MANAGEMENT OF TOMATO MOSAIC VIRUS THROUGH ALLIUM CEPA, CALOTROPIS PROCERA AND AZADIRACHTA INDICA IN RELATION TO EPIDEMIOLOGICAL CONDITIONS

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Recommended Pesticides or products or fertilizers or cure:

Some recommended for tomato mosaic virus – perfekthion 40 ES , Geolife No-virus biostimulant viro raze , vanproz v-bind viricide

Symptoms:

Mottling: The foliage of affected tomato plants shows mottling, with alternating yellowish and darker green areas1. These darker green areas often appear thicker and raised, giving a blister-like appearance12.

Distorted Leaves: The leaves tend to be fern-like in appearance with pointed tips1. Younger leaves may be twisted12.

Fruit Symptoms: The fruit may be distorted, with yellow blotches and necrotic spots occurring on both ripe and green fruit1. There may also be internal browning of the fruit wall1. Infected fruit appears mottled and develops raised "warty" areas2.

Stunted Growth: In young plants, the infection reduces the set of fruit and may cause distortions and blemishes1. The entire plant may be dwarfed and the flowers discoloured1.

Other Symptoms: Other symptoms include abnormally shaped fruit, fruit lesions, reduced fruit size, distorted growing points, abnormal color (often yellowing), form, and patterns on leaves, distorted stems, and distortion and dwarfing of the plant as a whole3.

What Is Tomato Mosaic Virus And How Does It Harms Tomato Plants?

Tomato mosaic virus (ToMV) is one of the oldest plant pathogenic viruses that is contagious. It is seed-borne and overwinters in seed coats, plant debris, and soil. It may affect the tomato at any stage of growth.

All parts of the plant, including stems, leaves, and fruit will be affected.

How To Confirm That Tomato Mosaic Virus Is Troubling Your Tomato Plants?

Inspect the leaves of tomato plants. A typical characteristic of ToMV is a pale green or yellowish leaf mottling effect on mature leaves. The leaflets may be fern-like in appearance, wrinkled, and reduced in size.

You may notice reductions in fruit size and internal brown lesions in the fruit.

Overall, growth will be stunted. It is recommended to get a firm diagnosis from a lab.

Natural Ways To Control Tomato Mosaic Virus On Tomato Plants

ToMV is highly contagious and is difficult to save the plant after severe viral infections. As it is spread by several insects, you may try sprinkling diatomaceous earth and neem oil to deter these insects around tomato plants.

Method 1- Sprinkle Diatomaceous Earth Powder

Diatomaceous earth is a natural, mined product that contains the fossilized remains of diatoms. It is a fine powder that is harmless to larger animals.

It kills the pests, including aphids, whiteflies, and mites, by dehydrating them.

- **Sprinkle DE powder**– Wear gloves and a mask while handling DE powder. Sprinkle food-grade DE powder around the base of the plant. You may also dust them on the leaves.
- **Repeat application**– Repeat the application as required or after rain. It helps in deterring pests that carry ToMV infection.
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Method 2- Spray Neem Oil

Neem oil is a natural measure that has been used to control many pests and diseases. It interferes with feeding, molting, mating, and egg-laying. It can be sprayed on tomato plants to deter the pests that carry ToMV.

- **Prepare the neem oil**– Add one teaspoon of dish detergent to a gallon of warm water. Mix thoroughly and add a tablespoon of neem oil. Shake well.
- **Spray the solution**– Spray the plant surfaces until completely wet. Avoid applying in the bright sun as it may burn the foliage.

Physical Ways To Control Tomato Mosaic Virus On Tomato Plants

The most effective way of controlling the spread of ToMV is pruning and removal of infected parts of the plants. It helps in preventing the spread of the virus to the rest of the garden.

1. Prune The Affected Parts

Remove the parts of the plant that you suspect may be infected with a virus. Destroy or burn them to prevent the spread of the virus.

- **Prune the affected parts**– Remove the infected parts using sanitized tools. Destroy the affected parts and do not throw them in the compost pile.
- **Disinfect the gardening tools**-Disinfect the gardening tools after every use in 1:9 dilution of germicidal bleach or an antiviral disinfectant. Also, wash your hands with soap and hot water.

- **Monitor the rest of the plants**– Monitor the other parts of the plant for infection.

Chemical Ways To Control Tomato Mosaic Virus On Tomato Plants

Currently, there are no chemical measures available to treat or manage TomV. Most chemicals used to reduce pest transmission are not feasible for home gardens. You may practice good sanitation practices to prevent the spread of infection to the rest of the garden.

How To Prevent Tomato Mosaic Virus In Tomato Plants?

Prevention is key to the control of the tomato mosaic virus. You may clear the infected debris, start the season with un-infected transplants or disease-resistant varieties, and practice crop rotation. Wash the hands and disinfect tools between working on the plant.

1. Practice Sanitation

Remove the infected plant debris and seedlings that appear stunted or distorted. Sanitize the tools by boiling them for five minutes and washing them with a strong detergent. Take care to remove weeds around the tomatoes.

2. Follow Crop Rotation

Practice crop rotation to prevent the ToMV infection. Do not plant tomatoes that are susceptible to ToMV in the same area again as these viruses may be overwintering in the soil.

3. Use Row Covers

As insects spread tomato mosaic viruses, you may cover the tomato plants with a floating row cover to prevent the insects from attacking your plants.

4. row Resistant Varieties

There are some resistant tomato varieties for ToMV and you may grow them, especially if your area has susceptibility to ToMV. You may check for resistance in a seed catalog.

Also, use the certified disease-free seeds or treat your seeds to get un-infected transplants.

What Causes Tomato Mosaic Virus Attacks In Tomato Plants?

The transmission of ToMV to healthy tomato plants is through contact and seeds. Also, it is spread via different insects, including aphids, leafhoppers, whiteflies, and cucumber beetles.

Leftover plant debris and weeds are important causes of disease spread.

Also, mechanical injury and divisions from infected plants may cause the virus attacks in tomato plants.

CHAPTER 1

INTRODUCTION

The family *Solanaceae* is an important source of vegetables and desert crops, which include potato (*Solanum tuberosum*), eggplant (*Solanum melangena*), various peppers (*Capsicum sp.*) and tomato (*Lycopersicon esculentum* Mill). The tomato is widely grown vegetable of the world after potato and ranked second in vegetables. Tomato is cultivated as perennials as well as annuals (Yamaguchi, 1983). The growth type of tomato include indeterminate lending itself to side shooting (vining), semi determinate, determinate forming a bushy and dwarf plant.

Botanically, tomato fruit is a berry with 2-9 loculi, containing seeds imbedded in a gelatinous matrix that soften a fruit reach maturity and seeds are fully developed. Ripe fruit may be red, pink, yellow or orange, round or plum shaped and of various sizes depending upon the cultivar. The ripe tomato contain 93 to 94% water and good source of vitamin A, B and C (Walts and Walts, 1944; Khoso, 1988).

Tomato is rich source of vitamin, mineral, essential amino acids, sugars and dietary fibers. It contain mostly lycopene which is an anti-oxidant that provide protection against various carcinogenic substances that are harmful for consumption. In maintaining good health and wound healing tomato has very important role (Cohn and Stompy, 1970). Tomato has also been reported to be suitable for diabetic patients (Mayer and Croll, 1921) and it exerts a better effect on urinary acidity as compared to orange juice (Saywell and Lane, 1933). These are used either raw or as salad or cooked with meat, fish and other vegetables. These are processed into soups, conserves, pickles, ketchups, sauces, etc. After processing, oil can be extracted from the seed and residual seed cake used for animal feeds (Helyes and Lugasi, 2006).

Tomato is native to Central, South and Southern America extending from Mexico to Peru (Smith, 1994). Initially the tomato was cultivated in Mexico and it was believed that in mid-16th century the Spanish explorer Cortez may had been the first that transferred it to Europe (Glick *et al.*, 2009). Tomato is native to Central, South and Southern America

extending from Mexico to Peru (Smith, 1994). It is used as a fruit in America and Europe whereas in Asian countries tomato is consumed as vegetable.

In Pakistan tomato nurseries in plains are generally raised in July/August or October/November and transplanted in the field is done in August/September or February/March depending on environmental conditions (Mehmood *et al.*, 1995). In the high hills, where tomato is grown as a summer crop, seeds are planted for raising nursery in March/April and the seedling are transplanted usually transplanted in May/June (Hameed, 1995).

The tomato is grown all over the Pakistan under diverse ecological, environment and soil conditions. Tomatoes grow well on fertile, well-drained soil (Splittstoesser, 1978). It occupies an area of 53.4 thousands hectares with the production of 561.9 thousand tons (Tahir *et al.*, 2012). The world total area under tomato cultivation is 3.7 million hectares with the production of 16.1793834 million tons, 144 countries are known for the tomato production. China is top most producer with area under cultivation (1255.1 thousand ha) and with the tomato fruit production of 30,102,040 million tons. United State is the largest producer of tomato in the world then China, Turkey, Italy and India are second third fourth and fifth producer of tomato, respectively (Engindeniz, 2007). According to Food and Agriculture Organization (FAOSTAT, 2012), tomato production in Pakistan is 0.56 million tons in 2012, while global trade enlarged to $4.3 billion.

The average yields of tomato in Pakistan is very low as compared to other countries of the world for which many factors are responsible. Among these, diseases of viral nature are of great importance because no viricides are available for their management. More than 20 viruses known to infect tomato in the world with losses up to 20-90 % by different viruses have been reported (Hameed, 1995).

At least seven viral diseases are known to be present in Pakistan; tomato mosaic virus (ToMV), tomato leaf curl virus (TLCV), potato virus X (PVX), cucumber mosaic virus (CMV), tomato yellow top virus (TYTV), tomato spotted wilt virus (TSWV) and tomato ring spot virus (TRSV) (Mughal, 1985).

Among the virus prevalent, tomato mosaic virus is the most important and commonly associated with tomato crop and distributed throughout Pakistan. Its symptoms can be found during any growth stage and all plant parts are affected generally, infected plants have a light or dark green mottling or mosaic with distortion of younger leaves, and stunting to varying degrees. Severely affected leaves may have "fernlike" appearance and may show raised dark green areas. Fruit set may be severely reduced in affected plants. There is the internal browning of the fruit wall, yellow blotches and necrotic spots may occur on green or ripe fruit. Some strains cause yellow mottling of the leaves, other cause dark necrotic streaks in stems, petioles, leaves or fruits. Symptoms are influenced by environmental conditions such as day length, temperature, and light intensity as well as by variety, plant age at infection, and virulence of tomato mosaic virus (ToMV) strain. On susceptible cultivars, symptoms may range from severe to none. ToMV tentatively identified on the basis of symptoms developed in the infected plants. An average incidence of 29.79 and 25.49% of ToMV was recorded in tomato leaves and seeds, respectively (Khan, 1997).

This disease is caused by a tobamovirus; virions consists of a capsid, which is not enveloped. Capsid / nucleocapsid is elongated with helical symmetry. The capsid is rod shaped, straight with a clear model length of 300 nm and a width of 18 nm. Axial canal is distinct, 4 nm is diameter. Basic helix is obvious. Pitch of the helix is 2.3 nm. The genome is not segmented and contain a single molecule of linear positive sense, single stranded RNA. The complete genome is 6383 nucleotides long and is fully sequenced. Virus has no known vector. But transmitted by mechanical inoculation; grafting; by contact between hosts and by seeds.

Tomato mosaic virus is transmitted by mechanical ways and not vector transmitted (Osmond, 2003). Certain control measures have been seen effective in reducing disease severity. In addition to resistant varieties, an alternative mean of protection should be taken into account to resolve the tomato mosaic virus problem suffered by tomato growers (Eraslan *et al.*, 2007).

Environmental factors act as a pin point in disease occurrence, spread and severity as there is a strong relationship between epidemiological factors and disease development. So

keeping in view favorable environmental conditions, suitable management practices could be applied to manage the diseases. Present studies were designed to investigate the effect of environmental conditions with tomato mosaic virus because the symptoms of the virus may appear at the conducive environmental conditions, during the growing season. So it is hypothesized that there may be correlation of epidemiological factors with ToMV.

Keeping in view the above mentioned facts, the present study was conducted with following objectives.

1) To evaluate tomato germplasm under natural field condition to identify the resistance / susceptibility against tomato mosaic virus.
2) To determine the environmental conditions conducive to ToMV in the field.
3) Evaluation of different plant extracts for the management of tomato mosaic virus disease.

To achieve the above objectives following line of work was followed:

1. Collection of tomato germplasm.
2. Establishment of disease screening nursery under field conditions.
3. Collection and making different concentration of plant extracts.
4. Data recording regarding the epidemiological factors and tomato mosaic virus on the basis of disease incidence.
5. Evaluation of plant extracts for the management of ToMV.

CHAPTER 2

REVIEW OF LITERATURE

Dorais *et al.,* (2008) stated that tomato (*Solanum lycopersicum*) is the second most significant vegetable after potato in the world. Tomato crop in Pakistan confronted with a number of biotic and environment stresses due to which crop yield far remain lower than the potential yield. The major biotic constrains are insects, diseases and weeds. Some insects and weeds could be managed both by the chemical and non-chemical means. However, disease is particular viral disease can be effectively managed through genetic mean. ToMV has attained the status of the second biggest threat to tomato production after tomato yellow leaf curl begomovius. Some reports are available which have covered the different aspects of ToMV, but this threat yet to be explored from different angles to build sound strategy for its management.

2.1. History of ToMV

El-Hammady *et al.,* (1983) described that virus disease were most serious diseases which reduced the quality and yield of such crops.

Hull and Davies (1992) stated that viral diseases of plants were known to cause enormous economic losses, particularly in the tropics and semi tropics, which provide ideal condition for the perpetuation of viruses. Viruses due to plant viruses had also been reported in temperate region of the world. Virus diseases are most responsible factors for reducing the production (Salazar, 1996).

Nono-Womdium (2001) stated that among various economically important diseases of vegetables, tomato mosaic virus, tomato spotted wilt virus, chili leaf curl virus, pepper veinal mottle virus and tomato leaf curl virus were considered as economically most significant in African and Asian countries.

Empress Koken in 752 AD described a virus disease in earliest literature by written a Japanese poem and translated by Inouye and Osaki in 1980 and recently reported by Saunders *et al.,* (2003).

Arli-Sokmen *et al.,* (2006) conducted surveys in 31 fields in Samsun province from May to August in 2002 and 2003. Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), Potato virus X (PVX) and Cucumber mosaic virus (CMV) were found in tomatoes after analyzing 186 samples through ELISA. ToMV, TSWV, PVX and CMV were detected 52.1, 12.9, 10.7 and 6.9% of the sample tested, respectively. Thirty samples (16.1%) were found to be mixed infected with these viruses. The most widespread and predominant virus (ToMV) also occurred in mixed infections with TSWV (6.4%), PVX (4.9%) and CMV (3.2%) in this study.

2.2. TOBAMOVIRUS

2.2. 1. What are Viruses

A virus is an infectious, submicroscopic (i.e. light microscopically invisible), filterable, non- cellular agent that multiplies only in living cells and often cause disease, it is an intra cellular obligate parasite with no energy producing metabolism of its own. Its particles (the virions or infectious unit) consists of a core of nucleic acid and composed of one or more molecules of either RNA or DNA, usually surrounded by a protective coat which composed of one or few protein and sometime an extra lipoprotein envelope.

On the bases of symptoms major tomato viruses which have been reported to occur in tropical Africa form two groups, i.e. Leaf curl-causing viruses, and mosaic symptom-causing viruses (Brunt *et al.,* 1990).

