. Usually, three-quarters of the tank is filled, so that adequate air pressure can be developed over the spray fluid in the tank. It is, however, desirable that the manufacturers should clearly mark the tank to indicate the maximum liquid charge that is recommended. To sustain the pressure, the containers are made of robust material. Also, they have to be made airtight for ensuring efficient operation. These sprayers do not have a provision for agitating the spray fluid. The movement of the operator provides a limited agitation. As such, pesticide formulations requiring much agitation should not be sprayed with these sprayers.

**a. Pneumatic or compression hand sprayer:** This type of sprayer consists of a tank of small capacity varying from 0.5 to 3.5 l. with a pump inside the tank. The tank itself acts as pressure chamber. The outlet pipe is suspended in the liquid in the container, the end running into the bottom, the other end or the outlet terminates in a nozzle. Before spraying, air is forced into the tank by action of the pump till sufficient pressure is built. Release may be made either through a stopcock or trigger. A continuous fine spray is obtained till the liquid is emptied out. Due to the provision of fine nozzles in these sprayers, solutions and emulsions can be effectively applied. Suspensions tend to clog the nozzle system. To prevent clogging, an easily removable strainer is provided. It is meant for small spraying jobs in and around the house, e.g., spraying small flower beds and vegetable plots in kitchen gardens.

**b. Pneumatic knapsack sprayers:** This sprayer works on the same principle as the pneumatic hand sprayer. The capacity of the tank, which is cylindrical, varies between 10 and 20 l.. It has to be fixed on the back, with suitable adjustable straps. A curved backrest is provided for easy carrying of the sprayer. Air is charged into the container or tank by action of the pump and a pressure gauge is provided to indicate the pressure. Air capacity and pressure are usually adequate to discharge the liquid contents

out in the form of fine spray without repumping. For discharge of the spray at constant pressure a pressure regulating valve may be provided. Pressure of 4-5 kg/cm<sup>2</sup> is considered sufficient for the purpose. It is necessary that the containers in such sprayers should be made of robust material and be leak-proof. Before each refilling of the tank, the pressure should be released slowly. These sprayers are used in agricultural crops and mosquito control operations.

**iii. Manually operated mist blowers:** The common flit gun or air atomizer is a small, handy and simple appliance. It works on the principle of air blast breaking up the spray droplets and carrying them to the target. It consists of a simple compression cylinder, usually made of tin, with an air pump. The pump is a flexible leather piston which, when moved up and down allows air to pass into the compression side of the cylinder on the return stroke. Hence valves are not necessary. The outlet of the cylinder is opposed at 90° to another orifice in a tube leading out of the liquid container and thus is modified into a nozzle. The container is a simple can with a filling hole and is usually suspended beneath the outlet and of the cylinder. It may have a capacity ranging from a few millilitres to a litre. On the compression stroke the flow of air draws up the spray fluid from the tank and the opposition of the high velocity air and liquid streams results in atomization into minute droplets of 15-50 microns. Continuous pumping is necessary though the spray is not continuous. These appliances are useful for spray treatment on household spraying against bed bugs, mosquitoes and house flies and for treating individual plants. It is meant for small spraying jobs in and around the house, e.g. spraying small flower beds and vegetable plots in kitchen gardens. Selection of the right kind of application equipment is an important factor for the success of control. The correct usage of equipment and proper maintenance are also important.

**iv. Power operated hydraulic sprayers:** A power operated hydraulic sprayer generally consists of a petrol engine and a framework in addition the other standard components of a sprayer; various types of booms with equidistantly fixed nozzles may be attached with these sprayers.

**a. Stretcher or pole-carried sprayer:** Small portable units are available and may weigh only 20 kg, when empty. They can deliver 23-27 l/min at pressures up to 10.5 kg/cm<sup>2</sup>. The sprayer is fixed on a stretcher-type frame with a pair of handles on each end. Alternatively, the sprayer is provided with lugs or loops for poles for carrying it on the shoulders. Since there is no tank, the suction hose is designed for use with a separate spray-fluid container.

**b. Wheel – barrow sprayers:** The wheelbarrow sprayers are designed to make possible the use of equipment too heavy and bulky to be carried by the operators. The sprayer is mounted on a single wheel or for greater stability on a pair of wheels. The sprayer is usually without a built-in tank which, if present, has generally a capacity of 50-80 litres. A capacity

greater than 160 litres is unusual. The 4-stroke petrol engine provides power for operating the hydraulic sprayer and for driving the sprayer in the field. The small general-purpose sprayers have discharge capacities 7-14 l/min at pressures ranging upto 18 kg/cm<sup>2</sup>. The power is furnished by an air-cooled engine of 1-3hp. The spray fluid in the tank may be mechanically or hydraulically agitated. Standard equipment includes an adjustable hand gun and one or more hoses of lengths up to 18m. Spray booms are available for some models. The height of the booms on such sprayers can be adjusted according to the height of the crop. The provision of hinges at the basal ends of the booms permits their vertical positioning during transportation. The wheelbarrow sprayers are used for spraying field crops, orchards and farm buildings.

**c. Traction sprayers:** The traction sprayer is a mounted row-crop sprayer. Power to drive the pump is supplied by the wheels carrying the machine. Each time the wheel stops and the clutch are disengaged, the power for the operation of the spray pump is cut off. This type of sprayer is, therefore, unsuitable for spraying trees, because a stop must be made at each tree for spraying it properly. To some extent, this drawback can be overcome by providing a pressure chamber. The main advantage of the traction sprayer over machines with an independent engine lies in their cheapness. There is no engine to buy, maintain, or repair. However, these sprayers need a source of power for traction. Horses and mules were used for hauling them in Europe and the USA, but these sprayers have, by and large, gone out of use with the replacement of animal power by tractors on the farms.

**d. Power take-off sprayer/tractor – mounted sprayers:** The tractor-mounted sprayer is attached to the tractor as a single unit and is completely carried on the tractor all the time. It is a power-take-off sprayer, because invariably the power for operating the sprayer is provided by the power-take-off of the tractor moving it. The spray-fluid containers have a capacity up to 680 litres. The plunger pumps are mostly used in these sprayers for developing high pressures up to 42 kg/cm<sup>2</sup>, and usually there are two or more pumps to give high-volume output. The spray fluid can be filled in the mounted tanks or drums with an auto-filling hose that can be suitably coupled with the sprayer.

Tractor-mounted hydraulic sprayers are used with spray lances, spray guns, or spray booms either vertical or horizontal. Usually, these sprayers are multipurpose ones, because their design permits the coupling of any of the said spray attachments. Basically, hydraulic sprayers incorporating blowers are also available. The spray is produced from hydraulic nozzles in the normal manner but is carried to the target by the air.

The use of these tractor-mounted sprayers is limited to low-growing crops, and to the early stages of tall crops. However, these crops must be grown in rows at proper distance. Otherwise, the crop damage

from the use of these sprayers would be excessive. As such, certain changes in agronomic practices may be necessary to permit their use. These sprayers are low-priced and can cover large areas. Further, the use of a tractor-mounted sprayer ensures a better utilization of the tractor and the driver.

The mobility of the modern tractor, and the facility of operating the pump of the sprayer from the power-take-off have, during the past several years, resulted in popularization of this spraying arrangement in countries where tractors have been used in agriculture for a long time. The use of the tractor power has, however, also increased the responsibility of the operator for this maintenance. Possibly, the main objection to the operation of a sprayer from a power-take-off is that the short turns normally required in orchard-spraying cause severe strain on the universal required in orchard-spraying cause severe strain on the universal joints and, sometimes, lead to their breakage.

**v. Power operated pneumatic sprayers:** In these pneumatic sprayers, the engine power is employed for creating a cushion of compressed air over the spray fluid in the tank. The sprayers are available in stretcher and wheel barrow models. Bigger and heavier sprayers meant for being carried on a tractor are also manufactured. Pneumatic sprayers can be used for spraying corrosive liquids, since there is no pump to be subjected to the corrosive action of the spray fluid. However, for such fluids, the containers must be lined with anti-corrosive material.

**a. Portable sprayers:** Compressed air from a single – cylinder air-cooled compressor, which is driven by a V-belt from a small air-cooled engine, is forced into the liquid container. This container holds up to 45 litres of the spray liquid. The containers are strong (of welded construction) and can stand a pressure of up to 14 kg/cm<sup>2</sup>. However, the sprayer is usually operated at a pressure of 5-7 kg/cm<sup>2</sup>. The outlet hose from the spray container may bear a lance or a gun, or may feed the liquid through a small boom. The agitation of the spray fluid in the tank is inadequate. Therefore, they are unsuitable for the application of wettable powders unless they are highly water-dispersible.

**b. Traction sprayers:** Traction sprayers are only a few pneumatic row-crop sprayers, which are powered from the wheels of a frame carrying the sprayer. The traction sprayer consists essentially of a portable compressed-air sprayer, a frame mounted on two ground wheels, a gear case, two pneumatic pumps and a spray boom. The wheels provide the power necessary for working the two pneumatic pumps. The compressed air from the pumps is led into the spray container through a rubber pipe. The capacity of the spray tank is 14 litres. It is provided with a pressure gauge and relief valve. The pressure at which the relief valve opens can be adjusted according to the requirements. A maximum of 2.8 kg/cm<sup>2</sup> pressure can be built up in the tank without the slipping of wheels. The spray tank is connected with a spray boom. Each half of the boom carries

3 nozzles. The position of the nozzles on the boom and the height of the boom from the ground can be varied to suit different row spacing and crop heights. The weight of machine (without beam) is 45 kg, when empty.

A pair of bullocks is employed for pulling the machine which has a draft of about 50 kg. Two rows of plants are sprayed from both the sides in each pass of the machine. It is, therefore, necessary to work only in alternate rows. The sprayer requires one person only for driving the bullocks. It can cover 2.4 to 2.8 ha in a day of 8 working hours.

**vi. Low volume sprayers:** The spray fluid is atomized with the help of an air stream at high velocity and hence they are called blower type sprayers. They are also known as air-blast sprayers, air-flow sprayers, air-carrier sprayers, air blowers, mist blowers, spray blowers, spray dusters etc. In some designs both spray and dust can be applied simultaneously and they are described as spray-dusters or wet dusters.

These sprayers have varying air capacity and velocity ranging from 140 m<sup>3</sup> of air/min. at 240 kmph to over 1600 m<sup>3</sup> of air/min at 160 kmph

**Motorized knapsack sprayer or Knapsack mist blower and duster:** The knapsack mist blower is commonly used in our country. The tank is made of a high density polyethylene and has a capacity of 10 to 12 litres. The fuel tank capacity is 0.75 to 2.27 litres. The blower is light, 12-20 kg inclusive of accessories, and is provided with 1.2 to 3.0 hp petrol engine. Fuel consumption varies from 0.6 to 1.86 litre per ha. About 3.0 ha of crop are covered by a power sprayer in a day of 8 hrs. This with suitable accessories can also be used for dusting and ULV spraying. During dusting the air blast enters into the tank through a tube with several holes.

Hopper made of high density polythene has a capacity of 7 to 12 litres. Besides the hopper there is a small tank of 0.75 to 2.25 l. capacity for fuel. These sprayers are operated by an air cooled engine with a power of 1.2 to 3 hp. These sprayers weigh 7 to 15 kg when empty. The machine is put on a suitable frame which is provided with a shock-proof cushion so that the operator does not feel any inconvenience when the machine is fitted on his back. Spray liquid is blown by an air current produced in the machine. The location of engine throttle on the delivery line enables the operator to control the air velocity. The nozzle is connected with the container through a flexible hose and control of discharge is effected through manipulation of a series of discs or restrictors with different borers. Normal discharge of air is 2.7 to 9.1 m<sup>3</sup>/min at a velocity of 175 to 320 km/h. Discharge rate of the liquid varies from 0.5 to 5 l./min and fuel consumption is 0.6 to 1.86 l. /h. Normally the tank should not be full, but a small space should be left for the air cushion to facilitate the uniform discharge of spray liquid.

**vii. Ultra low volume sprayers:** The pesticide, in ULV formulation, is used undiluted at a quantity less than 6 l/ha and usually at 0.5 to 2.0 l/ha



for field crops. The droplet size varies from 30 to 150 microns. Such small droplets cannot be forcibly propelled over a distance and their distribution, therefore, depends on gravity and air movement. With ULV spraying can be done quickly and in time. On the basis of active ingredient ULV formulations are cheaper than EC formulations. Spray deposits from ULV formulations persist longer than that from emulsions. The major disadvantage with ULV application is with the availability of special ULV formulations of pesticides; the qualities for such a formulation are low volatility, low phytotoxicity and high concentration. The ULV application is made by mist blowers fitted with restrictor nozzle or air craft with special nozzles. With ground spraying equipment for ULV spray an area of 8 ha can be covered in a day as against 3 ha in LV spray with power operated knapsack and 0.5 to 0.8 ha in high volume with manually operated sprayers.

#### **viii. Other sprayers**

##### **a. Electrodyn sprayer**

Sprayers based on the charging of droplets of the material to be dispersed have been developed recently. The operation of an electrodynamic type of sprayer is based on the droplets emerging from the delivery gun with an electric charge. Since the droplets have the same charge, the droplets repel each other causing them to form a reasonably wide spray. The total volume of liquid to be sprayed over a hectare is one litre or even less than one litre. Electrodynamic spraying uses rotary-atomization principle. The principle feature of electrodyn sprayer is the device for providing a high tension voltage in a small space through which the liquids emerge by gravity. The very source of energy in this sprayer is 4 standard torch light batteries with last for about 60.

##### **b. Sprayer for palm trees**

Coconut and palm are tall trees, so spraying insecticides on them is difficult. To overcome the problem a small sprayer consisting of a brass or aluminium container of 3 litres capacity covered with air-tight brackets at the top has been developed. A delivery lance and a nozzle are also attached. The spray fluid is put in the tank and air is compressed by an air pump. The sprayer is hung to the operator's back by straps and he climbs over the trees to apply fungicide.

##### **c. Sprayer for tall trees**

Air blast sprayers (air carrier sprayers) have been developed for applying sprays to tall trees. Air blast sprayers utilize an air stream to carry the droplets, rather than depending upon energy from hydraulic pressure. Consequently, they utilize smaller droplet sizes and obtain adequate coverage with less material per unit area. Their effectiveness depends upon the ability to displace air in all parts of the tree with spray-

laden air from the machine. Deposits on leaf surfaces decrease in proportion to the air velocities. Drift problem with air blast sprayers are comparable with those from aircraft spraying. Most of the large orchard-type air blast sprayers have axial-flow fans with guide vanes to direct the air radially outward through a partial, circumferential slot. Some have 2 opposed axial-flow fans blowing toward each other from either side of the slot. The discharge arrangement is such that one side of 1 row, or the adjacent sides of the 2 rows can be covered as the machine is driven along between the rows. The included angle of delivery on each side should be adjustable to accommodate different sizes of trees.

## **2. Dusters**

Fungicides dusts are made of very fine particles that may pass almost completely through a 325 mesh sieve of 44 micron aperture. Therefore, the dust particles when falling free in air either slowly settle down due to gravity or drift for long distances due to wind. The rate of deposition is directly proportional, and of drift inversely so, to the size of the particle. The settling velocity of the particles is also influenced by the density of the dust diluent and the presence of dust conditioners such as stabilizers and fluffing agents. The ability of dust particles to deposit on plant surface is influenced by the electrostatic charge on dust particles. Leaf surfaces generally have a negative electrostatic potential. The charge on the dusts can be increased by friction or by passing the dust particles through a flow of positive ions. Such charged dusts agglomerate less, adhere to the plant surface better and distribute more evenly on both sides of leaves.

The other significant properties of dusts that affect their storage, application, deposition and adherence are i. the bulk density, indicating the degree of fluffiness, ii. flowability, iii. hardness of particles causing abrasion of equipments, iv. shape of particles, for irregularly shaped particles flow slowly, and v. sorption resulting in caking due to absorption or adsorption of moisture.

Appliances that are used for distributing dust formulation are called dusters. The dusters may be manually operated or power-operated. All machines used for applying dusts consist essentially of a hopper (dust chamber) which usually has an agitator in it, an adjustable orifice or other metering mechanism, and delivery tubes. The rotary fan or a bellows supplies the air stream.

### **i. Manually operated dusters**

#### **a. Package or Container duster**

A package duster consists of a container provided with a rubber or plastic part which, when squeezed with fingers, provides a puff of air that ejects a quantity of dust. The capacity of an appliance may be up to 500 g of dust. Plastic bottles, similar to ones available for applying toilet

powder, are also used. Containers like cigarette tin with holes made in one side can also be used for similar purpose.

#### **b. Plunger dusters**

The plunger duster consists of an air pump of the simple plunger type, a dust chamber and a discharge assembly consisting of a straight tube or a small exit pipe whose discharge outlet can be increased or decreased by moving the lid provided at the end of the dust chamber. The container is generally cylindrical, with a detachable lid, for filling with the dust. In some models, the container is detachable and is coupled with the plunger barrel. In still others, the long cylindrical barrel of uniform width is partitioned internally to form an upper larger one, the plunger barrel, and a lower smaller one, the dust chamber. The air from the pump is directed through a tube into the container where it agitates the dust and ejects it from a discharge orifice or tube. The amount of the dust applied can be controlled by the speed at which the pump is operated.

The main advantage of the plunger dusters is that they are cheap and easy to operate. They are suited for dusting plants in small areas in households and kitchen gardens. They are especially useful for spot treatment in restricted areas, and for controlling ants, poultry pests and pests of farm animals.

#### **c. Bellows dusters**

As the name suggests, these dusters are operated through the expansion and contraction of a pair of bellows (made of leather, rubber or plastic) during which process dust is sucked in and then blown out into the delivery system. The bellows can be worked with a handle just like a blacksmith does. The dust is placed either in the bellows or in a separate container, made of wood, metal or plastic, attached to one end of the bellows. The air current that is created runs to one end of the bellows. The air current that is created runs through the container and drives the dust out through an opening.

#### **d. Rotary dusters**

They are also called crank dusters and fan type dusters. They vary considerably in design and may be shoulder mounted, back mounted or belly mounted. Basically a rotary duster consists of a blower complete with gear box and a hopper with a capacity of about 4-5 kg of dust. The duster is operated by rotating a crank and the motion is transmitted through the gear to the blower. Generally an agitator is connected to one of the gears. An adjustable feeding mechanism is also provided. The air current produced by the blower draws the dust from the hopper and discharges out through the delivery tube which may have one or two nozzles. Rotary hand dusters are largely used in India for dusting field crops, vegetables and small trees and bushes in orchards. The efficiency of these dusters is 1 to 1.5 ha per day.

#### **e. Knapsack dusters**

It is a bellows duster which is designed in the knapsack fashion. The hopper is of larger capacity and may contain 4 to 8 kg of dusting material and is mounted on a frame with straps for being fitted on the back of the operator. A pair of bellows are fixed on the top or back of the hopper and are operated by means of a lever or rod through an upward and downward movement extended on one side of the operator parallel to his body. A rotatable blade agitator also fixed inside coupled with this lever system helps in feeding the dust into the mixing chamber. The delivery system consists of flexible hose from the hopper which is adjusted or controlled by one hand of the operator. Leakage in bellows may make the machines inoperative. Leather bellows are liable to deterioration in moist hot climates due to action of moulds. They may be satisfactorily replaced by plastics specially developed for the purpose. The weight of the assemblage may be reduced by the use of light alloy metals, though it may increase the cost of the machine.

## **ii. Power operated dusters**

Power dusters are provided with petrol driven motor.

### **a. Engine operated power duster**

Engine operated power dusters are normally 1 to 3 hp. air cooled engines are used and the hopper capacity is usually 10 to 20 kg. Small engine operated dusters may be stretcher or wheel burrow type or may also be shoulder mounted. In shoulder mounted or knapsack types, engine and the fan housing are on the chest or belly whereas the hopper is on the back. Power dusters may have a single outlet or may be fitted with a series of four to eight outlets with flexible pipes and fitted with a boom with a large number, as many as 18 nozzles. Dusts can be discharged at the rate of 1 to 9 kg/min. A power duster with one outlet may cover 12 ha/hr. Motorized knapsack sprayers can be conveniently converted into dusters. The same container can serve the purpose of the hopper, only liquid feed tubes need to be replaced by appropriate dust feed tubes. The agitation of the dust may be carried out by diverting a part of the air generated by the fan. Dust flow may be regulated from 0 to 1.5 kg/min by adjustment of multi-hole discs.

Large sized dusters with hopper capacity of 50 to 100 kg operated by more powerful engines (up to 25 hp.) are in use in many countries of the world. These may be mounted on vehicles. In many cases, separate engines need not be provided. There is power take off from the tractors or vehicles on which they are mounted.

### **b. Traction dusters**

These dusters have high efficiency, for they develop enough power to revolve 30-40 cm fan at 2000-3000 rpm, which blows out the dust through as many as eight nozzles mounted on a boom. The fan is driven by the wheel or wheels on which the duster runs and hence the speed of the fan varies according to the rate at which the duster is drawn

through the field. They are mounted on wheeled structures. No separate power source is needed. The movement of the wheel in which the duster is mounted generates sufficient power to drive the fan to carry on dusting. The capacity of the hopper varies from 20 to 45 kg. As operation of the machine is dependent on the speed of the wheel, uniform discharge may not be maintained, in case movement of the vehicles is slowed down or impeded

### **iii. Other dusters**

#### **a. Wet dusters**

Dusts adhere and retain better on wet surfaces. Same will be the effect if wet dust particles are applied to target surfaces. This is accomplished by wet dusters, also called as spray dusters, which discharge simultaneously a fine mist of water or oil emulsion and a cloud of pesticide dust. During the process, the dust particles mingle with spray droplets and get wetted before reaching the target. By this method drift is greatly minimized. But it has been superseded by concentrate spraying.