Tomato is infected by 146 viruses worldwide (Green, 1991). These viruses are grouped into 33 genera, out of these 15 genera are most economically most important i.e. Tobamovirus, Alfalfamovirus, Begomovirus, Carlavirus, Crinivirus, Cucumovirus, Ilarvirus, Luteovirus, Nepovirus, Potexvirus, Potyvirus, Tombusvirus, Topocuvirus, Tospovirus and Tymovirus.

Nono-Womdim (1994) reported that major tomato viruses in tropical Africa fall into five genera, i.e. Tobamovirus, Cucumovirus, Tospovirus, Potyvirus and Begomovirus. More than 20 viruses infect tomato in the world and losses up to 20-90% by different viruses have been reported (Hameed, 1995).

There are about 650 known plant viruses (75% of the plant viruses which are economically important pathogen, possess single stranded positive sense RNA genome, other have genome of negative strand RNA, single stranded DNA or double stranded DNA (Harrison and Murrant, 1996).

No virus was found from the genera of Alfamovirus, Potexvirus or Clesterovirus, which have been recorded to occur on tomato in Europe (Wisler *et al.*, 1998).

Viruses may be undivided or monopartite genome i.e. one molecule of nucleic acid, mostly packaged in one or few types of protein. Several viruses have a genome consisting of a number of different nucleic acid molecules. They have divided or segmented genome (the genome then is multipartite). The segments may be enclosed together in one type of coat. Often, however they are packaged separately or in certain combination. Then there is no single type particle virion, such viruses are multipartite (Boss, 1999).

In Pakistan at least seven viruses are present, tomato mosaic virus (ToMV), tomato ring spot virus (TRSV), tomato leaf curl virus (TLCV), cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), and potato virus X (PVX), tomato yellow top virus (TYTV) (Imran *et al.*, 2013).

2.2.2. Genus Tobamovirus

Infection of many vegetables and ornamental plants with Tobamovirus cause economic losses in all parts of the world. Originally, the differentiation of the genus tobamovirus into the species was based on the amino acid composition of their CP (Tsugita, 1962).

Van Regenmortal (1975) demonstrated, that with the establishment of serological methods, the serological differentiation was correlated with the amino acid composition.

Genus tobamovirus species are classified to subgroups according to the location of their origin of assembly. The origin of assembly of one subgroup is located within the open reading frame (ORF) of the movement protein (MP) gene, whereas the origin of assembly of other subgroup is located within the coat protein gene (CP) ORF. Subsequently, the sub

genomic CP mRNA of viruses of the latter subgroup is encapsidated leading to the presence of short particles (Whitfield and Higgins, 1976). The genus Tobamovirus consists of 14 accepted and 5 tentative species (Fukuda *et al.*, 1981. Van Regenmortel *et al.*, 2000).

Tomato mosaic virus (ToMV) is the type species of this genus. Another species of this genus is tobacco mosaic virus (TMV). ToMV has been found as an aerosol in fog in USA and in nutrient solution used for crop cultivation in Apulia, Italy (Gallitelli *et al.*, 1982;Pares *et al.*, 1992) and in Spain (Cordero and Gaborjanyi, 1983). Heterologously reacting antibodies in polyclonal antisera are present in tomato mosaic virus (Van Regenmortal, 1986).

Tomato mosaic virus (ToMV) has been reported in Tanzania, Malawi and Zambia (AVRDC, 1987; Nono-Womdim, 1994). ToMV was also occurring in Uganda, where reports of its occurrence were actually based on symptomology (Hansen, 1990).

The subgroup 1 viruses' tomato mosaic viruses (ToMV), tobacco mosaic virus (TMV), pepper mild mottle virus (PMMV), tobacco mild green mottle virus (TMGMV) and odontoglossum ring spot virus (ORSV) mainly infect solanaceous plants except ORSV which is prevalent virus in orchards (Park *et al.*, 1990). Subgroup contains the species cucumber green mottle mosaic virus (CGMMV) and sunn-hemp mosaic virus (SHMV) infecting cucurbits and legumes, respectively.

These viruses have elongated particles of 300 X 18 nm and contain RNA molecule. Viral particles are found in trichomes and epidermal cells of infected plants and occur in hexagonal, crystalline array (Green and Kim, 1991).

In practical diagnostic work sometimes the detection of presence of a tobamovirus may be sufficient. However foe epidemiological investigations and for resistance breeding as well as for plant virus collection the determination of the species or even the differentiation in the pathotypes is desirable. Tenllado *et al.*, (1994) developed for this purpose a RT-PCR-RFLP to separate the pathotypes PMMV1, 2 (=PMMV-s) and PMMV1, 2, 3 (=PMMV-i), that are serologically indistinguishable.

Tomato mosaic virus (ToMV) is the type species of the genus tobamovirus. Another species of this genus is tobacco mosaic virus (TMV). ToMV has been found as an aerosol in fog in USA (Castello *et al.,* 1995). The distinction of the species within a subgroup depend on a careful selection of antisera therefore, it is time consuming and laborious (Lesemann and winter, 1998).

Jacobi *et al.,* (1998) developed a multiplex RT-PCR to distinguish TMV and ToMV, which are both members of the subgroup 1 and Eun *et al.,* (2000) described the simultaneous detection of two orchid viruses, the cymbidium mosaic potexvirus and the odontoglossum ringspot tobamovirus by real time RT-PCR. However, no RT-PCR was described yet which can distinguish all virus species within the subgroup 1.

2.3. Symptomology of ToMV

Broadbent (1964) studied that tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) are member of tobamovirus group and separated by in their serological affinities and protein composition. Although TMV infect tomato, ToMV is predominant virus worldwide in tomato crops. Infection of tomato mosaic virus (ToMV) caused up to 10-50% yield losses in fresh market. Yield was reduced because there were few fruit per plant as well as small fruit size. Time of infection is responsible to the extent of yield losses.

Tomato mosaic Tobamovirus (ToMV) is a stable and wide-spread RNA virus that infects plant species (Hollings and Huttinga, 1976), it generally causes mosaic and leaf curling on leaves, uneven ripening or internal browning or brown wall on fruits of certain varieties.

Broadbent (1976) reported that ToMV is a very stable and propagate mechanically with high efficiency. After symptoms appear on the initial source of infection, the control of ToMV was very difficult, as it spreads quickly as a result of cultural practices like pruning.

ToMV strains include those, which cause corky ring, crusty fruit, yellow streak and aucuba symptoms (Kang *et al.,* 1981; Jones *et al.,* 1991).

Van Regenmortal (1981) reported that tomato mosaic virus was mechanically transmitted from tomato to tomato with an efficiency of 100% and produced mild to severe mosaic symptoms in tomato. Infected tobacco leaves showed light and dark green mottling, together with distortion and smaller size. The virus produce necrotic local lesions on *Datura metel, D. stramonium, Gomphrena globosa, Nicotiana tabacum* cvs. White Burley, Riwaka-1, L-1158, CTRI special, *N. glutinosa* and *N. syveltris*. It produced local necrotic lesions on *Chenopodium amaranticolor*. TMV gives systemic infection of *N. sylvestris* whereas ToMV produced necrotic lesions.

Wetter (1984) identified four tobamoviruses which cause infection on pepper cultivars, through immunodiffusion tests and host plant reactions. The white mosaic virus strain (ATCC PV 230) was identified as a strain of type tobacco mosaic virus (TMV). It was very similar in host reaction to the yellow strain of TMV from *Nicotiana gluca* (ATCC PV 223), which was also shown to be a strain of type TMV. The South Carolina mottling strain of TMV (ATCC PV 228), was identified as a strain of para tobacco mosaic virus (PTMV = strain U2 = G-TAMV). McKinney's latent strain of TMV from South Carolina (ATCC PV 227) was found to be very similar if not identical to the newly described pepper mild mottle virus (PMMV) from Sicily. Another virus sample from Italy contained PMMV and tomato mosaic virus (ToMV).

Rast (1985) identified and isolated, the pathogenic strains of tomato mosaic virus by host passage. Three isolates of tomato mosaic virus, A.8, SJ-64 and SL a, assumed to contain the pathogenic strain 1 and 2, were each subjected to selection pressure by passage through different hosts on concurrent series.

Avgelis (1986) studied the virus diseases on tomatoes grown in plastic house in Crete. Plants with virus like symptoms were checked by sap inoculation to test plants and the isolated viruses were identified by host reaction and serology. The most common viruses were tomato mosaic viruses (ToMV), potato virus X (PVX), tomato bushy stunt virus (TBSV), potato virus Y (PVY) and cucumber mosaic virus. The large use of ToMV resistant cultivars reduces gradually the importance of ToMV while TBSV tends to become a serious problem of tomato in Crete.

Green *et al.,* (1987) reported that strains 0, 1, and 2 were the only naturally occurring ToMV strains detected on tomato during a survey conducted between 1980 and 1982 in the major tomato producing areas of Taiwan. Strains 0 and 1 were most frequently found, whereas strain 2 occurred very rarely. It was shown that ToMV persisted only up to 5 months in the soil after the tomato harvest, which occurs in late winter to early spring. Since the cropping pattern in Taiwan generally allows an interval of at least 6 to 7 months between tomato crops during which time usually non solanaceous crops are grown, treatment of the soil does not appear necessary. The major source of tomato mosaic virus infection for the tomato crops appears to be seed borne ToMV. More than 85% of different seed samples of locally grown commercial tomato cultivars were found to contain ToMV.

Among the numerous disease effecting tomato crop, the most serios and alarming are virus diseases caused by Tobamovirus not assigned to a family transmitted by contact and by seed and may survive on leaf and root debris in the soil for up to two years under favourable conditions (Green and Kim, 1991).

The common ToMV symptoms include mosaic, systemic chlorosis, local necrotic lesions, leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause death (Brunt *et al.*, 1990; Jones *et al.,* 1991). It is also sap-transmissible and composition (Brunt *et al.,* 1990).

Candilo *et al.,* (1992) studied that tomato mosaic virus Tobamovirus (ToMV) provokes a serious disease in tomato plants (*Solanum lycopersicon* L.), especially in susceptible cultivars. The yield of infected susceptible cultivars can be reduced by up to 25%.

Hassan *et al.,* (1993) studies the aspects of tomato mosaic viruses and tentatively diagnosed on the basis of characteristics and typical symptoms. These included Tomato mosaic virus, Cucumber mosaic virus, Tomato yellow leaf curl virus, Tomato yellow top virus, Potato leaf roll virus, Potato virus Y and X, Tomato bushy stunt virus, Tomato aspermy virus and Tomato ring spot virus. Both single virus and mixed virus infections were found. These viral infections were confirmed and verified through serological (Indirect enzyme-linked immunosorbent assay (ELISA) and double diffusion test) and biological (host

range, transmission to indicator hosts and transmission properties) infectivity essay. Tomato mosaic virus the most destructive and widely prevalent virus in the area.

Hassan, (1994) conducted experiment to determine the incidence, distribution and losses due to viral diseases in Malakand Agency. An average seedling infection of 30%, 15% and 20% of tomato mosaic virus, potato virus X (PVX) and potato virus Y (PVY) was recorded respectively in tomato nurseries. Mean incidence of ToMV, Cucumber mosaic virus (CMV), Tomato yellow leaf curl virus (TYLCV), Tomato yellow top virus (TYTV), Tomato bushy stunt virus (TBSV), Tomato aspermy virus (TAV), Potato leaf roll virus (PLRV) and Tomato ring spot virus (ToRSV) on the basis of symptom expression and serodiagnosis was 34.38, 12.92, 15.06, 8.26, 4.05, 1.48, 3.90 and 3.70 percent respectively. The predominant symptom observed were mosaic, mottling, curling, bushy growth, chlorosis, shoot proliferation and a variety of mixed symptoms. A reduction of 22.24% in fruit weight, 15.38% to 78.56% in fruit number and 22.77% in plant height recorded, resulting in significant decrease in total yield.

Schuerger and Hammer (1995) studied that tomato mosaic virus (ToMV) induced severe root, stem and foliar symptoms. When pepper plants were root inoculated during warm summer months as compared with when plants were root inoculated in cooler winter months. In growth chamber experiments, the effects of temperature on root or foliar inoculated peppers were similar the greatest difference between inoculated and non-inoculated plants occurred at 24 ^{0}C. Moderate or severe symptoms failed to develop on either root or foliar inoculated plants incubated at 18 ^{0}C but were observed on foliar inoculated plants incubated at 32 ^{0}C. The severity of foliar systemic symptoms and the rate of disease development were greatest in foliar inoculated pepper plants incubated at 24 ^{0}C.

Murphy *et al.*, (1995) reported that the mosaic of tomato crop caused by a specific virus species was classified in tobamovirus genus. The infected tomato plants show light and dark green mottled areas on the leaves and fruit may be reduced in size and number with uneven ripening. The disease disseminated through infected tools and seeds, which control was more difficult. It was very important in Europe. Three tomato mosaic Tobamovirus

strains (ToMV) were reported and one had spread out in Sao Paulo plantations where 30% of the tomato was harvested.

Samad and Khatijah (1996) observed a mosaic disease of tomato in Cameron Highlands, Malaysia and a tobamovirus was implicated as the caused based on virus particle morphology and reproduction of symptoms in *Lycopersicon esculentum* Mill. The virus was identified as tomato mosaic tobamovirus (ToMV) based on host range and serological properties.

Bachand *et al.,* (1996) studied that infection of ToMV on red spruce seedling caused 50% reduction in rate of increase of height, weight and root volume compared with that of non-infected seedling, but the freezing tolerance of the infected seedling was greater than that of non-infected seedlings.

Khan (1997) conducted survey in the tomato growing area and recorded the incidence of ToMV virus on tomato. An average incidene of 29.79 and 25.49% was recorded in tomato leaves and seeds, respectively. ToMV was transmitted to indicator host by rub inoculation and was purified in 0.2 M Phosphate pH 7.0, successfully. Identification of ToMV virus was confirmed through different serological techniques and electron microscopic studies. ToMV virus reacted with homologous antisera in gel diffusion tests.

Ganoo and Saumtally (1998) studied the incidence of viruses' diseases of tomato (*Lycopersicon esculentum*) in Mauritius. Two virus diseases, tomato mosaic virus (ToMV) and potato virus Y (PVY) were found to be widespread. The spread of two viruses was followed in a field at Medine on varieties Sirius and MST 32/1. Measures such a seed treatment, reducing plant density and frequent handling of plants and avoiding clipping of plants resulted into practically virus free plantations.

George *et al.,* (1998) studied the mechanism of infection and the seasonal pattern of tomato mosaic virus concentration in seedling roots and needles. One year old red spruce seedling were obtained from the nursery in April and June 1995 and August 1996 and tested for ToMV. Virus free seedling were divided into three treatments: control, root inoculated and needle inoculated. Two control, five root inoculated and five needle inoculated seedling

were sampled destructively at biweekly intervals for three months and then tested for ToMV by ELISA. ToMV was transmitted to seedling by root but not by needle inoculation. The virus was detected in 67 to 100% of roots but in less than 7% of needles of root inoculated seedling. The percent infection of root inoculated seedling differed significantly between the April and June and between the April and August inoculation periods. Virus concentration in infected seedling roots increased initially, peaked within 4 weeks post inoculation and steadily declined thereafter. Significant differences in ToMV concentration in roots also were detected among inoculation periods and sampling dates. Early spring may represent the optimal time for infection of seedlings, as well as for assaying roots for ToMV.

Cherian *et al.*, (1999) reported that tomato mosaic tobamovirus (ToMV) differ from the strain of tobacco mosaic virus (TMV) in producing local lesions instead of systemic infection on *Nicotiana sylvestris*. An isolate collected from Kolar district of Karnataka which produced this differential host reaction was propagated in the greenhouse on *N. tabacum* cv. Samsun and purified. The virus was a rigid rod shaped particle with a coat protein of molecular weight 18 KDa and genomic RNA of size 6.3 Kb.