#### **b. Electrostatic duster**

The objective of charging spray or dust particles is to increase the percentage deposition on plant surface. The electrostatic force generally has no great effect on the large particles and it does not affect the basic trajectory from the application equipment to the target. But if a charged particle reaches the plant, it increases the probability of deposition. Charging dusts has improved the control of insects and diseases on a number of different crops. The increased deposition efficiency, particularly for small particles, would reduce the amount of drift.

### **3. Other equipments**

#### **i. Soil injector / soil gun**

It consists of a cylindrical tank for the liquid fumigant, a pump barrel and plunger assembly, injector nozzle, thrust handle and injection handle. The length of injector nozzles may range from 12 to 22 cm for injecting the fumigant at different soil depths. Holding with thrust handle the equipment is thrust into the soil till the nozzle rod gets into the soil completely; then the injection handle is pressed to eject the calibrated quantity of the pesticide liquid into the soil. In some models the thrust handle is replaced by a foot thrust. Hand operated soil injectors are with 1 to 3.5 litres capacity and they cover about 0.5 ha in a day of 8. They are used to apply liquid nematicides.

Gumming and corrosion may occur if a soil fumigant is allowed to remain in the soil-injecting gun for a long period. It is therefore, advisable that on completing the fumigation work, the tank is emptied by lifting the filter. By depressing the injection handle several times, the fumigant remaining in the lance can also be ejected. Power-driven trailer-type fumigators and tractor-mounted soil-fumigating machines are used for covering extensive areas. They consist of hollow tires which are

drawn through the ground like a harrow. The liquid fumigant is fed into the tines through which it enters the soil.

## **ii. Granule applicators**

They are used to apply granular formulations of pesticides uniformly. There are two types. In one type, there is a plastic hopper of 1 litre capacity from which the granules flow by gravity to a nozzle. The output can be regulated by a variety of discs with apertures of different sizes. The second one is a knapsack type with hopper of 10 litres capacity.

They are used for scattering granules or for application in furrows or lines before or after planting, or for placing granules in leaf axils of plants.

## **iii. Seed treating drum**

It is also known as seed dressing machine. It consists of a drum fitted on a stand. The drum is fitted with a small door in the middle for filling the seed and the fungicide. It can be rotated or turned upside-down with a handle. For a thorough mixing of the fungicide with the seed, the inside of the drum is provided with baffles comprising small iron-plates fixed at right angles to the inner surface of the drum. Such a contrivance ensures a greater mixing and proper coating of the seeds with the chemical. About 30 to 40 rotations of the drum, taking about 2 min, will mix the seeds and the fungicide satisfactorily. The capacity of the drum varies from 20 to 60 kg. One machine can treat 200 to 800 kg of seed per day, depending on the capacity of the drum.

Power-driven models are also available. Such a machine has a treatment output of about 5,000 kg/h. These machines are suited for the large seed farms and for central agencies dealing with seed.

## **iv. Slurry seed treaters**

These machines are used for treating seed with a slurry formulation of fungicide. A significant feature of this machine is that the power-flow synchronizer assures a uniform synchronized cycle of weighing the seed and applying the chemical automatically. Slurry paddles are provided in the machine for agitation so as to prevent sedimentation. The machine is fixed on a ready-made stand and the hopper is larger. The capacity of the slurry tank is 120 litres, the machine is powered by a 3 to 4 hp electric motor or a suitable oil-engine, and the output is 3,000kg/h.

## **Calibration of an application equipment**

The application of the right pesticide at the right time and at the proper rate, is important to prevent contamination of the environment. To get the correct rate, application equipment must be properly adjusted and

operated. Accurate application of pesticides depends on accurate calibration of the application equipment. Calibration means to determine the output of the equipment under controlled and precise conditions. It is simply the adjustment of a machine to make it deliver the right amount of spray on a given area.

### **Calibrating sprayers**

Some of the factors that affect calibration and the rate of application are

**Equipment:** The equipment is designed to do the desired job according to the operator's manual or other information sources. It has to be set up accordingly. Select the correct orifices or nozzles for the flow rate and pressure to be used.

**Pressure in the spray tank:** It should be kept as constant as possible. The pressure on a nozzle or orifice will vary its output rate. A pressure gauge will be helpful but is not usually found on single lance sprayers since the pressure is determined by the spray man. This can be made more accurate by either maintaining the number of pumping made per unit of time, or maintaining the number of pumping in rhythm with the number of steps taken.

**Size of the orifice:** It regulates the amount of fluid passing through the nozzles. Both nozzle orifice and pressure affect the volume of spray material delivered per unit time.

**Spray swath:** It is directly affected by the distance between the nozzle tip and the top of the plant or ground level and on the degree of arm reach and body twisting by the spray man who must try to maintain a uniform spray swath while spraying with a single nozzle sprayer.

**Walking speed:** The speed of walking determines the area covered in a unit of time.

### **i. Procedure for calibrating a knapsack sprayer**

#### **a. Preparation of the sprayer**

- The nozzle should be removed and cleaned, and the sprayer rinsed and filled with clean water.
- Build up pressure and check up for leaks.
- Flush pump, hoses, and lance with clean water while the nozzle and strainers are removed.
- The nozzle and strainers should be replaced and the tank refilled and pressurized.

#### **b. Determination of nozzle discharge**

- Fill the sprayer with clean water and pump for pressure, while keeping it on the ground.
- Dip the nozzle in a bucket or a jar and spray water into the jar for a one minute period. Shut off the valve exactly at the end of one minute.

- Measure the quantity of water collected in a graduated cylinder in litres. This is the nozzle discharge or flow rate expressed in litres per minute.
- Repeat this calibration spraying three times to obtain the average nozzle discharge per minute which should be used in subsequent calculations.

**c. Determination of walking speed**

- Do this in the field planted with the crops that will be sprayed.
- Mark the starting point with a stake.
- Carry the sprayer on the back and operate by pumping while directing lance and nozzle within a spray swath. Walk exactly for one minute while someone else is reading the time on a watch. Walk at a normal and constant speed.
- Mark the stopping point with another stake and measure the distance between the first and second stake in metres.
- Repeat this action three times to obtain the average walking speed.

**d. Determination of the swath**

- Measure the width of the swath sprayed in metres, while keeping the distance between the nozzle and ground level constant. The width of the swath can clearly be seen on an asphalt road surface or on a dry path.

**e. Calculation of area sprayed in one minute**

Area sprayed can be calculated by the following formula

**f. Calculation of application rate**

$$\left. \begin{array}{l} \text{Area sprayed} \\ \text{speed} \\ \text{in one minute (square metres/ minute)} \\ \text{(metres / minute)} \end{array} \right\} = \begin{array}{l} \text{Width of spray} \\ \text{Walking} \\ \text{swath (metres)} \end{array} \times$$

**Example**

Given Nozzle discharge rate	= 0.4 litres / min
Area sprayed / min	= 20 m <sup>2</sup> / min
Area to be treated	= 4000 m <sup>2</sup> (equivalent to 1 ac)

$$\text{Application rate} = \frac{0.4 \times 4000}{20} = 80 \text{ litres ac.}$$

**g. Calculation of number of sprayer loads per hectare**

The following formula is used:

$$\begin{array}{l} \text{No. of loads} \\ \text{per hectare} \end{array} = \frac{\text{Rate of application per hectare}}{\text{Tank capacity of sprayer}}$$



Example : Given from the above example

Application rate

per hectare =  $80 \times 2.5 = 200$  l/ha.

Tank capacity = 20 litres.

$$\text{Number of sprayer loads} \frac{200}{20} = 10 \text{ loads / ha.}$$

#### **h. Calculation of the amount of pesticide to mix in each sprayer load**

The following equation is used. The amount equation is used. The amount of pesticide given in whatever units it was calculated, divided by the number of sprayer loads.

$$\begin{array}{lcl} \text{Amount of} & & \text{Amount of pesticide per area} \\ \text{pesticide per load} & = & \frac{\text{-----}}{\text{No. of loads per area}} \end{array}$$

#### **ii. Calibrating dusters and granular applicators**

- Fill each hopper to an easily determined level.
- Operate the equipment over a measured area or distance at normal walking speed. The area should be large enough to use  $\frac{1}{4}$  of the hopper contents.
- Refill the hopper to the same level, weighing the amount of pesticide needed to replace what was used.
- The amount of pesticide it takes to refill the hopper is the amount applied to the measured area. If the amount applied does not fall within 5 per cent of the recommended dosage per unit of area, reset the gate opening and repeat the previous three steps.
- Keep a record of the area treated with each filling of the hopper. This will indicate any slight change in rate of application and make the necessary adjustment.

#### **Pesticide calculations**

The success of spraying operation, whether you are spraying small areas or large fields, depends upon accurate control of the application rate. After the equipment is accurately calibrated to apply the volume of spray desired, it must be determined how much chemical to put into the tank to apply the correct dosage recommended. Before we can make pesticide calculations, the following four factors must be known:

- The recommended rate in kg. a.i. or litres a.i. or litres a.i. per hectare or per cent spray concentration to be applied.
- Amount of spray solution per hectare when applying sprays.
- The per cent active ingredient (a.i.) of the insecticide in the commercial formulation.
- Area in hectares to be treated.

### i. Calculations for foliar spraying

Foliar spraying is the most common form of insecticide application in field crops and orchards. The most common formulation types used for foliar sprays are wettable powders (WP) or emulsifiable concentrates (EC).

#### a. Example in solving a problem for emulsifiable concentrates

This example applies when a certain spray concentration is recommended for an EC formulation. The four factors mentioned above which we must know before hand are :

- Recommended concentration is 0.1%
- 500 litres spray solution is desired per ha (10,000 m<sup>2</sup>)
- EC formulation contains 50% a.i.
- Area to be treated is 0.5 ha. (5,000 m<sup>2</sup>).

Problem 1. How many litres of the commercial formulations are required to treat the 0.5 ha area?

Solution: First compute the total spray volume needed to treat the area in litres.

$$500 \text{ l/ha} \times 0.5 \text{ ha.} = 250 \text{ litres}$$

- Then use this formula :

$$\begin{aligned} \text{Litres of commercial formulation} &= \frac{\text{Amount of spray required} \times \text{\% spray concentration}}{\text{\% active ingredient}} \\ &= \frac{250 \times 0.1}{50} = 0.5 \text{ litres} \end{aligned}$$

Now that we know how much formulation we need, we must determine the number of sprayer loads needed.