Mayo *et al.*, (2000) described the mechanical transmission of potato leaf roll virus. They used the extract from infected leaves with PLRV and inoculated the *Nicotiana banthemae* and then post inoculated with PEMV-2. They observed the necrotic symptoms with same line patterning and vein yellowing.

Citovsky and Zambryski, (2000) reported that may vegetable and ornamental crops infected by tobamoviruses. Infections usually gave a characteristics mosaic symptoms and lead to considerable cosmetic damage and yield losses. Tobamoviruses, including tobacco and tomato mosaic virus (TMV and ToMV, respectively) belong to alpha like super group of viruses. They consist of a characteristics proteinaceous rod, made up of 2140 coat protein (CP) copies, which envelope the positive stranded linear RNA genome. After infection of the plant cell the RNA genome is uncoated and the viral gene products, the RNA dependent RNA polymerase (RDRp), the movement protein (MP) and the coat protein (CP) are produced. Infection of neighboring cells commences with the movement of RNA-MP complexes through plasmodesmata with MP-induced altered size exclusion limits.

Chen *et al.,* (2001) isolated the tobamovirus from Hibiscus (*Hibiscus rosa sinensis* L.) which was popular woody ornamental commonly used as fence or potted plants in Taiwan showing vein yellowing and mosaic.

Marathe *et al.,* (2002) reported that Tobamovirus can infect many vegetables and ornamental crops usually giving rise to characteristic mosaic symptom and growth reduction. Consequently, infection of susceptible agronomical important species, like tomato and tobacco, considerable yield losses. Because the virus particle were extremely stable and highly infectious prevention against virus infection is difficult. The most effective defense against infections is therefore, the introduction of genetic resistance from closely related resistant species.

Hadas *et al.,* (2004) described an indexing system for detecting tomato mosaic virus (ToMV) in commercial tomato seed lots. Factors associated with the procedure were analyzed and the following standard two-step working scheme is proposed: (1) mass screening by Elisa for the presence of the virus (2) evaluation of the virus infectivity within the infested seed lots.

Jan *et al.,* (2005) isolated a virus culture from infected Lisianthus (*Eustoma grandiflorum*) plants from Chiayi Country, Taiwan and established and maintained in systemic hosts *Nicotiana tabacum* and *N. benthamiana*. Chlorotic and necrotic spots developed on Lisianthus leaves 1-2 weeks after inoculation with the virus while symptoms became eventually systemic. Results from sequence analysis and diagnosis based on host reaction to virus inoculation indicated that the tobamovirus infecting Lisianthus in Taiwan was an isolate of tomato mosaic virus (ToMV). This was the first report on ToMV causing disease on Lisianthus in Taiwan.

Ssekyewa (2006) conducted a survey symptoms observed on tomato were categorized and screened for both ribonucleic and deoxyribonucleic acid tomato viruses. Genetic identify for one main virus disease was investigated. Major symptoms observed on tomato included leaf curl, mosaic and mottling in descending order of importance. Ribonucleic acid viruses i.e Tomato mosaic virus (ToMV), Cucumber mosaic virus (CMV), Alfafa mosaic virus (AMV), Pepper veinal mottle virus (PVMV), Potato virus Y (PVY), Potato virus X (PVX), Tomato

spotted wilt virus (TSWV) and Chili veinal mottle virus (Chi VMV), as well as deoxyribonucleic acid viruses, i.e Tomato yellow leaf curl virus (TYLCV-UG) and Tomato leaf curl virus (ToLCV-UG) were identified. Virus incidence varied in space and time, and with management practices, crop development stage and weather conditions.

Arli-Sokmen *et al.,* (2006) conducted surveys in 31 fields in Samsun province from May to August 2002 and 2003. Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), Potato virus X (PVX) and Cucumber mosaic virus (CMV) were detected in tomatoes after analyzing 186 samples by enzyme linked immunosorbent essay (ELISA). ToMV, TSWV, PVX and CMV were detected 52.1, 12.9, 10.7 and 6.9% of the samples tested, respectively. Thirty samples (16.1%) were found to be mixed infected with these viruses. The most widespread and predominant virus ToMV also occurred in mixed infections with TSWV (6.4%), PVX (4.9%) and CMV (3.2%) in their study.

Kamenova *et al.,* (2006) identified the tomato mosaic virus in jasmine. They observed virus like symptoms on leaves of landscape and nursery downy and star jasmine (*jasminum multiflorum*) and wax jasmine (*J. gracile)* in southeast Florida. Foliar symptoms included mottling, chlorotic ring spot and chlorotic line pattern. An agent was mechanically transmitted with difficulty from from symptomatic leaves of downy jasmine to *Nicotiana debneyi* and *N. tabacum* 'Xanthi' and subsequently from these hosts to *Chenopodium quinoa* and other herbaceous test plants. Virions were isolated from *N. tabacum* 'Xanthi'. A rod shaped particle (297 x 18 nm) similar to Tobamoviruses was observed in particularly purified virus preparations.

Duarte *et al.,* (2007) isolated ToMV from different hosts, including some ornamentals, from different geographical locations. The result indicate that *Hamerocallis sp.* and *i. hawkeri* infected by ToMV. This was the first report of the ocurance of this virus in ornamental species in Brazil. The tobamovirus was isolated from *Hemerocallis* sp. (tobomo-H) showing necrotic spots, and from *I. hawkeri* (tobamo-I) exhibiting mosaic and leaf deformation.

Pfitzner (2007) reported that tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) cause a serious disease in tomato, with systemic mosaic symptom and losses in fruit

yield and quality. Both viruses are closely related tobamoviruses, plus stranded RNA viruses with a rod like particle structure. The genomic structure of both viruses characterized, as a positive sense single-stranded RNA genome that encodes at least four proteins. In tomato TMV infection was more or less a rare event because the virus was soon competed out in tomato population by ToMV, which was more adapted to this host plant.

Balogun (2008) studied the seedling age at inoculation and infection sequence effect disease and growth responses in tomato mixed infected with potato virus X and tomato mosaic virus. The nursery days of after 14 days after germination, inoculation of 0.1 mg mL each of potato virus X (PVY, genus Potexvirus) and tomato mosaic virus (ToMV, genus tobamovirus) induced an acute symptomatic response and eventually death of over 90% of the plant population 15 days after inoculation. On the other hand, 28 days old plants inoculated either simultaneously, manifested only a synergistic disease response but no death were recorded.

Dawson (2008) cultured tomato mosaic virus derived from susceptible tomato plants (the standard virus) in resistant plants. Sap from non-inoculated leaves of resistant tomato plants infected with virus from the resistant host was more infective and contained more virus particles than leaf sap of resistant plants infected with the standard virus. Leaves of resistant tomatoes infected with virus from the resistant host also showed more obvious symptoms. Susceptible plants infected with virus from resistant plants not only shown fewer symptoms than when infected with standard virus, but samples were less infective and contained less virus up to 26 weeks when values for infectivity were similar.

Hirai *et al.,* (2008) reported that mosaic was a common disease symptom caused by caused by a virus infection in plants. Mosaic leaves of tomato mosaic virus (ToMV)-infected tobacco plants consists of yellow-green and dark green tissue that contain large and small number of virions, respectively.

Dawson (2008) studied the multiplication of tomato mosaic virus (strain of tobacco mosaic virus) in susceptible and resistant tomato plants by assaying the infectivity and virus content of sap from non-inoculated leaves on infected plants. In susceptible plants, infectivity and virus particle number increased rapidly to a maximum at about 14 days after inoculation;

leaves produced subsequently contained less virus. In resistant plant, no virus was detected in non-inoculated leaves until 5 weeks after inoculation. Infectivity then gradually increased at a somewhat higher rate than did number of virus particles. The concentration of virus in resistant plants remained lower than in susceptible ones. At first the ratio of infectivity to particle number was larger for samples from the susceptible than the resistant line increased slowly until it eventually equaled that from the susceptible one.

Alonso *et al.,* (2008) conducted survey during a four-year period (1982-1985) to study plant viruses' infection on pepper cultivars grown under plastic in the southeastern region of Spain, a tobamovirus was found to be the major disease agent of this crop. The virus produces slight or no symptoms on the leaves, but causes chlorotic mottling, malformation and reduction in size with occasional necrosis on the fruit and was able to infect all commercial pepper cultivars tested, including those resistant to other tobamovirus, causing a catastrophic disease.

2.4. Influence of Environment on Disease Intensity

Epidemiology of several plant diseases have helped researchers and extensions workers in many parts of the world to advice less capital intensive management strategies including supervised control operations. Research on epidemiological aspects to explain the dynamics of the disease under local condition was, therefore, of primary importance. Weather is one of the important parameter that influences plant disease epidemics. Understanding of climatic and weather conditions is required to provide base line information for developing simple and reliable disease prediction systems. There is not so much work done in relation to environmental conditions conducive for the tomato mosaic disease development. The impact of environmental conditions and their fluctuation in relation to inoculum build up and spread of disease is not quantitatively studied.

Hassan *et al.,* (1993) studied the epidemiology of tomato viruses and found that nursery plants were the initial and primary source of tomato mosaic virus (ToMV) infection due to high percentage of the infected seeds.

Hassan (1994) conducted experiment to investigate incidence, etiology, epidemiology of viruses infecting winter tomatoes in Malakand agency. Viruses identified on the basis of

serology and biology were tomato mosaic tobamovirus (ToMV), Potato Y potyvirus (PYPV), Potato X potexvirus (PXPV), Tomato yellow top virus (TYTV), Tomato yellow leaf curl bigeminivirus (TYLCV), Tomato ring spot nepovirus (TRSP), Tomato spotted wilt tospovirus (TSWV) and PLRV (Potato leaf roll luteovirus), mosaic, mottle, severe stunting, ern leaf and shoe string leaf lamina, leaf curling and rolling, ring spots and asymmetrical fruits were the characteristics symptoms of viral infections of tomatoes. Both single and mixed infections were prevalent into tomato crops. ToMV virus was detected in all seed lots and ToMV, PVY and PYV were found in tomato nurseries. Chillies *(Capsicum*), Okra, squashes, cucumber and several wild solanaceous and cucurbitaceous species served as alternative and reservoir hosts for tomato viruses.

Khan *et al.,* (1998) reported that weekly air temperature, rainfall, relative humidity and wind movement were regressed against percent plant infection by leaf curl virus on eight varieties of cotton. Relationship of weekly air temperature (max./min.), relative humidity and movement to cotton leaf curl virus disease (CLCuV) development was explained by linear regression in most of the varieties. Percent plant infection by CLCuV increased on all varieties at maximum and minimum air temperature of 33^0C-45^0C and 25^0C-30^0C respectively. There was poor correlation of rainfall to CLCuV disease development.

Colvin *et al.,* (1998) reported that African cassava mosaic disease incidence was highest on the edges facing the prevailing wind direction. So it was suggested that the use of ACMV resistant guard rows to protect a mainly susceptible crop.

Khan (2001) studied the disease severity of potato virus X, Y and potato leaf roll virus and aphid population recorded on weekly basis was subjected to correlation analysis with the maximum and minimum air temperature, relative humidity, rainfall, clouds and wind speed. The overall correlation of maximum (28^0C-42^0C) and minimum (15^0C-22^0C) air temperatures and wind speed (4-8 km/h) with disease severity of PVX, PVY and PLRV was significant. Rainfall (0.00-2.9 mm), relative humidity (40-70%) and clouds (0.00-3.43) has no correlation with disease severity. Maximum temperature, solar radiation, dew point and wind speed were significantly correlated with PLRV. When the data were split by variety the degree of correlation decreased and it differ greatly according to each variety.

Yu *et al.,* (2004) studied that tobacco mosaic virus and tomato mosaic virus are two closely related viruses belong to the genus tobamovirus, but they cause different sizes of necrotic lesions in tobacco plants comprising the N gene.

Ali *et al.,* (2005) determined correlation of environmental conditions (maximum and minimum temperature, relative humidity, rainfall, clouds and wind velocity) with okra yellow vein mosaic virus (OYVMV) disease severity on commercial growing varieties of okra i.e. Pahuja, Safal, Subz peri and Surkh Bhindi. Minimum temperature and relative humidity had significant correlation with OYVMV disease severity. Disease incidence increase with the rise temperature.

Broadbent *et al.,* (2008) studied the epidemiology of tomato mosaic. Tomato plants infected with Tomato Mosaic Virus by contact with infective clothing. Tomato Mosaic Virus persisted for more than three years on infective clothing that stored in a dark enclosed place, but it was inactivated within a few weeks in daylight, often being invisible after a month. Sometime persisting at low concentration for two months. They stated that the commercial glasshouse structures survey showed Tomato Mosaic Virus occasionally over wintered. Mostly tomato mosaic virus (ToMV) persisted in dry tomato leaf debris. That debris should be removed from structures by washing.

Tomlinson (2008) reported the ecological and epidemiological aspects that determine viral infection of vegetable crops. These include the reservoir and spread of viruses including horticultural and some agricultural practices that influenced their occurrence. In contrast, vegetables grown protectively are most important for the mechanically transmitted tobamovirus (tomato and tobacco mosaic, capsicum mosaic and cucumber green mottle mosaic).

2.5. Disease Management

Viral diseases are more difficult to manage than those caused by other pathogens because of the complex disease cycle, efficient transmission and lack of viricides. The effective management of viral diseases required integration of management practices such as

avoidance of source of infection and modification of cultural practices and resistance of host plant (Verma, 1976).

Broadbent (1976) reported that ToMV was the predominant virus worldwide in tomato crops. Infection by ToMV reduced yield in fresh market tomatoes by 10 to 50%. Yields were reduced because there were fewer fruits per plant as well as smaller fruits. The extent of the yield loss depends on the time of infection. The use of three ToMV resistance genes Tm-1, Tm-2 and Tm=2^2 , in commercial cultivar has been the most effective control measure against losses in fruit yield and quality caused by ToMV.

Fraser and Loughlin (1980) reported that the Tm-1 gene in tomato inhibits development of mosaic symptoms and multiplication of tomato mosaic virus (strain isolates). A virus isolate of strain 1 type caused mosaic symptoms on Tm-1 hosts almost as almost as severe as those it caused on susceptible hosts. However, multiplication of strain 1 virus (measured as accumulation of virus RNA or coat protein) was still partly in habited in Tm-1 hosts. Thus the two end effects of the Tm-1 gene were to some extent separable.

Fraser (1990) reported that virus disease cannot be cured and so searchers were focusing on controlling the infection by different ways, i.e., removal of weed hosts, starting with virus free planting materials and breeding of plant resistant to virus infection.

El-Afifi *et al.,* (2005) conducted experiment to establish a sample controlling system for tomato mosaic tobamovirus (ToMV) and potato Y potyvirus (common strain) PVY viruses depending on the production of tobacco transgenic plants expressing viral coat protein. Plants resistance was tested by challenging with the viruses under study, remarkable success was obtained as 20% and 36% of transformed tobacco plants blocked of transformed tobacco plants blocked viral infection for ToMV and PVY.

Strasser and Pfitzer (2007) studied that tomato mosaic virus (ToMV) causes a serious loss of yield and fruit quality in tomato crops. To control ToMV, three resistance genes, Tm-1, Tm-2 and Tm-2² from wild tomato species were introduced into commercial tomato cultivars.

2.5.1. Screening of resistant varieties

Gates and Mckeen (1972) studied the resistance or tolerance of tomato genotypes to tobacco mosaic virus (TMV) with 27 TMV isolates. Genotypes containing gene Tm-2 or Tm-2 resisted all the isolates. Those containing Tm-2 were agronomical better than those with m-2, and the performances of some cultivars with h1-2 that have been released for use were assessed. Although resistance of these cultivars to TMV was usually satisfactory, small plants developed systemic necrosis when inoculated and kept in unusually hot greenhouse conditions.

Fraser and Loughlin (1980) studied the gene Tm-1 in tomato plants is dominant for suppression of mosaic symptoms caused by tobacco mosaic virus isolates designated as tomato strain 0. Virus multiplication (measured either by virus RNA content or by virus coat protein content) was inhibited in plants containing Tm-1. Inhibition was greater in hosts homozygous for Tm-1 (90 to 95%) than in hosts heterozygous for Tm-1 (65 to 75%). Thus, inhibition of tobacco mosaic virus multiplication is Tm-1 gene dosage dependent suppression of visible symptoms is not.

Boukema (1980) studied that plants of *Capsium* spp. have resistance against tobamovirus such as tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV), which are manifested by the appearance of necrotic local lesions at the infection site. The resistance is conferred by four different genes at L-locus known as L, L2, L3 and L4 respectively.

Barden *et al.*, (1986) conducted the experiments for screening of tomato somaclones for resistance to tobacco mosaic virus and tomato mosaic virus out of 370 somaclones, 6 somaclones found resistance to TMV/ToMV.

Smith and Murakishi (1987) screened tomato somaclones for resistance to tomato mosaic virus. Out of 370 somaclones found 6 were resistance to ToMV.

Hameed *et al.*, (1992) screened 15 tomato lines against tomato viruses to find out the level of resistance in these lines. On the basis of symptoms expression, biological essay and ELISA, line 442 and 838 appeared to be moderately resistant and line 943 was tolerant. Rests of lines were susceptible.

Resistant varieties have been considered as the most efficient control of the plant diseases especially those caused by viruses (Tewari and Ramanujam, 1994).Sowing resistant varieties has been found the most effective way to control the virus diseases (Green and Kallo, 1994).

Mirza *et al.*, (1994) screened cotton cultivars against natural infection by CLCuV in the fields in the district Faisalabad, for finding source of resistance against the disease. Studies indicated that of the ten cultivars, none was immune or resistant or even moderately resistant. Cultivar S-12 was found to be susceptible whereas rest of the cultivars were highly susceptible under the prevailing natural climatic conditions.

Bashir *et al.*, (1996) evaluated 80 advanced line of mash against MYMV and ULCV. Ten lines were free of MYMV and ULCV symptoms viz., 9011, 92011, 92014, 92020, 92048, 92050, 92054, 92055 and 9080. Thirty and sixty lines were resistant to MYMV and ULCV respectively. The rest of the lines were either tolerant or susceptible to MYMV or ULCV.

Parrella *et al.,* (1997) screened Lycopersicon accessions for resistance to alfalfa mosaic virus. Tomato and wild related species were inoculated with AMV under artificial conditions in order to evaluate their conditions in order to evaluate their resistance. *L. hirsutum f. typicum* LA 1777*, L. hirsutum f. glabratum* P1 134417 and 'Bruinstoa' accessions were found to be resistant.

Monma and Sakata (1997) screened eighty three sweet or hot pepper accessions (*Capsicum annum L.*) and 57 other *Capsicum* spp. accessions developed symptoms after the first inoculation with CMV but 45 plants in 22 accessions were symptomless. When the letter were re-inoculated, 15 symptom less plants in 10 accessions were selected for progeny testing. The resistance for some progenies derived from symptom less plants was higher than that of the parents. The resistant progenies are preserved as seed stocks.

Kanjilal *et al.,* (2000) studied the disease potential of desi and hybrid cultivars of tomatoes in Coochbehar, Nadia and Murshidabad districts of West Bangal, India. Results shoes heavy incidence of disease on hybrid cultivars compared to desi cultivars. Predominant

diseases of hybrid cultivars were virus and bacterial. The desi cultivar showed little or no infection due to the above pathogen.

Ragupathi and Narayanaswamy (2000) screened out one hundred and sixty germplasm enteries of tomato against tomato viruses during summer in Coimbatore Tamil Nadu, India. Under natural conditions only 2 wild species, namely *Lycopersicon hirsutum* (LA 1353) and *Lycopersicon hirsutum f. sp, glaboratum* (LA 1223) were free from the virus infection. The remaining 157 cultivars had more than 50% infection.

Ali and Hassan (2002) screened 12 commercial tomato varieties against ToMV, PVX, PVY and CMV. Two varieties were (florist and forest) were resistant to four of the viruses including ToMV, for which the highest incidence was recorded in nurseries and field. Two varieties represent a previously undescribed and potentially useful source of resistant to the four inoculated viruses.

Bashir and Zubair (2002) evaluated 132 breeding lines/cultivar against mungbean yellow mosaic virus (MYMV) and Urdbean leaf crinkle virus (ULCV) under field conditions. They found that 53% urdbean genotypes were highly resistant to MYMV and 26% to ULCV. More than 60% lines expressed multiple disease resistance to both the viral diseases.

Safdar *et al.,* (2004) evaluated four okra cultivars (Pahuja, Safal, Sabaz Pari and Surkh Bhindi) in a field to check their response to okra yellow vein mosaic virus (OYVMV) and to evaluate neem extract, against OYVMV. Surkh Bhindi was found highly resistant, Subz pari and Safal were moderately resistant an Pahuja was tolerant to OYVMV. Neem extract were also found effective against OYVMV as compared to control.

Kaya *et al.,* (2004) conducted experiment to find out the virus diseases in tomato plants growing at the protected production areas in Mugla province between 2002 and 2004. During the studies tomato cultivars were tested for the presence of tobacco mosaic tobamovirus (TMV), tomato mosaic tobamovirus (ToMV), Cucumber mosaic cucumovirus (CMV), tomato spotted wilt tospovirus (TSWV), tomato yellow leaf curl bigeminivirus (TYLCV) and potato Y potyvirus (PVY). The obtained results revealed that all of tested

seeds and seedling samples were free from the viruses mentioned above. In the examinations throughout the cultivations periods, it was seen that main virus like symptoms on the tomato plants were mosaic, mottling, upward curling and deformation in leaves, the formation of cup shaped leaflets and stunting in plants. Some leaf samples were taken from the suspected tomato and tested foe the existence of TMV, CMV, TSWV, TYLCV and PVY by DAS-ELISA. The incidence od TMV, TSMV and CMV in the leaf samples were 30.00, 3.85 and 2.56%, respectively.

Micznski, *et al.,* (2004) screened 28 accessions of wild tomato belonging to the species *Lycopersicon peruvianum, L. glandulosum, L. chilense, L. pimpinellifolium, L. hirsutum* and *L. pennellii* for resistance to infection following mechanical inoculation with tomato mosaic tobamovirus (ToMV), cucumber mosaic cucumovirus (CMV) and tomato spotted wilt tospovirus (TSWV). All plants of *L. hirsutum* showed complete resistance to ToMV and one accession of *L. peruvianum* remained uninfected by TSWV. Two other accessions of *L. peruvianum* also exhibited high level of resistance (97%) to TSWV. Resisitance in the other accessions was completely resistant to all three viruses, but some showed relatively high resistance to combinations of two viruses.

Shad *et al.,* (2005) assessed 254 lines of mungbean germplasm gainst mungbean yellow mosaic tobamovirus (MYMV) under natural field conditions conducive for development of disease. Majority of the lines were infected within 2-3 weeks and the disease increments monitored over a period of six weeks were 2.36%, 18%, 48%, 74%, 83% and 95%. Disease severity followed similar trend i.e. 0.48, 1.85, 3.82, 4.74, 4.94, and 4.99, repectively. None of the lines appeared to be resistant of any category; 7 lines were classified as “Susceptible” and 247 as “highly susceptible” indicating that resistance was scare in mungbean germplasm. Some lines in spite of higher disease incidence produced significantly good yield, therefore, tolerance rather resistance against MYMV should be a preferred criterion.Resistant varieties still have been recommended against almost all plant viral diseases (Kumar and Poehig, 2006).

Leiva-Brondo *et al.,* (2006) reported that tomato mosaic virus (ToMV) restricts Pepino, extension due to delay in fruit ripening and loss of quality of fruit caused by the

virus, which makes the fruit non-marketable. Their accession of pepino identified as resistant through hypersensitive reaction (HR) against mechanical inoculations and characterized for their resistance behavior in three different ranges of temperature which gives resistant reaction at low and medium temperatures, while at high temperature they behave as susceptible. Hybrids between resistant accessions and the susceptible accession 'sweet round' showed different segregations patterns depend on the resistant pattern used. Due to the vegetative propagation system used with pepino, successful selection among resistant hybrid could quickly yield resistant commercial varieties. These varieties combine with suitable prevention measure will avoid damage to the pepino crop caused by ToMV.

Sana *et al.,* (2007) identified resistance or tolerance in mungbean germplasm. A disease screening nursery comprising of 108 test entries, was developed. Screening was done under natural environmental conditions against yellow mosaic disease (YMD). All the test entries showed a highly susceptible response. Despite being highly susceptible, some test entries produce good yield and showed tolerance to YMD.

Ling and Scott (2007) identified the sources of resistance to Pepino mosaic virus in tomato accessions. These accessions included 23 *Solanum lycopersicum,* 8 *S. pimpinellifolium,* 33 *S. peruvianum,* 18 *S. chilense* and 27 *S. habrochaites.* The results showed that all plant in the accessions corresponding to *Solanum lycopersicum,* and *S. pimpinellifolium,* were susceptible to PepMV-US infection. On the other hand, two accessions of *S. peruvianum,* (LA107 and LA1305) and *S. chilense* (LA1971 and LA2748) appeared to have some level of moderate resistance. However, the most promising segregated in three *S. habrochaites.* accessions (LA1731 and LA2156, and LA2167).

Soler-Aleixandre *et al.,* (2007) screened different solanum species to find sources of resistance to pepMV. All plants of *S. Lycopersicon, S. Lycopersicon var. carasiforme, S. penelliic Corell, S. cheesmaniac* (L. Rilley) Fosberg, *S. habrochaites,* S. Knapp & D. M. Spooner, *S. pimpinellifolium* L., S. *basendopogen Bitter*, S. *canense* Rydb., S. *caripense* Humb & Bonpl. Ex *Dunal,* and *S. muricatum* Aiton accessions showed a 100% systemic infection rate, high viaral accumulation and apparent symptoms. As a result, *S. chilense* and *S. peruvianum,* were the most promising species as a source of resistant to PepMV.

Okoro *et al.*, (2008) screened four exotic varieties of tomatoes impoted from the Netherlands against natural pathogenic infection and yield advantage 2004 and 2005 growing season. Results showed that five disease attacked the tomato varieties. They include tomato mosaic virus disease, Sclerotial root rot, leaf spot disease, Sclerotial fruit rot and blossom end rot diseases. Topson variety performed better than other varieties in yield even though it suffered more attack of diseases which apparently did not affect its productivity. All the four varieties screened were found to be resistant to mosaic and leaf spot diseases, however Ronco variety recorded the lowest leaf spot and mosaic severity of 0.1 and 0.3 score respectively, showing that it may be the most resistant among the four varieties while BSS 281 and Sultan varieties were found to be tolerant to sclerotial root rot. The peak of attack of the fruit diseases was found to be the 17^{th} week after transplanting the tomato plants. All the four exotic four varieties screened adapted to the environmental well and disease attack did not affect the yield significantly.

2.5.2. Effect of various plant extracts on disease intensity

Awasthi and Mukerjee (1980) used plant extracts for protection of potato virus X infection. Extracts from the root of *Boerhaavia diffusa* L. stems of *cuscuta reflexa* or leaves of *Euphorbia hirta* L. have shown a potential protective effect on the infection of potato virus X, in hypersensitive and systemic hosts. The inhibition by these extracts was systemic and sensitive to actinomycin D.

Chowdhury and Shah (1985) stated that pre-inoculation spray of onion extract exhibited maximum inhibition of UI.CV in vivo and in vitro while the extract of ginger and turmeric inhibited the ULCV to an extent of more than 50%.

Dohroo and Gupta (1995) reported that Azadiratchin and other limonids were quite effective in controlling plant diseases of diverse nature. Neem extract and oil were the most potent in reducing the virus.

Baranwal and Ahmad (1997) studied the effect of *Clerodanrum aculentum* which was applied as a powder to the soil or in liquid form as a foliar spray. There were eight treatments and disease progress was monitored at fortnightly intervals from the seedling stage. Plant height and tomato yield per plant were also recorded. Tomato plants who received CA as a

soil application, spray or both showed delayed incidence of virus infection and comparatively higher yields of tomato. Foliar spray combined with soil application was the most effective treatment. Symptoms in CA treated plants were less compared with untreated plants.

Siddiqui and Khalid (1998) evaluated the pepper lines against tomato viruses under control condition to study the incidence of virus infection and comparatively higher yield of tomato. Foliar spray combined with soil application was the most effective treatment. Symptoms in CA treated plant were less compared with untreated plants. Plant seed oil were mainly used to control viral pathogens (Jayashree *et al.*, 1999).

Aslam and Naqvi (2000) tested the efficacy of neem product (Phytopesticide IWB) which was compared with prefekthion (dimethoate) against sucking pests (jassid, aphid, thrips and white fly). Perfekthion 40EC proved to be more toxic and its effect lasted for 4 days only while neem product (FWB) was less toxic but its effect lasted for 6 days. In addition, neem product was much safer and environmentally friendly.

Botanical extracts have gained importance in modern days for crop protection against pest and diseases because of their safety and target specificity. Plants extracts or products have also been found effective against a wide range of pathogens (Manickam and Rajappan, 2001).

Thirumalaisamy *et al.,* (2003) conducted a greenhouse experiment to identify plant extracts as potential inhibitors of plant viruses and to evaluate the different methods of application of the plant extracts. Some physical properties of the selected potential plants extracts against ULCV were studied. The plants species and parts used for the extract comprised of *Allium cepa* bulbs, *Allium sativum* cloves, Aloe vera (*Aloe barbadensis*) leaves, *lantana camara* leaves, *Dhthura stramonium* leaves, *Bouganvillea spectabilis* leaves, *Curcuma longa* rhizome, *Mentha arvensis* leaves, *Ocimum sanctum* leaves, *Phylanthus niruuri*, *Piper longum* leaves, *Piper longum* fruit, *Polyalthia longiflora* leaves, *Solanum nigrum* leaves and *Zingiber officinale* rhizome. The extracts from *Z. officinale* and *Prosopis juliflora* diluted from 1: 1 to 1: 5 and *Piper longum* diluted from 1: 1 to 1: 10 showed high percentage of disease inhibition.

Kumar (2004) studied the response of pre inoculation spray of different fraction of plant extracts from *Mirabilis jalapa* (roots), *Asparagus adscendens* (roots), Vitex negundo (leaves), *Symphytum perigrinum* (roots) in inhibition of TMV. The extracts were sprayed at 3 different intervals (24. 48 and 90 hours) at different concentration 1, 3, 7 and 10 respectively. Pre inoculation spray with coold water fraction of *mirabilis jalapa* (roots), when spray thrice at 24 hour interval results in maximum inhibition of virus (89.97%). Irrespective spray interval of alcohol extract of *vitex negundo* (leaves) proved to be effective by inhibiting virus up to 67.12%.

Madhusudhan *et al.*, (2005) studied the effect of inducers against tobamovirus infection in tomato and bell peppers. When the seedling were sprayed with salicylic acid (50mM) and neem oil (5%), the concentration of tomato mosaic tobamovirus (ToMV) and tobacco mosaic tobamovirus (TMV) was assessed based on the number of local lesions on *Nicotiana glutinosa*. The results showed that the seed or seedling treatment with inducers reduced the number of local lesions when compared to untreated ones. Salicylic acid was an effective inducer.