Suppose the sprayer being used for the treatment holds 10 litres.

Problem 2. How many loads are required to spray the 0.5 ha. area.

Solution:

$$\left. \begin{array}{l} \text{No. of sprayer loads} \\ \text{required to treat} \\ \text{the 0.5 ha. area} \end{array} \right\} = \frac{\text{Total spray volume (litres)} \quad 250}{\text{Capacity of sprayer (litres/ load)} \quad 10} = \frac{250}{10} = 25 \text{ loads.}$$

Now determine further how much of the formulation goes into each sprayer load.

Problem 3. How many litres of the commercial formulation are required in an 10 litre sprayer for a 0.1% spray?

Solution: Litres formulation required per sprayer load.

$$\frac{\text{Amount of spray required} \times \% \text{ spray concentration}}{\% \text{ a.i. in commercial formulation}}$$

$$= \frac{10 \times 0.1}{50} = 0.02 \text{ litres or } 20 \text{ ml/tank.}$$

so, 20.0 ml of the commercial formulation is required per sprayer load. It could also have been determined by simply dividing the 0.500 litres by 25.

#### b. Example in solving a problem for wettable powders:

We now want to consider the calculations used when the foliar spray recommendations are based on kg.a.i/ha required. The same formula as used for the % concentration problem can also be used here if the following four factors are known.

- The recommended rate is 1.0 kg /ha.
- Volume of spray solution is 500 l/ha (10,000 m<sup>2</sup>)
- The WP formulation contains 80% a.i.
- Area to be treated is 0.5 ha. (5,000 m<sup>2</sup>).

Problem : How many kg of the commercial formulation are required to treat the 0.5 ha. area?

Solution:

$$\text{Kg of commercial Formulation required} = \frac{\text{Recommended rate per hectare} \times \text{Area to be treated in hectare} \times 100}{\% \text{ a.i. in the commercial formulation}}$$

$$\text{@ To be changed} = \frac{0.75 \times 0.5 \times 100}{70} = 0.536 \text{ kg.}$$

So 0.625 kg or 625 g of commercial formulation is required.

We have already calculated that 250 litres of spray fluid are needed for 0.5 ha which is delivered through 25 sprayer loads using 10 litres /sprayer load.

Problem: How many kg of the commercial WP formulation are required in one 10 litres sprayer load ?

Solution:

$$\begin{aligned}
 \left. \begin{array}{l} \text{Kg of WP formulation} \\ \text{required per sprayer} \\ \text{load} \end{array} \right\} &= \frac{\text{kg formulation required for total area treated}}{\text{No. of sprayer loads}} \\
 &= \frac{0.625}{25} = 0.025 \text{ kg or 25 g}
 \end{aligned}$$

Thus 25 g of the WP formulation are required per sprayer load.

### c. Calculating the amount of dust for field application:

In this problem the weight of an insecticidal dust required to treat a certain area will be calculated.

- The recommended rate is 0.75 kg a.i./ha.
- You need to treat 1,000 m<sup>2</sup>
- The % a.i. in the commercial formulation is 5%.

Problem: How many kg. of the commercial formulation are required to treat the 1,000 m<sup>2</sup> area at the recommended rate of 0.75 kg a.i./ha?

To solve this problem we use the same formula as in the previous calculation for granular application.

Solution:

$$\left. \begin{array}{l} \text{kg commercial dust} \\ \text{formulation} \\ \text{required to dust 1,000 m}^2 \end{array} \right\} = \frac{\text{Recommended Rate in kg a.i./ha.} \times \text{Area to be treated in ha} \times 100}{\% \text{ a.i. in the dust commercial formulation}}$$

**Type calculation (see p. 285)**

## Precautions in using plant protection equipment

### i. Before using the equipment

- Before putting the pesticide application equipment into service, the recommendations for lubrication, operation and maintenance should be carefully read. The operator should endeavour to familiarize himself with the mechanism and working of the machine.
- The lubrication instructions should be followed faithfully using the specified lubricant. Special precautions should be exercised to see that dirt or other foreign substances do not accumulate in the bearings and other critical areas where the lubricant is needed.
- Before placing the appliance into service, it should be given a careful general inspection for cracks, loose connections or other malfunctions that may have resulted from vibration or earlier usage. The inspection should include the checking of the machine for any leakage, worn-out washers, etc. The nut of the plunger rod should be tightened, if loose.

- In case of 2-stroke engines, only correct fuel (petrol-oil)\_ mixture should be used. The oil and petrol should not be poured separately into the tank but first mixed thoroughly in a separate container before pouring into the tank. It may be again emphasized here that a multigrade motor oil should not be mixed with petrol as fuel for a 2-stroke engine.
- In order to apply the correct dose of the chemical per hectare, the machine should be calibrated. In the case of dusting machines calibration should be done for each of dust. Sprayers can, however, be calibrated for all spray fluids, viscous materials, by employing water as a test liquid.
- Clean water should be used for preparing spray fluids, which should, anyway, be strained. It is important to mix wettable powders with a small amount of water to form a paste adding the remaining water to it. This practice prevents the formation of lumps. In the case of motorized knapsack sprayers-cum-dusters, the spray fluid should be filled into the tank, using a funnel. The fluid must not fall on the carburetor and the spark plug. Similarly, in the case of dusts necessary precaution should be taken to see that no twigs, dry leaves, etc. enter the blower assembly, because they will put an additional strain on the engine and damage it. It would be better if the dust is sieved.
- Only those formulations should be applied with a machine for which the machine is designed. Further, to prevent the packing and forming of lumps, the dust should be dry. Otherwise, the output may be adversely affected and uniformity of the flow may be disrupted.
- Before it is applied, the spray fluid in the tank should be agitated to mix it well. After every interruption in the spraying operations, the spray fluid should be again agitated. This precaution is necessary when there is no agitator in the spray tank, or it is not effective enough.
- In case there is difficulty in fitting a plastic hose or parts, it is advisable to dip the parts into hot water for a short while before trying a second time. This will prevent the cracking of parts.

## **ii. While using the equipment**

- Nozzle-setting is important with hydraulic sprayers, because this influences the spray pattern. The working of a hydraulic-energy nozzle can be suitably adjusted. A wide hollow cone of the spray is suitable for close-up work, but it has little carrying power. The carrying power can be increased by narrowing the cone, e.g., by making the eddy chamber deeper. The height of the nozzle should be adjusted to suit the crop and wind conditions.
- Nozzles should be watched for any deformity or inconsistency in the spray pattern, as also for their blockage. If the erosion of the nozzle

orifice has increased the output by more than 10 per cent, then a new tip or disc should be fitted to avoid over-dosing.

- Lance-spraying should be carefully planned to avoid waste of time and materials. Trees and bushes should be treated in a logical sequence down each row, so that when the spraying of one tree is completed, the operator does not find that he has to unwind the lead of the lance from around a tree before proceeding to the next.
- During the spray operation, close watch should be kept on the pressure-gauge. This is necessary to ensure that the required pressure is being maintained. A reduction in pressure can occur owing to shortage of the spray fluid in the tank, blockage of the filter, leakage of air into the suction line between the tank and the pump, obstruction of relief valve, or leakage of the hose.
- When changing from one dust to another, the machine should be cleaned out and the application rate rechecked. The time setting will not necessarily give the same rate with a new material.
- When changing from one chemical to another it may be essential to thoroughly clean the spray system to remove the chemical used earlier. Water can be used for flushing the machine. Sometimes it is necessary to use a detergent. e.g. washing soda at the rate of 500 g in 50 litres of water. Washing with a detergent is essential when the history of the previous use of the sprayer is not available, or when a herbicide had been applied with it previously and the sprayer is now required for applying a non-herbicide chemical, e.g. fungicide or an insecticide.
- If the working of the machine is heavy, force must not be applied as it may result in some breakage. On the other hand the machine should be carefully examined to find the fault, so that necessary adjustment or correction can be carried out.
- Hoses should not be bent at angles because it results in reduced pressure. Also it puts additional strain on the tubes and shortens their life.
- Engine should not be run continuously for long period because they get overheated. An overheated engine cannot be expected to do an efficient job.
- The operator should walk at a constant speed for ensuring an uniform distribution of the material. A slight alteration, especially in the concentrate sprays may lead to uneven coverage that may give disappointing results in pest control. Further, in the case of spraying with non-systemic insecticides for the control of insects found all over the foliage, care should be taken to spray both the surfaces of the leaves.

### iii. After using the equipment

Pesticide – application equipment, like other machines, needs periodic repair and replacement of parts. The maintenance, after pesticide application, falls into two categories. First, there is the daily routine of cleaning, adjustment, greasing and lubrication. Freedom from breakdown and long life of the machine depend largely on this routine. Second, at the end of the season, all wearing parts, such as nozzles, pumps, valves, nuts and ball-bearings, should be inspected. A similar examination should also be made of hoses and plastic parts which crack or break due to prolonged use and ageing.

In order to prevent untimely breakdowns and delay in the middle of the season of heavy demand, it is desirable to arrange necessary replacements well before the start of the season. If major repairs are needed, a machine can be sent to the manufacturer or his agent for a complete overhaul, so that it will be in a first class condition once again.

Due care should be taken while replacing hose and valve fittings. In this connection, it is important to use wrenches of proper size on the hexagonal fittings to prevent the rounding off of the edges. Similarly, threads may be destroyed and gaskets crushed by over tightening. Consequently, spray fluids may start leaking. Further, broken gaskets may clog the discharge assemble. Tightening should, therefore, be done carefully – just sufficient to prevent leakages.

### Faults and their remedies

In finding faults and to remedy defects of the application equipment, the operator should have with him a pair of pliers, a set of screw drivers, an adjustable wrench, a knife and string. The common defects in application equipment, their cause and remedy are given in the Tables.