Pun *et al.* (2005) determined the efficacy of six plant extracts against Okra vein yellow mosaic virus (OVYMV) under field conditions. Neem oil, neem seed kernel extract and leaf extracts of Bougainvillea spectabilis proved most effective in reducing disease and also increasing the yield.

Ferguson (2005) reported that there were no available chemicals for controlling virus diseases in infected plants. However, milk as a spray or dip for seedling has often been suggested as means of reducing the incidence of virus infections. The idea originated from several older studies 1940s and 1950s that have demonstrated milk's effectiveness in reducing infection due to tobacco mosaic virus (TMV) in pepper, tomato and tobacco. Research in field tomatoes in Mississippi in the late 50's suggested that milk, as a dip for fingers prior to handling diseased seedling, reduced TMV incidence from over 50% to 0% in pepper, and from 90% to 15% in tomato. Although the majority of these studies focused of on TMV, infection due to other viruses can also been reduced, but to differing degrees. Such viruses include pepper mild mottle mosaic virus (PMMV), cucumber mosaic virus (CMV), bean mosaic virus (BMV) and tobacco ring spot virus (TRSV).

Virus infection cause great damage to economical crops, this loss is so clear especially in developing countries. Investigators were aiming to control such incurable pathogen uning an alternative controlling strategy depending on a clean agriculture system (Fletcher *et al.,* 2006).

Reddy *et al.,* (2006) reported that six plants extracts viz; *Alirabilis jalapa, Carthamus roseus, Daiwa metal, Bougainvillea pcclabilis, Boerliaavia dijfusa,* and *Azadirachta indica* caused the reduction in the incidence of urdbean leaf crinkle virus (ULCV) in urdbean crop at field levels.

Eraslan *et al.,* (2007) studied the effects of foliar sprayed calcium source on tomato mosaic virus (ToMV) infection in tomato plants grown in green house. The solutions containing 0.3% ca prepared from four calcium sources were foliar sprayed on greenhouse grown tomato plants, infected with the tomato mosaic virus tobamovirus (ToMV). ToMV infected and uninfected control groups were sprayed with distilled water. ToMV reduced the fresh and dry weight and Ca concentration of tomato plants but significantly raised P concentration in the tissue. Neither virus inoculation non foliar Ca applications affected N and Mg concentrations in tomato plants. The foliar applied Ca from all the sources gave K concentrations similar to those of control plants.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Material

The seeds of ten tomato lines / varieties (Nagina, Naqeeb, Rio Grande, Savana, GHT-2, Baby Red, SBS-292, VRI- 575, Nemador and GSL-198) were obtained from the vegetable section, Ayub Agriculture Research Institute (AARI), Faisalabad. These lines / varieties were sown in natural conditions in field to evaluate their resistance or susceptibility against tomato mosaic virus (ToMV) disease.

3.2. Establishment of Tomato Nursery and its Transplantation

The seeds of ten varieties / lines were sown in one small plot (m^2) and maintained at experimental area, Department of Plant Pathology, University of Agriculture Faisalabad to raise tomato nursery. After 40 days, when seedlings were 15-25 cm tall with 3-5 true leaves, transplanted on the beds in the field, spacing between plant to plant (30 cm) and row to row (70 cm) was maintained according to the varietal growth habit, soil type, cropping system and also weather conditions.

3.3. Growing Tomato Seedlings in Green House

Growing tomato seedlings in green house is an easy, cost effective and healthy method. Plants of five tomato varieties / lines (SBS-292, Baby Red, GSL-198, Nemador and Nagina) were raised in earthen pots (12.50 cm × 12.50 cm) containing mixture of sand, soil and farm yard manure in the ratio of 1:1:1. Seeds were covered lightly with potting compost then pots were placed in a warm (up to 27 °C) and dark place, and appropriate conventional agronomic practices were performed to maintain crop. The seedlings were emerged in 7-10 days, when their roots appeared through the base of pots, and then plants were transferred to the larger pots. Potted plants were kept and maintained in an insect free environment (green house) for pot experiment for the confirmation of virus through mechanical transmission.

Figure 3. 1: Field Preparation and transplanting of tomato seedling
(a), (b) showing the field preparation
(c) Irrigation before tomato seedling transplantation
(d) Tomato seedling transplanting

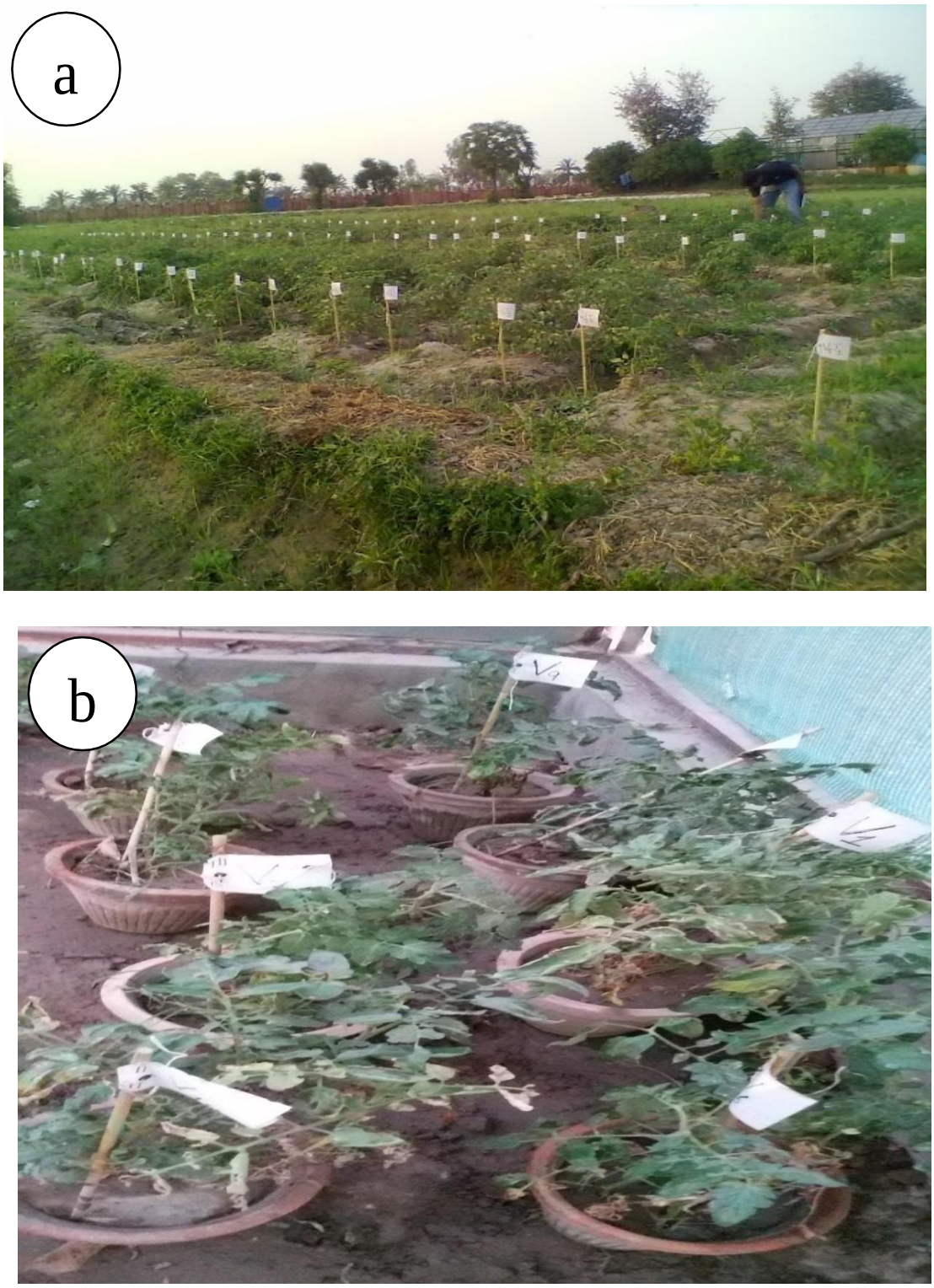


Figure 3. 2: Tomato germplasm grown under field conditions and in green house

(a) Tomato plants in Field
(b) Tomato plants in green house

(a)

(b)

(c)

(d)

Figure 3. 3: Tomato plants showing typical symptoms of ToMV (a), (b), (c), (d) showing the ToMV symptoms observation in the field

3.4. Transmission Studies

3. 4.1. Mechanical transmission

Tomato plants were maintained in pots, in the green house used for the confirmation of ToMV. Transmission of virus through mechanically inoculation was carried out following the procedure described by Mughal and Khan (2006). ToMV was mechanically inoculated to the tomato plants 21 days after seedling transplantation. For virus transmission experiment, plants of three tomato lines / varieties (Baby Red, GHT-2 and Savana) were raised in earthen pots (diameter 12.50 cm) containing mixture of sand, soil and farm yard manure in ratio of 1 :1 :1. There was one plant in each pot. The pots were maintained in an insect free environment (Green house) to ensure that these remain free from other viruses. Leaves from diseased tomato plants supposedly showing the symptoms of ToMV were ground in 0.02 M sodium phosphate buffer (pH. 7.0) in a pestle and mortar. The homogenate was filtered through two layer of muslin cloth. Corborundum powder was dusted on the leaves of healthy test plants and the inoculum was applied on the leaves with cotton swab. ToMV was confirmed by the appearance of characteristics symptoms on these plants.

3.5. Confirmation of ToMV on Indicator plants

Tomato Mosaic Virus was also confirmed through indicator plants because these plants show typical symptoms in case of virus attack. Plants showing characteristics symptoms of ToMV were inoculated on indicator plants such as Jasmine (*Jasminum multiflorum*) and Pigweed (*Chenopodium album* L.). The plants were placed in screen house to avoid insect attack. Leaves from diseased tomato plants supposedly showing the symptoms of ToMV were ground in 0.2M sodium phosphate buffer (pH. 7.0) in a pestle ad mortar. The homogenate was filtered through two layer of muslin cloth. Carborundum powder was dusted on the leaves of indicator plants and the inoculum was applied on the leaves with cotton swab. These plants were kept under observation for the appearance of diagnostic symptoms of ToMV. The symptoms were also observed and recorded after 14 and 21 days. These symptoms were compared with photographs indicating ToMV on indicator plants.

(a)

(b)

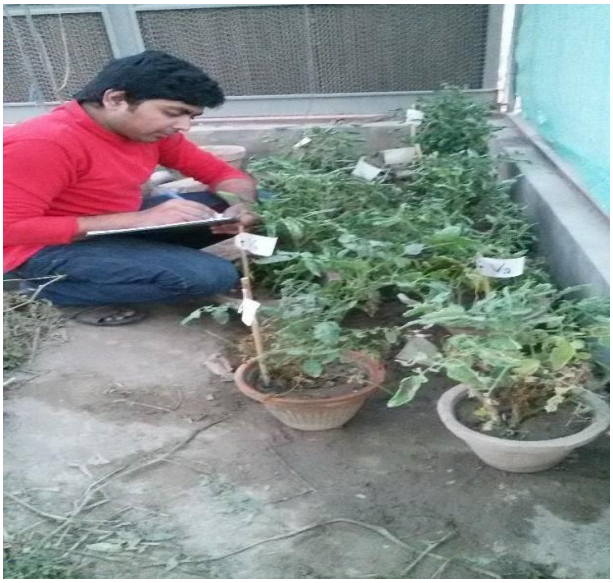
(c)

(d)

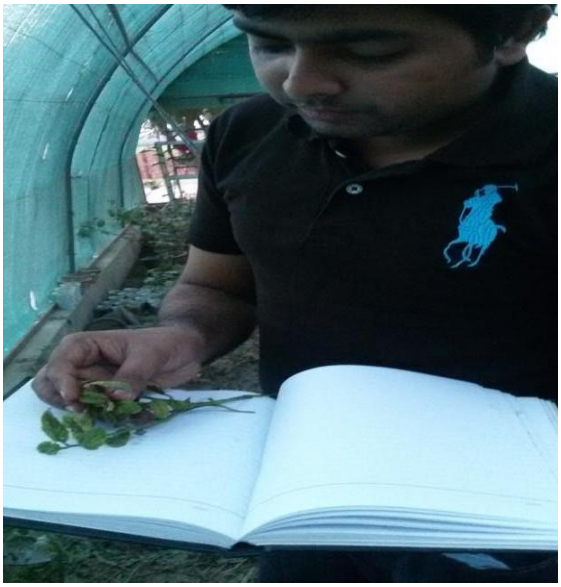
Figure 3.4: Research Scholar performing confirmation experiment of ToMV in gree house

(a) Healthy Tomato plants in pots **(b)** Rubbing of Tomato leaves

(c) Inoculum **(d)** Applying of inoculum on the tomato Leaves



(a) **(b)**



(c) **(d)**

Figure 3. 5: Data recording and ToMV symptoms observation

(a), (b) showing the data recording

(c), (d) showing the symptoms observation for the ToMV

3. 6. Varietal Screening

The ten tomato lines / varieties were sown in the research area of Department of Plant Pathology, University of Agriculture Faisalabad. The conventional agronomic practices were performed to maintain plants. The plant to plant distance was maintained 30cm and row to row distance was maintained 70cm. Data regarding to the ToMV disease was recorded on the basis of disease incidence by the following formula

.

$$\text{Disease incidence of ToMV} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

(Imran *et al.*, 2013)

1. NAGINA
2. NAQEEB
3. RIOGRANDE
4. VRI-575
5. SAVANA
6. GHT-2
7. SBS-292
8. NEMADOR
9. GSL-198
10. BABY RED

The Data on ToMV disease incidence was taken according to the following disease incidence scale (Bashir *et al.*, 2005).