#### Faults with sprayers and dusters and their remedies

Type/ Name of Sprayer	Defects	Cause and remedy
Hand compression sprayer	a. Plunger rod is pushed up automatically after the downward stroke. b. On the downward stroke strong resistance is felt	This is due to leakage of the air-check valve. Clean it. If defect persists, replace the valve. A faulty valve has let the spray fluid into the pump barrel. Since liquid cannot be compressed, resistance is encountered. Replace the valve.
Knapsack sprayer (with single pump and a	Spray fluid is properly discharged only during the pressure stroke	Repair or change the delivery tube.

pressure barrel)		
Foot sprayer	a. spray fluid leaks along the plunger rod during operations	Tighten the gland nut that touches the plunger rod. If necessary, replace the packing twine of the gland nut. Change the pedal-return spring.
	b.The pedal does not move upwards automatically after the downward stroke	
Rocking sprayer	a. Water leaks along the sides of the plunger during spraying.	Tighten the locknut of the piston.
	b.PVC piston does not move freely the pump barrel.	Loosen the locknut
	c.Pressure chamber does not retain pressure required for efficient spraying	Replace the rubber washer below the pressure chamber.
Rotary duster	a.Dust not discharged on operating the duster	It may be due to blockage of the suction pipe or stoppage of movement of the feeding-brush. Clean the suction pipe and tighten the feeding brush on the shaft by tightening the nut
	a.Blower touching the case	It is due to the bush or ball-bearings getting worn-out. Replace the worn-out part(s)

#### Defects with manually operated sprayers and their remedies

Defects	Possible causes	Suggested remedies
Pressure drops	a. Clogged suction screens.	Clean them
	b.Loose connections or missing gaskets in suction line or partially opened suction valve; broken or leaky suction hose suction pipe; drain cocks not closed	Find out the specific cause and rectify the fault.
	a. Imperfect sealing due	Cleaning the ball and its seat usually improves the performance of the pump. If necessary, replace the valve seat



	to deposition of grains of sand under the ball valve or certain solids on the surface of the ball or wearing-out of ball valve and its seat	or valve, or both Replace the disc Often increasing the pump speed is all that is needed to regain pressure.
	b. Worn-out nozzle disc.	
	c. Pump speed slow	
Leakage	Loose nuts and clamps or worn-out gaskets	Tighten the nuts and replace the gaskets.
Lack of suction	a. Cup leather (plunger bucket) is either wrinkled or worn-out or it is completely dried.	Clean the suction assembly set. Replace the cup leather, if wrinkled or worn-out. If cup leather, is hard and dry lubricate with a vegetable oil, e.g., castor oil, groundnut oil, coconut oil or palm oil. Put water through the inlet, so that it reaches the suction valve to loosen it. If necessary, open the valve.
	b. Suction valves temporarily stuck	
Failure to retain pressure	Lid of the tank may be loose or the gaskets may be spoiled.	Tighten the lid and check the gasket and replace it, if necessary.
Difficult working of the plunger	Plunger may be bent through the full length of the barrel.	Straighten the plunger rod, if found bent.
Uneven discharge of spray	Nozzle obstructed	Open the nozzle and clean its various parts, especially the orifice.

### **Faults with engine of the motorized knapsack sprayers and dusters**

Defects	Possible causes	Suggested remedies
1. Engine does not start	a. Fuel cock closed (not opened) or blocked (Ensure fuel is present in the tank). If no flow, remove cock, clean and replace. b. Lack of fuel in the tank	Open the fuel cock  Fill the fuel tank

2. Engine runs irregularly or stops	c. Air-vent in the fuel tank cap clogged	Open or clean the air vent
	d. Main jet blocked in carburetor is	Remove it and clean it by blowing with air, and not with a needle or a wire and replace.
	e. Engine flooded with the fuel	Close the fuel cock; open the throttle lever completely and turn the engine over a few times. Unscrew the spark-plug clean, and dry it
	f. Spark-plug sooted or damaged	Clean the spark-plug and see whether the gap is proper. If the lead gives a spark but the plug does not change the spark-plug
	g. Carburetor dirty	Clean the carburetor
	h. short circuit	The lead meant for stopping the engine may be in contact with the frame. Break this contact
	i. Ignition wire loose or damaged	Fasten it or replace it, as need be
	j. Contact-breaker points dirty	Clean the points and make the contact-breaker point opening equal to 0.3-0.4 mm, i.e. equal to the thickness of a post card
	k. Air-cleaner dirty	Clean it with petrol, dry and refit
	l. Starting difficult in winter	Remove the air-cleaner and close the air passage in the carburetor with the hand to stop the intake of air. Start the engine with the rope and immediately fit the air-cleaner.
	m. Float needle sticking and stopping petrol supply	Remove needle, check for burns or rough surface. Clean off rough surface, if not possible, replace with a new one.
	n. Water in carburetor float bowl	Remove and clean, check also that fuel in tank is not contaminated with water.
	o. Thimble filter in carburetor is blocked	Remove filter, clean and replace
	a. spark-plug loose	Tighten the spark-plug

	b. Dirty fuel-pipe screens	Clean the fuel-pipe filter at the fuel cock or in the carburetor supply nipple, or both. Ensure that there is no air in fuel line.
	c. Main jet clogged	Clean the jet
	d. Spark-plug sooted	Clean or replace it
	e. Lead to spark-plug loose or disconnected or insulation broken or burnt	Fasten the lead tightly to the plug or replace it, if badly damaged
	f. Contact-breaker point oily or frozen together	Clean and adjust the contact-breaker points or replace them, if necessary
	g. High tension ignition lead loose or of shorting on metal parts of the engine	Check that lead is firmly affixed to spark plug. Where lead has chafing on base metal either cover base wire with insulation tape or replace with a new lead
	h. Fuel running low in the tank. Engine vibration or irregular movement of operator leaves outlet pipe uncovered, or irregular resulting in fuel starvation	Refill tank with correctly mixed fuel
3. Engine does not gain momentum	a. Air-filter partially choked	Clean the air-filter element
	b. Choke closed	Open the choke
	c. Carburetor partly blocked	Clean the carburetor
	d. Exhaust port or silencer choked	Remove the carbon deposit or replace with a new part
	e. Oil seal of the crank-case leaking	Change the oil seal
	f. Cylinder bore and rings worn-out	Change the barrel and the rings
	g. Ignition timing incorrect	Correct the ignition timing
4. Misfiring	a. Plug dirty or defective	Clean or replace the plug
	b. Silencer faulty	Replace the silencer
	c. Poor connections to the coil	Tighten the connections

5.Engine overheating	d. Water in the fuel	Change the fuel
	a. Engine overspeeding	Run at the speed recommend by the manufacturer
	b. Fuel incorrect	Drain of the tank refill with fuel mixed in the correct ratio
	c. Mixture lean	Check for dirt in the jet and set the carburetor needle jet.
	d. Incorrect size of main jet	Remove and refit one that complies with manufacturer's specification
	e. Ignition setting incorrect	Correct the ignition setting with a competent person.
	f. Air-flow to the engine obstructed	Clean the surface of fins and fan blades
6. High fuel consumption with a strong smell of unburnt fuel	g. Exhaust of silencer choked with carbon	Remove the carbon deposit
	a. float needle damaged	Replace the defective part
	b. needle seating damaged	
	c. Float punctured	
	d. Main jet too big	
	e. Air-cleaner dirty	
		Clean the air-cleaner

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## Safety Measures to be Followed During Handling of Fungicides

The various measures to be observed as precautions during handling of fungicides are given below.

### **During mixing and before applying fungicides**

Having purchased the right fungicide for the job, following guidelines for safety in the fungicides handling should be followed.

Know the disease and how much damage is really being done. Apply the chemical where there is economic damage.

- Seek advice on the proper method of control.
- Use only the recommended fungicides for the disease control.
- Read the label on the container and leaflet carefully and follow the instructions there in.
- Make necessary calculations for the required dilution.
- Obtain the required application equipment including the personal protective clothing and device.
- Take the most needed parts or tools to the field.
- Wear clean, appropriate, protective clothing before handling the fungicide.
- Never allow children or other unauthorized persons near the mixing.
- Never work alone when handling a high toxic fungicide.
- Never allow children to handle or mix fungicide.
- Check that fungicides on the farm in a dry, locked store. Avoid inhaling fungicide mists or dusts especially in confined spaces such as fungicide store.
- Warn neighbours of your spray programme especially to those people maintaining apiaries.
- Take only sufficient fungicide for the day's application from the store to the site of application.
- Do not transfer fungicides into other containers, especially beer and soft drink bottles. Keep the products always in its original container.
- Never leave fungicide unattended. Inquisitive children or animal may be affected.
- Mix chemicals outside in an open area or in a well ventilated area.

- Carefully open the factory sealed containers and take care that nothing should spill on the body. Open bags or sacks with scissors, knife or blade to prevent dusting. Do not tear the bag which is bound to cause spillage and undesirable dusting.
- Never position any part of the body below the fungicide when pouring out.
- Always stand with the wind on your back when mixing or loading fungicide.
- Measure and mix quantities accurately.
- Drain the container completely by allowing it to stand for 30 seconds in a inverted vertical position.
- Fill dilution water (used in the spray tank) to 1/3<sup>rd</sup> of the container volume. Cap tightly and shake. Pour this rinse water into the spray tank and drain container completely again by allowing to stand vertically for 30 seconds. Repeat this operation twice or more.
- Do not keep food, drink, tobacco, cigarette or cooking utensils in the work area.
- Never eat, drink, smoke, rub your eyes or face while working with fungicides. Avoid inhalation of chemicals, dusts or fumes etc.
- Do not use the mouth to siphon a fungicide from a container.
- Harvest fruits and vegetables before application. Before applying the fungicides, if the crop needs irrigation do it before spraying.
- When filling the spray tank, do not allow the delivery hose below the highest possible water surface to avoid back siphoning.
- Verify for any ill-effect in combining two chemicals. Use only compatible fungicides in combination.
- Clean up spills immediately. If the body is accidentally contaminated immediately wash. Change clothes if required.
- Commercial operators using large quantities of organophosphorus fungicides should visit their doctors and have a blood cholinesterase test and have repeat checks during the season.
- Always have plenty of water available for washing.

### **During mixing and application of fungicides**

The person applying the pesticide can be exposed to it during his work. If he is careless he may also expose other people or farm animals to the fungicide. It is therefore essential to use safe working practices in the application of the fungicide.

- Wear appropriate personal protective equipment as recommended. If it is contaminated remove and replace with clean clothing.
- Never use children or untrained persons for application.
- Spray crops with the wind. In other words spray with the wind coming from the back.

- Ensure that there are no animals, people, food or animal feed downwind i.e., in the direction in which the wind is blowing.
- Check sprayer and equipments for leaks. Leaking or poor quality spray equipment can seriously contaminate the person.
- Never allow children or other unauthorized persons near the mixing.
- Do not blow out blocked spray nozzles with the mouth. Apply fungicide where needed. Do not walk with running sprayers on roads / pathways.
- Avoid spraying when crops are in flowering stage. Risk to bees is reduced if sprays are applied in the evening when they are no longer foraging. Never spray if the wind is blowing towards growing livestock or regularly used pastures.
- Apply the correct dosage. Do not use higher dosage than those recommended as it becomes phytotoxic. Also a lower than recommended dosage will make fungicide ineffective.
- To apply the correct amount of fungicide, the application equipment must be properly calibrated. Knowledge of output of selected equipment is essential to calculate the amount of fungicide needed in the mixture to treat a great area.
- Apply fungicide at the correct time allowing sufficient time for harvest. This will help to prevent undesirable residue remaining in the farm produce. Many pesticide labels state the number of days (interval) between the last fungicide application and the time of harvest.
- Use properly maintained and functioning equipment. Repairing defective equipment during the spray operation increases time of exposure to the pesticides.
- Take care to prevent the applied pesticide from contaminating nearby streams, ponds, lakes or wells.
- If during spraying the person feels ill or notices any irregular body symptoms, work should be stopped and medical attention should be sought immediately.
- Provide proper supervision of those assisting with the pesticide application and have adequate rest periods.
- While these precautions need to be observed during application, elimination of hazards requires proper care even after the spraying operation is over.