Table 3.1: Disease Rating Scale for ToMV

Disease Rating	ToMV Disease Incidence (%)	Host Reaction
0	All plants free of virus symptoms	Highly resistant (HR)
1	1-10% infection	Resistant (R)
2	11-20% infection	Moderately resistant (MR)
3	21-30% infection	Moderately susceptible (MS)
4	30-50% infection	Susceptible (S)
5	More than 50% infection	Highly susceptible (HS)

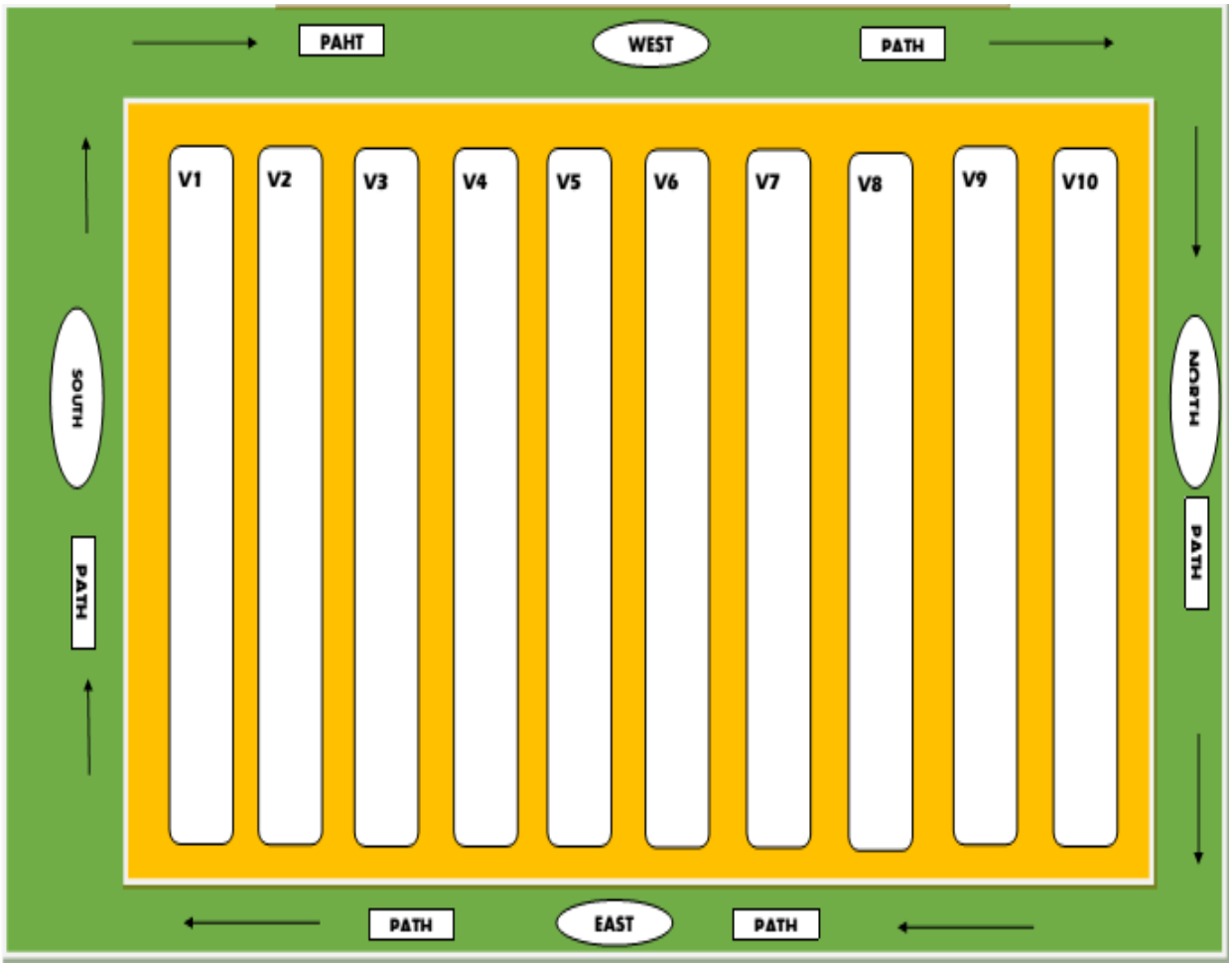


Figure 3. 6: Layout plan for screening tomato lines / varieties against ToMV

3. 7. Epidemiological Studies

To study the epidemiology of ToMV an experiment was conducted in the research area of Department of Plant Pathology, University of Agriculture Faisalabad. Five varieties Nagina, Nemador, SBS-292, Baby Red and GHT-2 were used for epidemiological studies. The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. Each variety was planted in a sub plot with row length 3cm, row to row 2 ft and plant to plant spacing 1 ft. Data regarding the disease development was recorded on weekly basis and these were correlated with environmental parameters i.e. Maximum and minimum temperature, relative humidity, rain fall and wind speed (Steel *et al.*, 1997).

3. 8. Collection of Environmental Data

The data of different environmental factors (Maximum temperature, minimum temperature, relative humidity, rain fall and wind speed) during the experimental period was obtained from the department of Crop Physiology, University of Agriculture and Faisalabad. The weekly averages of these parameters were calculated and correlated with disease incidence (Steel *et al.*, 1997).

Figure 3. 7: Extracts Material

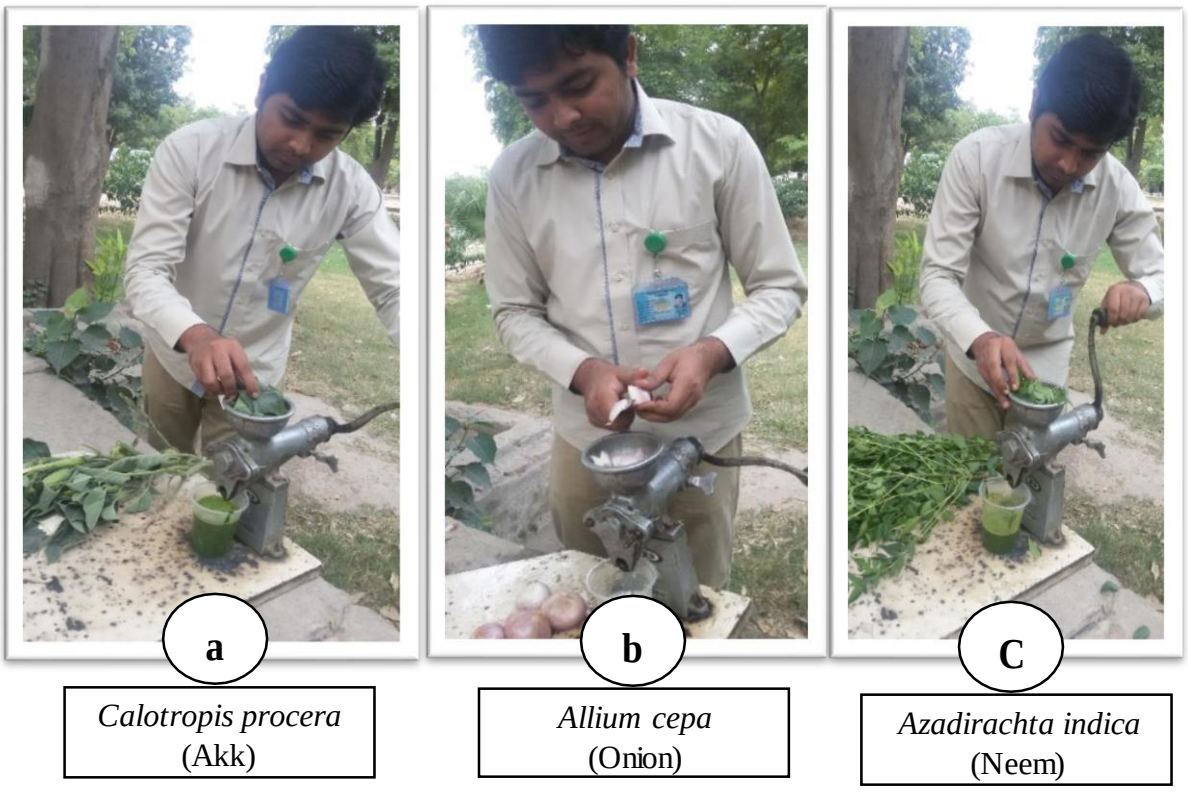


Figure 3. 8: **(a), (b), (c)** showing Extraction of different plant extracts

3. 9. Management of ToMV through Plant Extracts

Five lines/varieties (Nagina, Nemador, SBS-292, GHT-2, Baby Red) were sown in field. The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications and treatments were randomized on varieties and replications. The data of ToMV disease incidence was recorded one day before spray by selecting ten plants at random from each block.

Sr. #	Treatments		Common Name	Scientific Name	Stock Solution
1	T1	Neem	Neem	*Azadirachta indica*	1500 ml
2	T2	Akk	Akk	*Calotropis procera*	1500 ml
3	T3	Onion	Onion	*Allium cepa*	1500 ml

The plant extracts of *Allium cepa* (Onion), *Calotropis procera* (Akk), and *Azadirachta indica* (Neem) were used (1%) to manage the disease. One treatment sprayed with distilled water was considered as control. Extracts were prepared by grinding leaves of these three plants in equal quantity of water in a grinding machine. The juice was filtered through muslin cloth in three separate jars one for each extract and the jar were labelled. 10 ml extract was mixed in distilled water to make volume up to 1000 ml. This gave 1 percent final extracts that ware used for management studies.

To= Control (distilled water)
T1= *A.* cepa
T2= *C. procera*
T3= *A. indica*

These extracts were sprayed in the months of March and April at four different dates that were 15-03-2015, 30-03-2015, 14-04-2015 and 29-04-2015. The treatment were repeated after 15 days and total four sprays were applied (Hilje et *al.*, 2003).

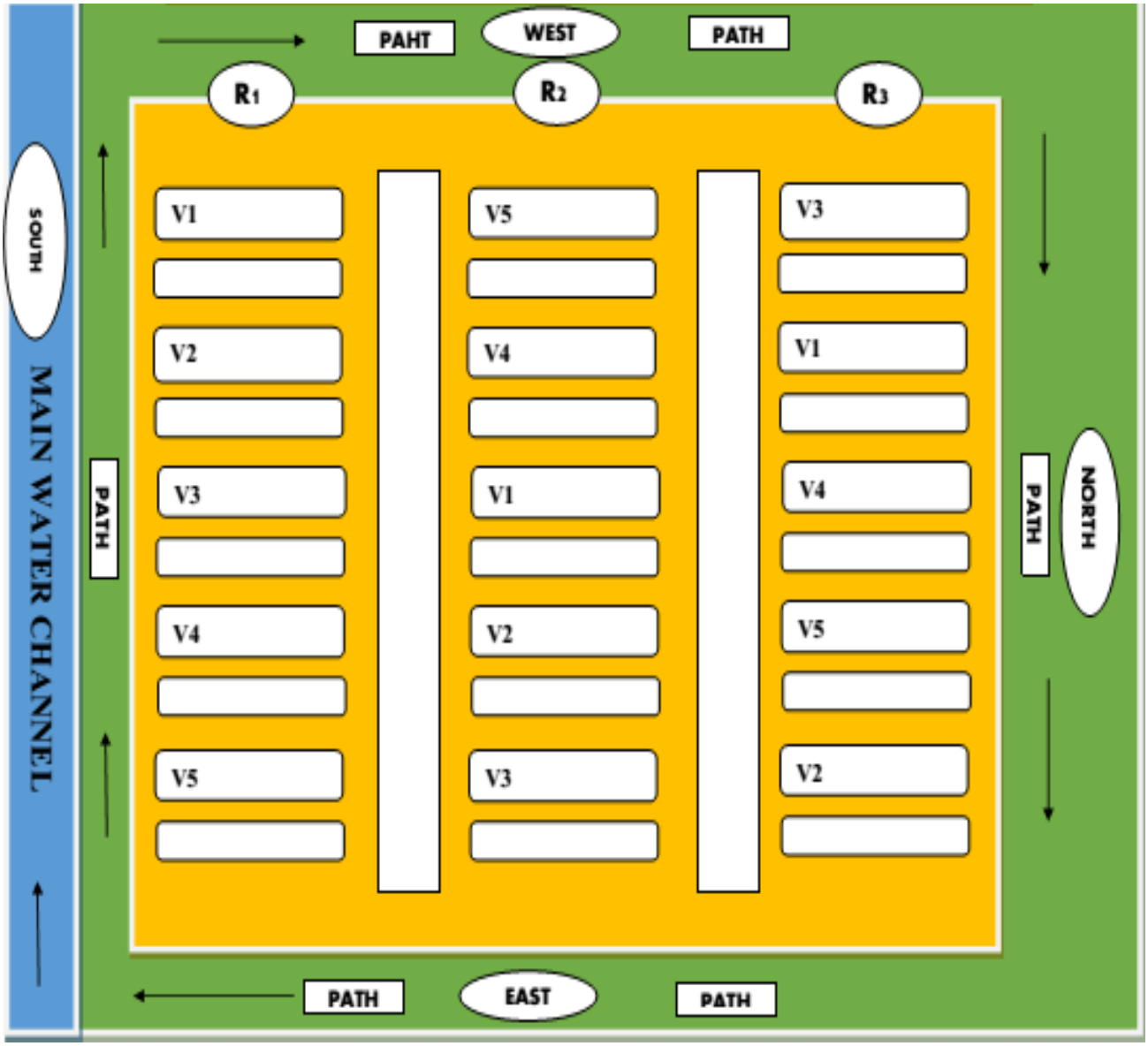


Figure 3. 9: Randomized Complete Block Design (RCBD) for management trial

Figure 3. 10: Research Scholar applying plant extracts for the management of ToMV on tomato crop in the field

CHAPTER 4

RESULTS

4.1: Screening of tomato varieties/ lines against Tomato Mosaic Virus disease (ToMVD) under field conditions

Experiment for the screening of tomato lines/varieties was conducted in the research area of Department of Plant Pathology University of Agriculture, Faisalabad under natural field conditions. The experiment was conducted in three replications in RCBD. The data of disease incidence recorded from these varieties/ lines on weekly basis, the results are described below.

The data was recorded to find out resistant lines of tomato against tomato mosaic virus (ToMV). On ten lines/varieties i.e., Naqeeb, VRI-575, Riogrande, SBS-292, VRI-575, Baby Red, GHT-2, Nemador, GSL-198, and Nagina screened against tomato mosaic virus (ToMV).

Naqeeb appeared to be highly resistant to disease whereas line VRI-575 was found resistant. Riogrande and Savana these two varieties exhibited mild resistance against tomato mosaic virus. Lines SBS-292, GHT-2 and variety Baby Red were moderately susceptible. While one variety Nemador and one line GSL-198 were susceptible. Whereas the variety Nagina was highly susceptible to ToMV.

Table: 4.1. Reaction of tomato varieties/ lines to Tomato Mosaic Virus

Rating	Varieties/ lines	Disease incidence (%)	Level of resistance/ susceptibility
1	Naqeeb	0%	Highly Resistant (HR)
2	VRI- 575	1-10% infection	Resistant

			(R)
3	Rio Grande and Savana	11-20% infection	Moderately Resistant (MR)
4	SBS-292, Baby Red, GHT-2	21-30% infection	Moderately susceptible (MS)
5	Nemador, GSL-198	30-50% infection	Susceptible (S)
6	Nagina	More than 50% infection	Highly Susceptible (HS)

4.2: Mechanical Transmission and pathogenicity studies

Inoculum prepared from naturally infected tomato plants exhibiting conpicuous symptoms of ToMV was mechanically inoculated on healthy plants. Prominent symptoms developed and observed after two week of inoculation. Inoculated plants produced characteristics mosaic symptoms of ToMV.

4.3: Relationship of maximum temperature, minimum temperature, relative humidity, rain fall and wind speed with ToMV disease incidence

The effect of some environmental parameters with ToMV disease incidence (%) was highly significant on all the varieties / lines (Nagina, GSL-198, Nemador, GHT-2 and SBS-292). The relationship of ToMV disease incidence with maximum temperature was positive because an increase in ToMV disease incidence was observed with the increase in temperature. Whereas relative humidity showed negative correlation with ToMV disease incidence, ToMV disease incidence was decreased as relative humidity increased. It was best explained by linear regression model as explained by high r values.

Table: 4.2 Over all correlations of meteorological parameters with ToMV disease incidence (%)

Treatments	Maximum temperature	Minimum temperature	Relative humidity	Rainfall	Wind speed
ToMV disease incidence (%)	0.751**	0.778^{**}	-0.766^{NS}	-0.128^{NS}	0.475^{**}
P- value	0.001	0.012	0.56	0.59	0.013

*= Significant when P-value < 0.05

**= Highly significant when P- value < 0.01

NS= Non-significant when P- value > 0.05

ToMV disease incidence (%) changes were observed during the period of tomato crop growth. The relationship of maximum temperature minimum temperature and wind speed was significant, but the relationship of relative humidity and rainfall was non-significant with ToMV disease incidence (%) on all the varieties/lines (Nagina, GSL-198, Nemador, GHT-2, SBS-292).

The correlation of maximum temperature with ToMV disease incidence for all the varieties was highly significant. With the increase in maximum temperature from 20ºC to 31ºC the disease incidence (%) on all five varieties increased as shown in Figure: 4.1. The relationship of maximum temperature with disease incidence was positive (maximum temperature was positively correlated with disease incidence) i.e. disease incidence increased as maximum temperature increased and it was explained by linear regression model as indicated by r values 0.84, 0.79, 0.80, 0.77 and 0.83 for the five varieties Nagina, GSL-198, Nemador, GHT-2 and SBS-292, respectively. Similarly in Figure: 4.2 it is clear that with increase in minimum temperature from 10ºC-16ºC the disease incidence (%) also increased.

The relationship of minimum temperature with disease incidence was positive (minimum temperature was also positively correlated with disease incidence) i.e. disease incidence increased with the increase in minimum temperature. The relationship was explained by linear regression model as explained by r values 0.88, 0.87, 0.87, 0.85 and 0.88. The relationship of relative humidity with disease incidence of ToMV as shown in Figure: 4.3. Indicates that change in relative humidity also influenced the disease incidence of ToMV and a decrease in disease incidence (%) was observed on the five varieties as the relative humidity increased from 55% to 70%. The relationship of relative humidity with disease incidence was negative (relative humidity was negatively correlated with disease incidence). The correlation was explained by linear regression model as indicated by r values -0.90, -0.84, -0.85, -0.83 and -0.87. The relationship of disease incidence with rainfall as plotted in Figure: 4.4. Indicates that with the increase in rainfall from 0 mm to 5 mm the disease incidence (%) on all varieties decreased. There was no correlation of rainfall with ToMV disease incidence and it was explained by linear regression model as indicated by r values -0.03, -0.05, -0.02, -0.02 and -0.04. The relationship of disease incidence with wind speed was positive. Its mean that ToMV disease incidence was positively correlated with wind speed and it was demonstrated in Figure: 5.5. It is clear that with increase in wind speed from 4.5 Km/h to 7.5 Km/h the disease incidence (%) on all five varieties increased. The correlation was poor and it was explained by linear regression model as explained by r values 0.38, 0.38, 0.35, 0.32 and 0.40 for the five varieties Nagina, GSL-198, Nemador, GHT-2 and SBS-292 respectively.

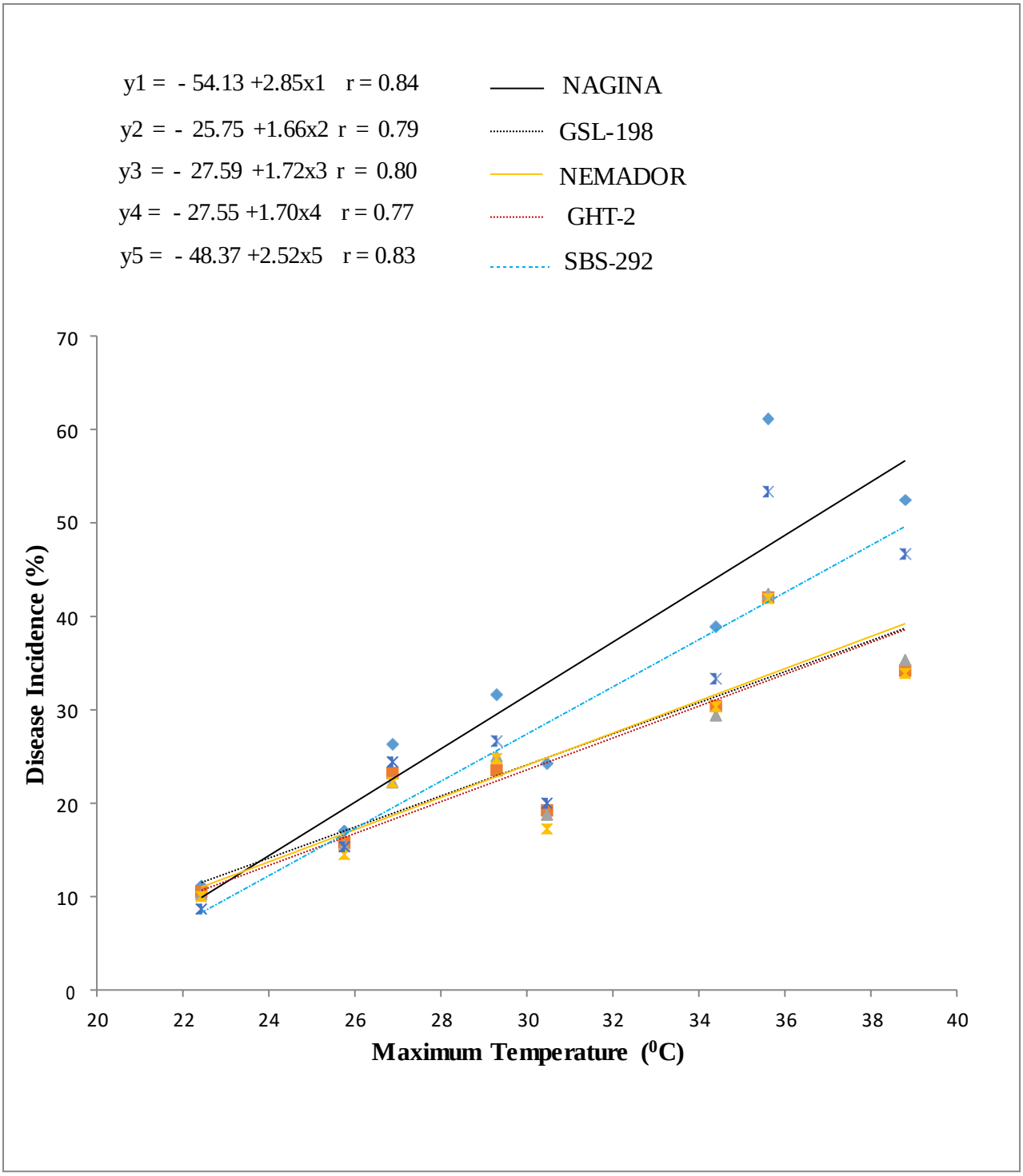


Figure: 4.1 Relationship between maximum temperatures with tomato mosaic virus (disease incidence) recorded on NAGINA (y1), GSL-198 (y2), NEMADOR (y3), GHT-2 (y4), SBS-292 (y5), respectively.

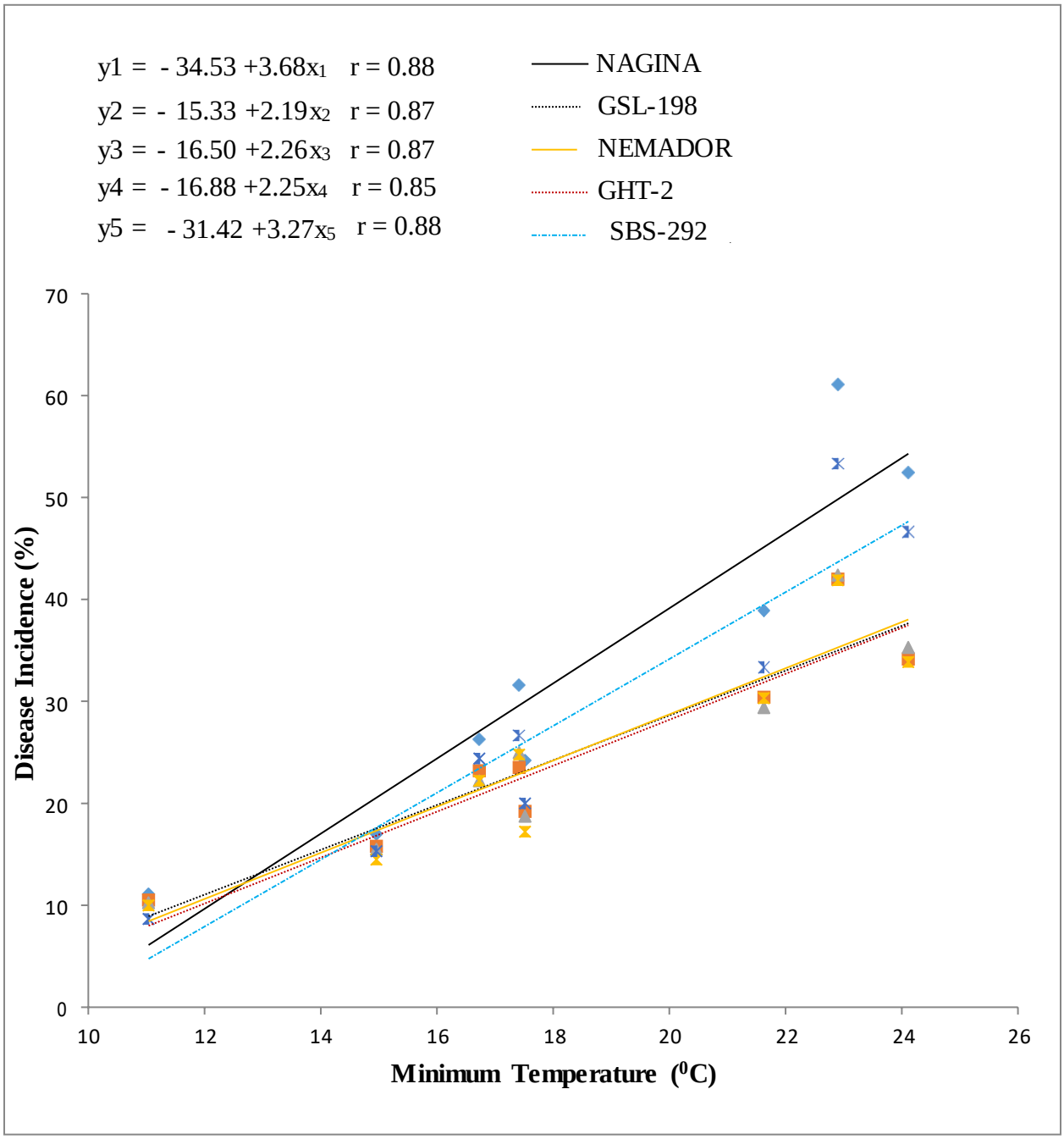


Figure: 4.2 Relationship between minimum temperatures with tomato mosaic virus (disease incidence) recorded on NAGINA (y1), GSL-198 (y2), NEMADOR (y3), GHT-2 (y4), SBS-292 (y5), respectively.

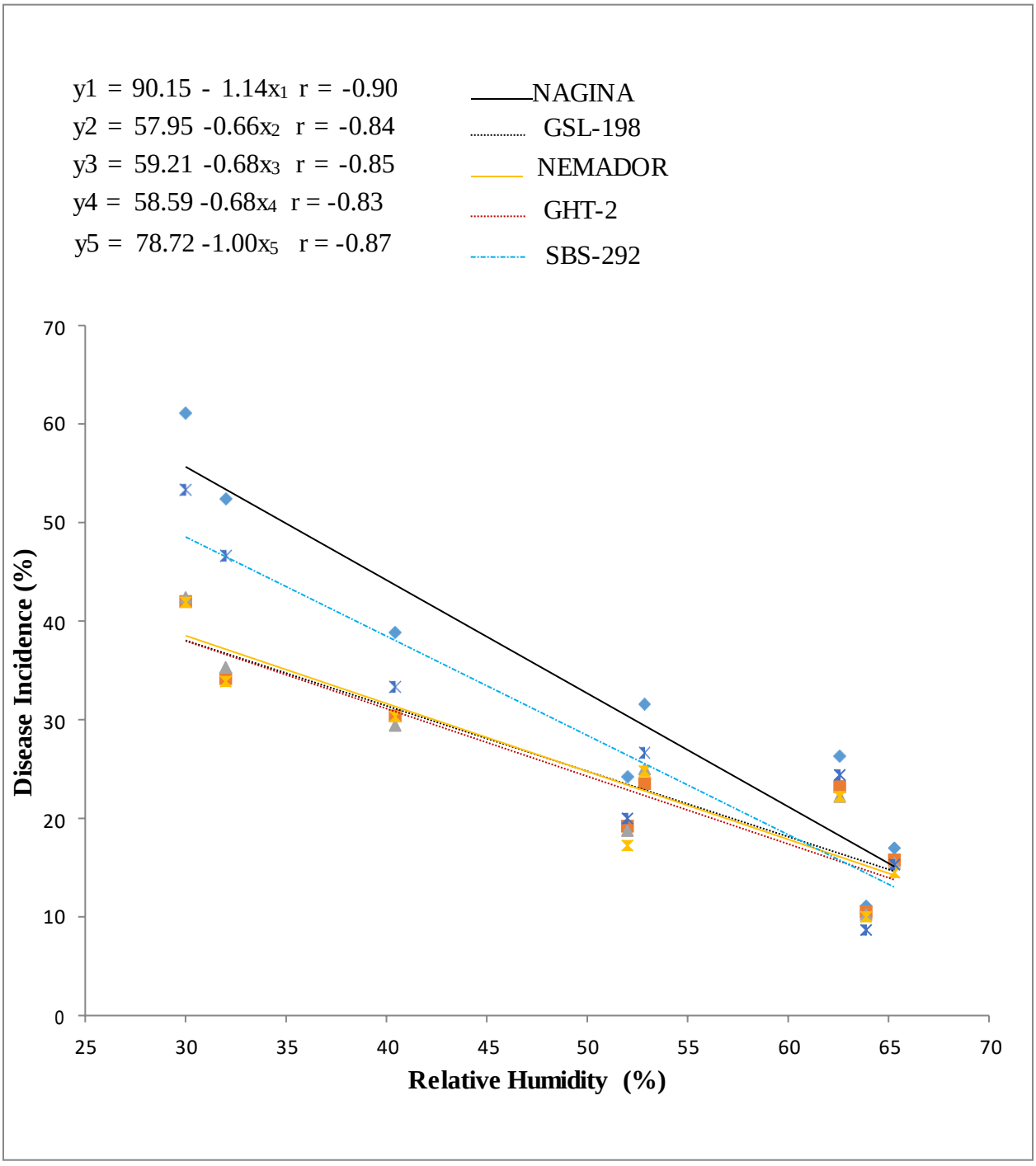


Figure: 4.3 Relationship between relative humidity with tomato mosaic virus (disease incidence) recorded on NAGINA (y1), GSL-198 (y2), NEMADOR (y3), GHT-2 (y4), SBS-292 (y5), respectively.