#### **After application of fungicides**

- Empty the spray tank completely during the spraying. If not empty, dispose the contents. After each day of spraying the equipment must be washed with water. Do not allow a used sprayer to dry off. Sprayers must be washed thoroughly immediately after use.

Otherwise nozzles can get choked. Cleaned equipments should be returned to store.

- Return unused fungicide to the store.
- Safely dispose all the empty containers. As it may be difficult to use empty containers after each day of spraying operations. They should be kept in the fungicide store until a convenient number are ready for disposal. It is absolutely impossible to clean out a container sufficiently well to make it safe for use for storage of food or as a cooking utensil. If any containers are burnt, never stand in the smoke.
- Never empty the spray tanks into irrigation canals, water ways, ponds or wells.
- Do not leave dirty spray equipment unattended. Immediately clean up and decontaminate.
- Decontaminate or destroy device such as buckets, sticks, measuring cups etc. used in the preparation of the spray solution.
- Decontaminate all protective clothings and footwears.
- Avoid contamination of the skin, especially, the eyes and mouth. Liquid formulations should be poured carefully to avoid splashing. Avoid powder formulations, “puffing up” into the face. If contaminated with the concentrate, wash immediately. Take a bath with plenty of clean water and soap after fungicide application.
- Wash the clothes separately
- Mark the sprayed field and prevent unauthorized entry into the treated area.
- Keep a record of the use of fungicides.
- Do not allow other persons to enter the treated area for the required period. Do not allow cattle to graze in the treated area for a specified period.
- The fungicide should be stored in a separate room which has enough ventilation.
- Farmers should avoid storing chemicals for more than 18 months. Containers left longer than this may corrode or the active ingredient may become less effective.



# 21

## Mushroom Cultivation

Mushrooms are large reproductive structures of edible fungi belonging to either Ascomycotina or Basidiomycotina. These could be either epigeal or hypogaeal. The vegetative parts of the mushroom mainly consist of thread-like long thin mycelia, which under suitable conditions form fruit bodies or sporocarps (basidiocarps). Mushrooms are non-green fungal plants occurring seasonally all over the world in various habitats varying from sandy plains to thick forest or green meadows to roadside pathways. The mushrooms comprise a large heterogeneous group having various shapes, size, colour, appearance and edibility. Of more than 2000 edible species, about 70 genera having 300 species are reported from India. However, only a few have been brought under cultivation on commercial scale. About 80 mushrooms have been grown experimentally, 20 cultivated commercially and 4-5 species produced on industrial scale throughout the world.

Table 1. Important mushrooms grown in India

Sl.No.	COMMON NAME	SCIENTIFIC NAME
1.	Button/European/temperate mushroom	<i>Agaricus bisporus</i>
2.	Hot weather mushroom	<i>A. bitorquis</i>

3.	Oyster mushrooms	<i>Pleurotus sajor caju</i> , <i>P. flabellatus</i> <i>P.ostreatus</i> , <i>P.florida</i> , <i>P.citrinopileatus</i> , <i>P.comucopiae</i> , <i>P.sapidus</i> , <i>P.membranaceous</i>
		<i>P.eryngii</i> , <i>P.fosssulatus</i> , <i>P.eous</i> and <i>P. platypus</i> .
4.	Paddy straw / Chinese / tropical mushrooms	<i>Volvariella volvaceae</i> and <i>V. diplasia</i>
5.	Black ear mushroom	<i>Auricularia polytricha</i>
6.	White milky mushroom	<i>Calocybe indica</i>
7.	Brown cap or Giant mushroom	<i>Stropharia rugoso annulata</i>
8.	Shiitake mushroom	<i>Lentinus edodes</i>

## IMPORTANCE

Mushrooms have been recognised as the alternate source of good quality protein. They are capable of producing the highest quantity of protein per unit area and time from the worthless agro-waste which are available to the tune of more than 300 million tonnes per annum in our country. The major advantages of growing mushrooms are:

1. They are good source of high quality proteins and are rich in vitamins and minerals.
2. These have got medicinal properties.
3. They are capable of degrading agro-waste and thus avoid pollution.
4. It is an indoor crop, grows independent of sunlight and does not require fertile land.
5. They have huge potential for export as global market is expanding very fast.

6. Their cultivation is labour intensive and offers vast employment opportunities in rural areas.
7. In addition to floor air space is also utilised resulting in higher production (upto 20-30% bio efficiency in *Volvariella*, 30-35% in *Agaricus*, 80-100% in *Pleurotus* and 143% in milky mushroom).

### FOOD VALUE / NUTRITIVE VALUE

Mushrooms were believed to be the “Food for God” by the Greeks. Now they are cultivated and consumed as delicious food by people across the world. Edible mushrooms are many in temperate and tropical countries. Among them the following are cultivated in larger quantities.

- White button mushroom - *Agaricus bisporus*
- Oyster mushroom - *Pleurotus* spp.
- Paddy straw mushroom - *Volvariella* spp.

Composition of edible mushrooms

Mushroom	Moisture (%)	CHO (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Calories
<i>A. bisporus</i>	90.1	5.0	2.9	0.3	0.9	0.8	36
<i>P. sajorcaju</i>	90.2	5.2	2.5	0.2	1.3	0.6	35
<i>V. volvaceae</i>	90.1	4.7	2.1	1.0	1.1	1.0	36

- Mushrooms contain 20-35% protein (dry weight basis), which is higher than in vegetables and fruits and is of high quality.
- Mushrooms are very rich in lysine and tryptophan, the two essential amino acids deficient in cereals.
- Mushrooms contain good amount of vitamin C and vitamins of B complex group (thiamine, riboflavin and niacin). They also

contain appreciable amounts of fat soluble vitamins like A, D, E and K.

- They are rich in K, P and Na and contain low but available form of iron.
- Potassium:Sodium ratio is very high which is desirable for patients of hypertension.
- It is a low calorie food with very little fat.
- It has no starch and contains very low sugar.
- It contains high fibre content.
- Cholesterol is absent and ergosterol is present.

#### **Nutritive value of APK 2, the milky mushroom**

<b>NUTRIENT</b>	<b>PER CENT</b>
Moisture	85.58
Crude ash	8.89
Crude protein	32.29
Crude fibre	41.10
Crude fat	0.67
Total carbohydrates	9.85
Iron	0.03
Calcium	0.12
Phosphorus	0.80
Sodium	0.95
Potassium	4.52

This mushroom contains increased fibre content and is useful to patients suffering from peptic ulcer constipation and heart ailments.

#### **MEDICINAL VALUE**

Mushrooms have traditionally been used in China and Japan for the medicinal and tonic properties. Both edible and poisonous mushrooms have medicinal properties and are used in specific diseases.

They possess antibacterial, antifungal and antiviral properties. *Pleurotus* has antibacterial effect. *Agaricus campestris* has antiviral activities against human diseases. Cosmetic products and tonic beverages have also been produced in China from *Ganoderma* mushrooms. Pharmaceuticals worth \$700 million are produced annually in Japan alone from *Lentinus*, *Coriolus*, *Schizophyllum* and *Ganoderma*.

The medicinal values of important mushrooms are listed below

Anaesthesia	-	<i>Calvatia gigantean</i>
AIDS	-	<i>Grifola frondosa</i> , <i>Lentinus edodes</i>
Anticancerous	-	<i>Lentinus edodes</i> , <i>Flammulina velutipes</i>
Antidiabetic	-	<i>Coprinus comatus</i>
Eye diseases	-	<i>Auricularia auricula</i>
Improving digestion	-	<i>Agaricus bisporus</i>
Lengthening of life period	-	<i>Ganoderma lucidum</i>
Mental disorders	-	<i>Psilocybe mexicana</i>
Purgative	-	<i>Armillaria mellea</i>
Reducing high blood pressure	-	<i>Agaricus bisporus</i> , <i>Lentinus edodes</i> <i>Ganoderma lucidum</i> , <i>Volvariella volvacea</i>
Strengthening health, healing piles- and stomach ailments	-	<i>Auricularia polytricha</i>

## **BIOCONVERSION OF ORGANIC WASTES IN TO DIGESTIBLE FEED**

Huge quantities of lignocellulosics and other organic waste residues are generated annually through the activities of agricultural, forest and food processing industries. More than 300 million tonnes of

agricultural waste is available annually in India and about half of this residue remains unused. Most of the mushrooms possess the enzyme complexes, which enable them to attack and degrade these industrial and agricultural byproducts thereby resulting in a high valued food protein suitable for direct consumption.

**Table 2. Comparison of different mushrooms**

CHARACTER	OYSTER MUSHROOM	BUTTON MUSHROOM	MILKY MUSHROOM	PADDY STRAW MUSHROOM
Species	<i>Pleurotus</i> spp.	<i>Agaricus</i> spp.	<i>Calocybe indica</i>	<i>Volvariella</i> spp.
Substrate	Paddy straw	Paddy straw compost	Paddy straw	Paddy straw
Growing temperature.	20-30°C	15-25°C	25-35°C	30-35°C
Crop cycle	30-45 days	85-100 days	45-50 days	25-30 days
Days for first harvest	10-25 days	60-70 days	24-28 days	25-30 days
Yield per bed	500g/500g bed	3.5kg/tray	356 g/bed	5kg/30kg bed
Shelf life	1-3 days	3-5 days	3-5 days	1-3 days
Bioefficiency	80-100%	30-35%	143%	20-30%
Prodn. Cost /kg (Rs.)	15	30	20	20
Market price /kg (Rs.)	40	80	80	40
Net profit /kg (Rs.)	25	50	60	20

Spawn preparation is important pre-requisite in mushroom cultivation. Methods of spawn preparation and cultivation of oyster and milky mushrooms are given below.

## **SPAWN PREPARATION**

Spawn is nothing but the mushroom fungal growth maintained on grain based medium. This is the **Seed** material for cultivating mushroom. The first generation fungal culture is called the **mother spawn**. Normally, from the mother spawn, further spawn can be produced up to third or fourth generation. Continuous sub culturing may reduce the efficiency of the spawn.

### **Materials required**

1. Sorghum grains: Select good quality well filled bold grains free from pests and moulds coated grains
2. Polypropylene bags 200 gauge 30 x 15 cm.
3. Cotton wool (non-absorbent)
4. Used papers 10 x 10 cm
5. Jute thread (10-15 cm long bits)
6. Vessel (Aluminum or stainless steel)
7. Hessian cloth / Jute beg

### **Method of preparing spawn**

1. Put the sorghum grains in water to remove chaffy and damaged grains
2. Half cook the sorghum grains for 30 minutes in a vessel
3. Test the cooked grains by gently pressing them between fingers. Grains should slightly break and should not be sticky.

4. Remove the half cooked grains, drain out water and spread over a hessian cloth evenly to allow excess water to evaporate (approximate time 60 minutes). Soak hessian cloth in solution containing 5 g Bavistin + 10 g thiram or mancozeb dissolved in 10 litres of water before use
5. Mix thoroughly 20 g of calcium carbonate for every kg of sorghum grains.
6. Fill the grains in glucose bottles (which are previously cleaned with soap water, rinse with fresh water and sun dry) upto  $\frac{3}{4}$ th height (300 g / bottle)
7. Tightly plug the mouth of the bottles with non-absorbent cotton.
8. Cover the cotton plug with the paper and tie it around the neck of the bottle using twine.
9. Keep the bottles in an autoclave or pressure cooker
10. Sterilize the bottles with grains at 20 p.s.i. for 2 hours or one hour per day on 2 consecutive days.
11. After cooling, the bottles are ready for use

### **Method of inoculation**

1. Keep required number of sterilized bottles containing grains in culture room
2. Punch out 6-8 mm diameter discs of fungal growth from Petriplates using a sterilized cork borer in inoculation room. The stock culture maintained on potato dextrose agar slants can also be used.
3. Transfer with the help of sterilized inoculation needle, four or five discs into each bottle. From one Petridish containing good growth of the mushroom fungus about 10 bottles can be inoculated.
4. Incubate the spawn bottles at room temperature outside the inoculation room.



5. Observe the growing mycelium. In about 15 days complete growth of white mycelium covering the entire bottle can be seen. Allow the spawn to mature for 4 to 5 days more. Now the mother spawn is ready for use.

#### Preparation of planting spawn or bed spawn from mother spawn

1. Always use well-grown mother spawn (18-20 days old). From a single mother spawn bottle 30 spawn bottles can be prepared. Two persons are required to transfer the inoculum from the mother spawn to new bottles (to be done in inoculation room).
2. One person should hold the spawn bottle in his left hand and open the plug with right hand by keeping the mouth of the bottle near the flame.
3. With the help of a hooked sterilized 5 mm iron rod stir the spawn to get individual grains with the fungal growth.
4. Second person, now, can open the sterilized bottle with sorghum grains in the same way holding the cotton plug in his right hand.
5. Now the first person holding mother spawn bottle should transfer about 10g of sorghum grains with the fungus to the new bottle.
6. Heat the mouth of the inoculated bottle and quickly close with the same cotton plug. All the above steps should be done near the flame to avoid contamination.
7. Incubate the spawn bottles at room temperature. In about 15 days time full growth could be seen.

**Note:** Use 18 to 20 days old spawn for bed preparation. (Expiry time for spawn bottle. Spawn when stored under normal room temperature, it can be used up to a maximum period of 30 days from the date of inoculation. Under refrigerated conditions it can be stored for 3 months. Temperature below 15°C and above 30°C is not favourable for growth).

## MUSHROOM BED PREPARATION

The cultivation procedures of both oyster mushroom and milky mushroom are given below.

### 1. Oyster mushroom –*Pleurotus* spp.

Oyster mushroom (*Pleurotus* spp) is shell, fan or spatula-shaped. It forms clusters of caps one above the other. They are with shades of white, cream, grey, pink or light brown depending upon the species. Pink mushrooms become slightly white at maturity. The stipe is strongly eccentric to lateral. The flesh has a mild taste. Oyster mushroom is edible and can be cultivated throughout the year in Tamil Nadu. Different types of oyster Mushrooms grown in Tamil Nadu are given in table. They are grown indoors and require a mushroom house (shed).

1. **Mushroom house:** Thatched shed is preferred for mushroom growing. Sheds are built in east west direction to avoid direct effect of sun and to reduce the temperature inside the house. On the top covering with chicken mesh prevents entry of rats, squirrels, snakes etc. The sides are covered with coconut plants. The floor of the shed is filled with sand to a uniform height of 15 cm. Racks are built to accommodate mushroom beds. Inner side of the shed is covered with jute gunny bags. Water is sprinkled twice in a day on the floor and gunny bags to maintain the required temperature and relative humidity.

- a. **Spawn running room:** Spawn running room is one where the beds are kept for running of spawn. Temperature in the room should be maintained between 25 to 28°C. No light is required. But ventilation is needed.

- b. **Cropping room:** Cropping room is one where the opened mushroom beds after completion of spawn running are kept. Between 23 to 25°C relative humidity should be maintained above 75-80% with diffused light and aeration.
2. **Spawn:** Suitable substrates for spawn are cholam (sorghum), maize, wheat grains. **Preparation of spawn:** Half cook the grains, air dry in hessian cloth, with calcium carbonate at 2% level (20g/kg of seed), fill the grains in empty glucose drip to  $\frac{3}{4}$  capacity bottles, plug with non-absorbent cotton and sterilize in an autoclave at 15 psi. for 2 h. Inoculate with pure culture of the fungus and incubate at room temperature for 15 days. Use 15-18 days old spawn for spawning.
3. **Substrate for mushroom cultivation:** Paddy straw is cheap and easily available and is used as substrate. Hand thrashed and fresh paddy straw is cut into 3 to 5 cm length.
4. **Pasteurization of substrate:** Paddy straw bits are soaked in potable water for about 6 hrs. Pre-soaked bits are boiled in water for 30 minutes and removed and air dried in hessian cloth (which was sterilized with carbendazim solution 0.1%) to 65 per cent moisture. No water should drip when the bits are squeezed between fingers.
5. **Preparation of mushroom bed (cylindrical bed method):** Preparation of bed (spawning) use 60x30 cm polythene bags. Tie one end of bag, put two holes of 1 cm dia in the middle to ensure aeration. Put the processed straw in the bottom of the bag to height of 5 cm, sprinkle 25 g of spawn. Place the straw to 10 cm height. Repeat the process to get four layers of spawn and 5 layers of straw. The last layer of straw is of 5 cm height. Tie the mouth with twine and arrange

beds in tiers in the spawn running room. After 15-20 days of spawn running period, cut and remove the polythene bag and transfer the beds to cropping room. Maintain cropping conditions. Keep the beds moist by periodical spraying with water.

#### 6. Management of pests and diseases:

- a. **Weed moulds:** *Trichoderma*, *Penicillium*, *Aspergillus* and *Sclerotium* are common weed moulds appearing on beds. Use good quality spawn and straw, pasteurize the straw properly and maintain optimum moisture (65%) and high level of cleanliness.
- b. **Phorid flies:** Provide 35 mm mesh to windows, maintain cleanliness in and around mushroom house.
- c. **Bacterial rot:** Avoid excess spraying of beds with water. Use chlorinated water to control rotting of mushrooms (2g of stable bleaching powder in 10 l of water).

7. **Harvest and yield:** Mushroom pin-heads appear on 3<sup>rd</sup> or 4<sup>th</sup> day of opening of beds. Matured mushrooms can be seen 3-4 days after pin head formation. Harvest matured mushrooms before spraying water. Second and third harvest can be obtained after scraping the surface of beds to 1 to 2 cm deep after first or second harvest. The entire cropping will be over in 35-40 days.

**Table 1. Characters of oyster mushroom varieties (*Pleurotus* spp.)**

S.No	Characters	Co 1 (White oyster)	M2 (Grey oyster)	APK 1 (Pink oyster)	MDU 1	Ooty 1
1	Species	<i>P.citrinopileatus</i>	<i>P.sajor – caju</i>	<i>P.eous</i>	<i>P.djamor</i>	<i>P.ostreatus</i>
2	Year of release	1986	1975	1995	1993	1998
3	Colour	White	Grey	Deep rose	Bright	Bright

				buds & white at maturity	white	white
4	Texture	Fleshy	Fleshy	Fleshy and tough	Fleshy	Fleshy
5	Spawn run (days)	15	20	7-12	12-16	33
6	Crop cycle (days)	35-40	40-45	35-40	35	55-60
7	Yield in g. /bed of 500 g substrates	395	328	910	538	531
8	Bio-efficiency (%)	79.0	65.6	182	122.8	116
9	Shelf life (hrs)	48	48	72	24	36

## 2. Milky mushroom- *Calocybe indica*

Milky mushroom (*Calocybe indica*) is a edible mushroom. It is also known as mil white mushroom or white summer mushroom or dudh chalta. It is popular in West Bengal and adjoining states in India. It is collected from natural habitats and sold in the local markets. The fruit bodies are large sized and delicious. The sporosore is milky white robust and attractive on an average a mushroom weighs 55 to 60 g (max 472g). Milky mushroom can be grown at temperatures between 25 °C and 35 °C and relative humidity above 80 percent. It can be cultivated throughout the year in Tamil Nadu. A variety **APK 2** was released by Tamil Nadu Agricultural University and it contains the following nutrients.

### Substrates

Milky mushroom can be cultivated on a wide range of cellulosic substrates namely, paddy straw, maize stalks, sorghum stalks, pearl millet

stalks, palmrosa grass, vetiver grass, sugarcane bagasse, soy bean hay, groundnut haulms etc.

### **Mushroom spawn**

Half-cooked sorghum grains or paddy chaff are mixed with 2 per cent calcium carbonate and filled in empty glucose bottles or in polypropylene bags. They are autoclaved at 1.4kg/cm<sup>2</sup> pressure for 1.5 to 2.0 h. The bottles / bags are aseptically inoculated with pure cultures of mushroom fungus maintained in PDA medium and incubated at room temperature. The spawn run will be completed in 10-12 days and these serve as mother cultures. From each bottle of mother culture 25 additional spawn bottles can be prepared.

### **Cultivation chamber**

Beds after preparation may be kept under normal room temperature ( 25- 35C) for spawn run. After completion of spawn run and after casing, the beds are to be incubated over racks in a partially sunken chamber lined with blue coloured high density polythene sheet as roofing material. Inside the chamber the temperature should be around 30-35C and the RH more than 85 %. Light intensity of about 1600-3200 lux is essential in the cropping room. Proper ventilation for gaseous exchange is also essential in this chamber.

### **Mushroom bed preparation**

Polythene bags of 60 x 30 cm size are used for mushroom bed preparation. Chaffed paddy straw bits of 3-5 cm in length are soaked in cold water for 4 hrs. After draining the excess water, the straw bits are boiled for 30-45 minutes in a separate drum. Sometimes, steam treatment of substrate for 1 hr. or chemical treatment with carbendazim 75 ppm + formalin 500 ppm (soaked for 16 h.) may be followed. Comparatively hot

water or steam treatment is safe and best. After substrate treatment they are shade dried to remove excess moisture and used for bed preparation. At the time of bed preparation the substrate should contain around 60% moisture (can be tested by squeeze method) . Sorghum grains or paddy chaff spawn may be used and cylindrical beds are prepared following layer method of spawning as we do in case of oyster mushroom. With each bottle of spawn 2-3 cylindrical beds can be prepared. The beds are then incubated for spawn run under semi-dark conditions in a clean room. Spawn run will be completed in 10-12 days.

### **Casing**

Unlike oyster mushroom cultivation, milky mushroom production involves an additional process called casing. After the completion of spawn run the cylindrical beds are cut horizontally into two equal halves. Over the each half bed casing soil is applied to a height of 1-2 cm. For casing steamed (for 1 h) garden soil (clay loam, pH around 8.0) is useful. In some cases, red soil mixed with sand and calcium carbonate (2%) or any other porous medium with good WHC, moderate CEC and low EC are also found useful.

### **Cropping**

Beds after casing are kept in cultivation chambers and sprayed regularly with water to maintain 50-60% moisture level in the casing medium. Pin heads appear in 8-10 days after casing and the first harvest can be made in 6-8 days after pin head formation. After obtaining the first harvest the casing medium is gently ruffled, slightly compacted back and sprayed regularly with water. Second and third harvest may be obtained within 45-50 days of bed preparation. Then the beds are removed and fresh beds may be kept for cropping.

### **Yield**

Mean yield is 356 g per bed (contains 250 g of paddy straw on dry weight basis) which accounts to 143 percent bio-efficiency. On an average single mushroom weighs 55-60 g and sometimes a maximum of 472 g/ button has been recorded.

A variety of recipes like soup, pickle, chips currey, briyani, samosa, kuruma, bread-mushroom sandwich can be made with this mushroom at the time of cooking a piece of ginger or castor oil may be added to the mushroom for improving softness and texture.

### **3. White Button Mushroom –*Agaricus bisporus***

The white button mushroom *Agaricus bisporus* is called as European mushroom. This mushroom requires comparatively a cooler climate. In Tamil Nadu this is cultivated successfully in the hilly tracts like Nilgris, Kodaikanal, Yercard, etc.

**Table 2. Requirement of climatic elements**

<b>Growth phase</b>	<b>Duration (days)</b>	<b>Temperature (°C)</b>	<b>RH (%)</b>
Spawn running	12-15	25-27	95
After casing	Upto 18	23-25	85
Fructification and picking	Upto 18	18-20	85

**Substrate:** It requires a special type of compost.



**Preparation of compost:** The most important problem in cultivation of this mushroom is the correct preparation of compost. It requires a seed bed of special compost. Two types of composts are used for this purpose.

1. Natural compost
2. Synthetic compost

**Preparation of Natural compost:** The natural compost is prepared from horse dung collected from horse shed along with abundant wheat straw litter with urine. The manure consists of one part of wheat straw and 3 parts of horse dung with abundant urine. To this 100 kg of chicken manure and 3 kg of urea, one tonne of horse manure are added. The manure mixture is heaped to 1 m width and 1 to 1.5 m height. The length may vary according to the availability of substrate. It is under an open shed or under a roof. After 3-4 days, ammonia will begin to evolve. The heap is again stirred in shade 3-4 times at 3 days interval. Twenty kg of gypsum is sprinkled in 2 equal splits at 2<sup>nd</sup> and 3<sup>rd</sup> turning. The compost will be ready in 12-15 days. The compost will be done under a roof at room temperature. Compost will contain 18-20 % total nitrogen, ammonia 0.1 to 0.2 % and the pH will be 7.5 with a moisture of 65 %.

Nitrogen in the finished compost is in the form of ammonia, amino acids, hexamine, protein and lignin. The mycelia of *Agaricus* contain phenol oxidase enzyme, which break down the lignin. A good compost will contain actinomycetes, *Humicola* and other thermophilics. It will contain less bacterial population. The prepared compost is almost a selective medium for the growth of *Agaricus*.

**Synthetic compost:** The following ingredients are required:

Wheat straw	300 kg
Wheat bran	10 kg
Saw dust	15 kg
Ammonium sulphate	10 kg

Urea	2 kg
K <sub>2</sub> SO <sub>4</sub>	4 kg
Gypsum	7 kg
Lime	2 kg

### **Procedure**

The wheat straw is chopped into piece of 10-15 cm length and soaked in clean water for 3 days. The straw is processed on a hard or cement floor. The straw is spread in one sq.m. area up to 20 cm height. The ingredients other than wheat straw are mixed and sprinkled over the straw surface. Similarly, the wheat straw and fertilizer mixture are alternated up to the height of 1.5 m, compacting the heap on all sides. After adding fertilizer at every stage, water is sprinkled. The heap is turned 6 times on 6,11, 15,18,20 and 23<sup>rd</sup> days to ensure uniform pasteurization of the materials. Water is sprayed over the compost up to 3<sup>rd</sup> turning and not afterwards. The temperature gradually rises till 3<sup>rd</sup> turning and later more rapidly up to 65°C. After 4<sup>th</sup> turning, it comes down to room temperature. At the time of fifth turning, 2 kg of lime is added to the compost and incorporated uniformly to bring the pH 7.5 to 7.7. Now the pasteurized compost is ready for spawning on 25<sup>th</sup> day.

### **Pasteurization and Conditioning**

The special compost is filled in wooden boxes of 90 x 60 x 15 cm to a height of 10 cm and pressed uniformly. Pasteurization is done by keeping the mushroom house at 60°C for 10 hours to kill the nematodes and reduce the bacterial population. Composting is continued by 8 days at 58°C. Now, the compost is brought to the condition for better growth of mushroom and also for the growth of thermophilic fungus, actinomycetes, Humicola. That is the pasteurization and conditioning.

### **Spawning**

Spawn is spread in a single layer of seeds over the surface of the compost. Again, a thin layer of compost up to 2 cm is added over which another layer of spawn is broadcast. Again, the spawn is covered by a thin layer of compost to avoid speedy evaporation and spawn will not dry. One bottle of spawn is sufficient for seeding 2 boxes of the above size. A newspaper is spread over the box and mancozeb at 0.2 per cent is sprayed on the paper just to moisten and also control any other fungal spores. The fungicide spraying is repeated on the next day. Afterwards, the paper is kept moist by spraying clean water. The heaping may be arranged one over the other in a clean room. The maximum temperature is recorded daily.

### **Casing**

Casing is done after 12-15 days of spawn running with sterilized soil. Sterilization of soil is done with formalin 1 % under a polyethylene cover for 3-4 days and bringing the soil pH to the neutral level by adding  $\text{CaCO}_3$  to the soil. After a week, the polyethylene sheet is removed and the sterile soil is aerated at room temperature. The formalin eliminates any other organism. The soil is spread over the compost up to a height of 2 cm. After casing, mancozeb at 0.2 % is sprayed over the cased soil and the surface is kept moist after 2 fungicidal spraying. Water spraying is followed whenever necessary. Pin head of mushroom begins to emerge out 3 weeks time after casing and they attain full growth in 46 days.

### **Harvesting**

Harvesting is done a day before the pileus opens. Three to four pickings are possible at an interval of 4-6 days. The buttons so harvested are cleaned thoroughly by pressing gently to remove the adhering soil or compost. The harvested mushrooms are packed in perforated

polyethylene bags. The normal yield may be 10-15 kg of mushroom in 1 sq. m. compost area.

#### **4. Paddy straw mushroom – *Volvariella volvacea***

Paddy straw mushroom or the straw mushroom or the Chinese mushroom is most common in South East Asia. It requires a temperature range of 28 to 36°C and relative humidity of 75 – 85 %. It is distinguishable from *Agaricus* that the ring on the stipe is absent and it possesses a conspicuous cup like structure called volva at the base of the stipe. The genus takes its name volva, means a wrapper which completely envelop the main fruiting body during the young stage.

#### **Substrate preparation**

It grows best on paddy straw and several other substrates like wheat straw, sorghum straw, composted cotton waste, coconut waste, water hyacinth, oilpalm bunch waste, sugarcane baggasse, banana leaves and saw dust can also be utilized for growing. Paddy straw is tied into bundles of 1.2 m long and 20 cm dia. They are soaked in water in a cement trough for 18-24 h. This would soften the straw sufficiently for the mycelium to penetrate. Then the bundles are taken out from the trough and kept in hot water at 80°C for 2 h. Remove the bundles and allow the excess water to drain off in a shade.

#### **Bed preparation and spawning**

Mushroom beds are prepared on a bamboo frame supported on bricks or on a raised brick platform inside a thatched hut. The soaked wet bundles are placed lengthwise side by side on the platform with their butt ends on one side and the loose ends are on the opposite sides. The

number of bundles so placed should be such that it approximates the length of the straw so that it makes a square layer (approximately 4 numbers). A second series of bundles (4 nos.) similarly placed lengthwise with their butt ends on the opposite side. An arrangement of this type makes one layer. The loose straw extending the layer is cut with the help of scissors. Small bits of straw spawn are placed 7-10 cm inside the margin leaving a spacing of 5.0 cm from each other. If the grain spawn (80 – 100 g) is used it is sprinkled. A small quantity of redgram powder (20 g) is applied along with the spawn about 15 cm away from the outer edge. On the top of the first layer, another series of bundles is placed with their butt ends at right angles to the previous layer i.e., in a criss-cross fashion and another series of bundles placed with their butt ends just opposite. This forms second layer (approximately 8 bundles), which is also spawned similarly as done in first layer. The third layer (8 bundles) is again laid on the top of the second layer and spawned. The final layer of 8 bundles is place on the third layer and spawned. Then it is covered with thin layer of straw. This (32 bundles) makes a single bed. This bed is gently pressed to make it compact for effective spawn running and to avoid rapid water loss and provide favourable humidity it is covered with polythene sheet or gunny (bag) sheets.

### **Spawn run**

The individual beds are watered daily with a rose-can once or twice depending upon the climatic conditions. Mostly no watering is required for the first 3 or 4 days. The bed temperature should remain between 30 and 35°C with 85 to 90 % relative humidity. The spawn run will be completed within 10-15 days. Then the polythene sheets are removed.

## **Harvest**

The spawn run beds are exposed to fresh air and the mushroom starts appearing as pinheads within few days. They remain in the button stage for 4-5 days and then grown into full size. Mushrooms are picked at button stage early in the morning by gently twisting of fruiting body. The correct stage of picking is when the volva is about to rupture or just ruptured.

Mushroom production continues for a period of two to three weeks. Total crop period is 30 to 40 days. Each bed of 10 kg paddy straw can produce 1-1.5 kg of fresh mushroom i.e., 10-15 % bioefficiency. When the mushroom production is stopped the straw can be composted into manure. This mushroom is known for its excellent aroma and very good taste.