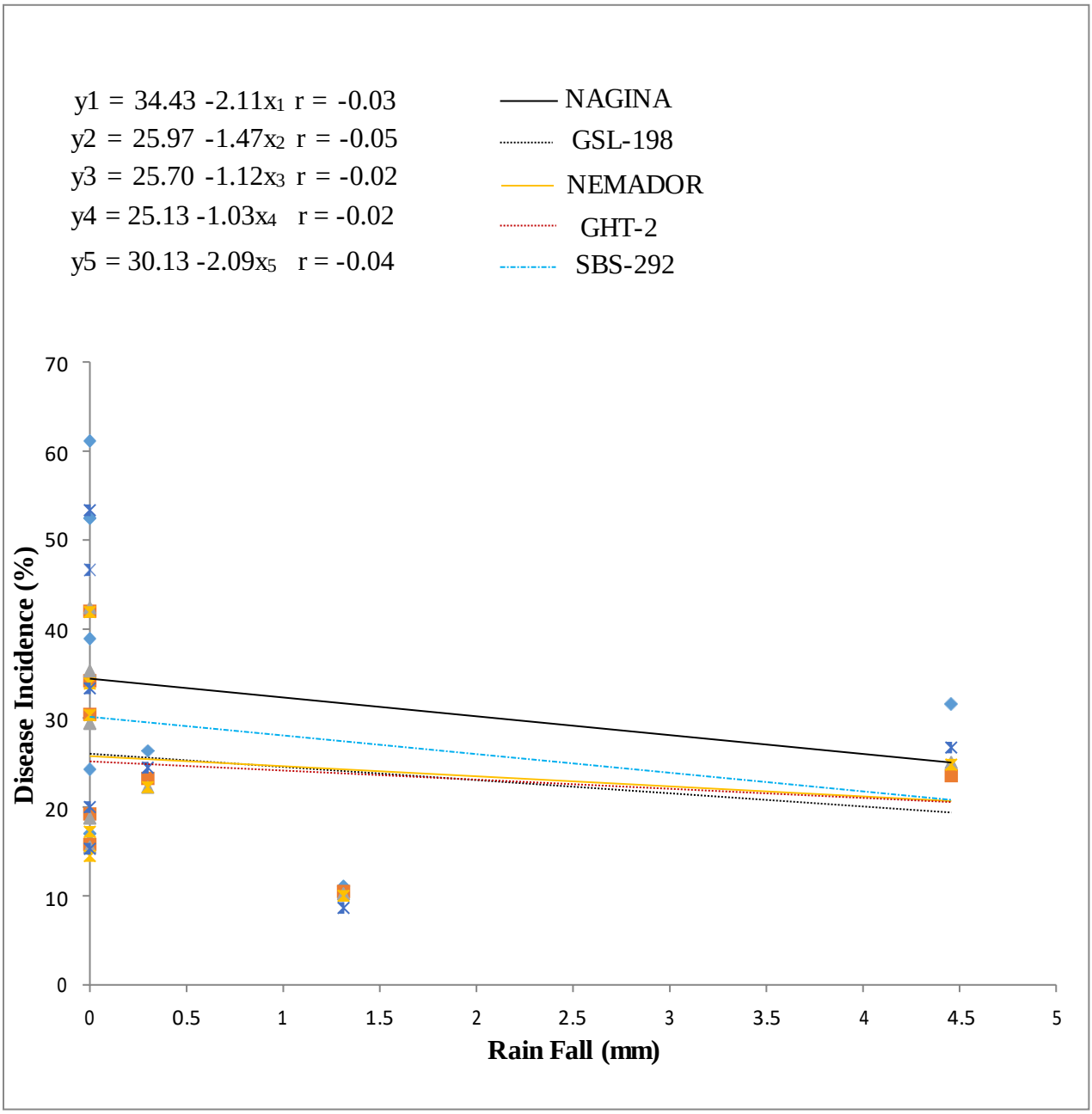


Figure: 4.4 Relationship between rainfall with tomato mosaic virus (disease incidence) recorded on NAGINA (y1), GSL-198 (y2), NEMADOR (y3), GHT-2 (y4), SBS-292 (y5), respectively.

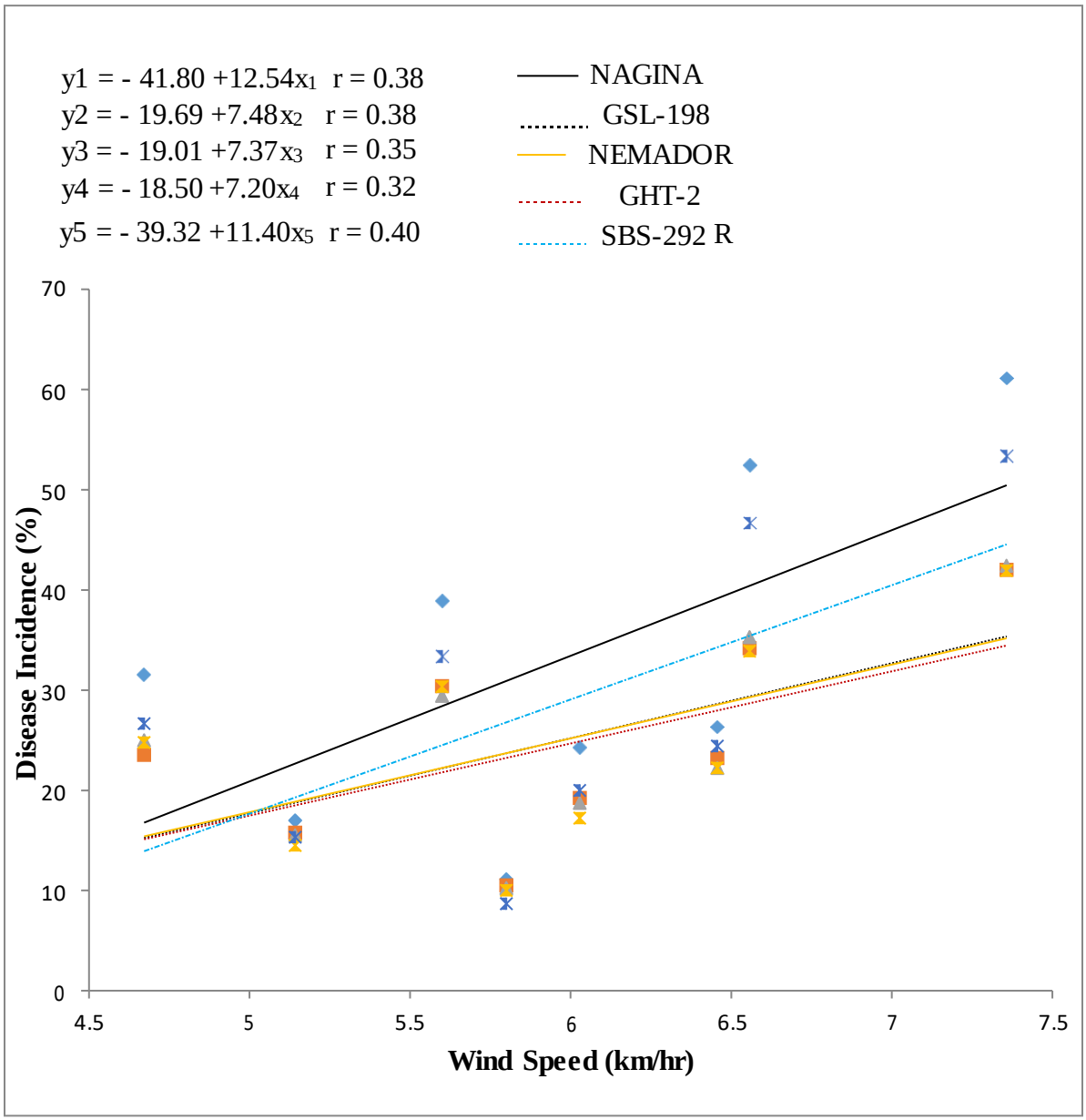


Figure: 4.5 Relationship between wind speed with tomato mosaic virus (disease incidence) recorded on NAGINA (y1), GSL-198 (y2), NEMADOR (y3), GHT-2 (y4), SBS-292 (y5), respectively.

4.4 Effects of plant extracts on ToMV disease incidence

The individual effect of all the extracts was highly significant, whereas the effect of Varieties was significant and interaction between varieties and treatments was non-significant. The effect of date of spray on ToMV disease incidence (%) and two way interaction between treatments and dates of spray was highly significant. While two way interaction between varieties and dates of spray was non-significant and the three way interaction of dates of spray, treatments and varieties was also non-significant.

Table: 4.3 Analysis of variance ToMV Disease incidence (%)

Source of variation	DF	SS	MS	F-value	P-value
Treatments (T)	3	11808.412	3936.137	279.309**	0.001
Varieties (V)	4	311.925	77.981	5.534*	0.020
Interaction (V X T)	12	216.130	18.011	1.278 ^{NS}	0.236
Dates of spray (D)	3	11181.195	3727.065	264.474**	0.012
Interaction (D x T)	9	2624.999	291.667	20.697**	0.011
Interaction (D x V)	12	161.768	13.481	0.957 ^{NS}	0.493
Interaction (D x V x T)	36	143.935	3.998	2.84 ^{NS}	1.000
Error	158	2226.598	14.092		
Total	240	324676.936			

*= Significant when P-value < 0.05

**= Highly significant when P- value < 0.01

NS= Non-significant when P- value > 0.0

4.5. Comparative efficacy of different treatments on ToMV disease incidence

The data given in the Table: 4.4 indicates that there was significant difference among treatments on the ToMV disease incidence (%). So ToMV disease incidence was greater in untreated/ control as compared to the chemicals treatments (*Azadirachta indica, Calotropis procera* and *Allium cepa*). *Azadirachta indica* and *Calotropis procera* proved to be more effective as compared to the *Allium cepa*. The most effective treatment was T_1 where *Azadirachta indica,* was applied.

4.4 Comparison of mean values of disease incidence

Treatments		Varieties					Means
		SBS-292	GHT-2	NEMADOR	GSL-198	NAGINA	
T1	NEEM	24.18	26.05	26.69	28.22	29.57	26.94d
T2	AKK	28.58	30.31	31.46	33.34	34.95	31.72c
T3	ONION	34.62	35.63	36.48	37.51	38.22	36.49b
T4	Control	42.13	43.33	44.07	45.22	46.67	44.28a
Means		32.37e	33.83d	34.67c	36.07b	37.35a	

LSD (Treatment) = 4.01

LSD (Varieties) = 1.22

The effects of different plant extracts (*Allium cepa, Calotropis procera and Azadirachta indica*) were different on five varieties/ lines as given in table: 4.4. *Azadirachta indica* was most effective minimum disease incidence was observed on variety SBS-292 where mean value of disease incidence was 24.18% and in Nagina variety *Azadirachta indica* showed lower effect and mean value of disease incidence was 29.57%. Similarly in case of *Calotropis procera* disease incidence was 28.58% in variety SBS-292 that was its maximum effect. Maximum effect of *Allium cepa* recorded in variety SBS-292 where mean value of disease incidence was 34.62% but Allium cepa was least effective as compared to the *Azadirachta indica* and *Calotropis procera* and *Azadirachta indica* was most effective.. These results indicated that all extracts suppressed the ToMV disease incidence but *Azadirachta indica* was the most effective, compared to untreated control.

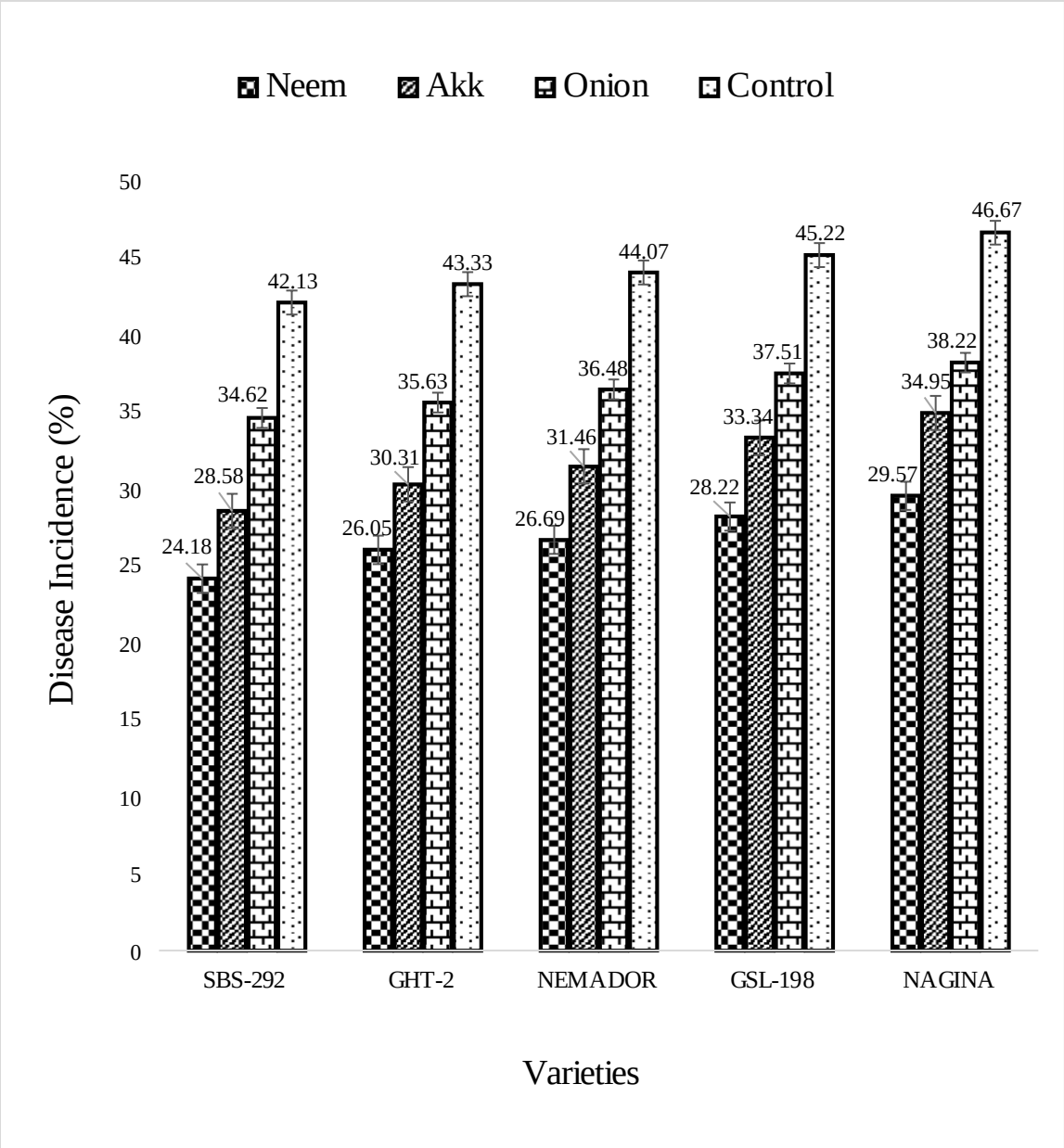


Figure: 4.6 Comparison of mean values of ToMV disease incidence (%)

Table: 4.5 Comparative efficacy of different treatments on ToMV disease incidence

Treatments		Dates of spray				Means
		15-03-2015	30-03-2015	14-04-2015	29-04-2015	
T1	NEEM	19.39	26.69	32.79	28.11	26.74d
T2	AKK	23.24	31.25	38.22	33.41	31.53c
T3	ONION	27.39	35.21	43.99	39.40	36.49b
T4	CONTROL	28.54	41.25	53.82	59.18	45.69a
Means		24.64d	33.60c	42.20a	40.02b	

LSD (Extracts) = 4.01

LSD (Dates of Spray) = 2.35

The ToMV disease incidence was greatly reduced in all the treatments as compared to the control (Table: 4.5). In the table letter "a" represent maximum disease incidence and letter "d" indicate minimum disease incidence. At the first date of spray, mean value of disease incidence in tomato plants where *Azadirachta indica* was applied was only 19.39% whereas mean value of disease incidence in control was 28.54%. Similarly at second, third and fourth dates of spray percent disease incidence was 26.69, 32.69 and 28.11, respectively but in control percent disease incidence was much higher which was 41.25, 53.82 and 59.18 at second, third and fourth dates of spray, respectively. *Azadirachta indica* spray resulted in reduction of disease incidence as compared to the other treatments. At first date of spray disease incidence in tomato plants where *Calotropis procera* was sprayed mean disease incidence was 23.24% while in control it was 28.54%. Similarly at second, third and fourth dates of spray mean value of percent disease incidence in plants where *Calotropis procera* was sprayed was 31.25, 38.22 and 33.41, respectively while in control it was 41.25, 53.82 and 59.18 percent at second, third and fourth dates of spray, respectively. Efficacy of *Allium cepa* was less as compared to the *Azadirachta indica* and *Calotropis procera*. At first spray of *Allium cepa* disease incidence was 27.39% whereas in control it was 28.54%. Similarly at second, third and fourth dates of spray mean value of percent disease incidence in plants where *Allium cepa* was sprayed was 35.21, 43.99 and 39.40, respectively while in control it was 41.25, 53.82 and 59.18 percent at second, third and fourth dates of spray, respectively.

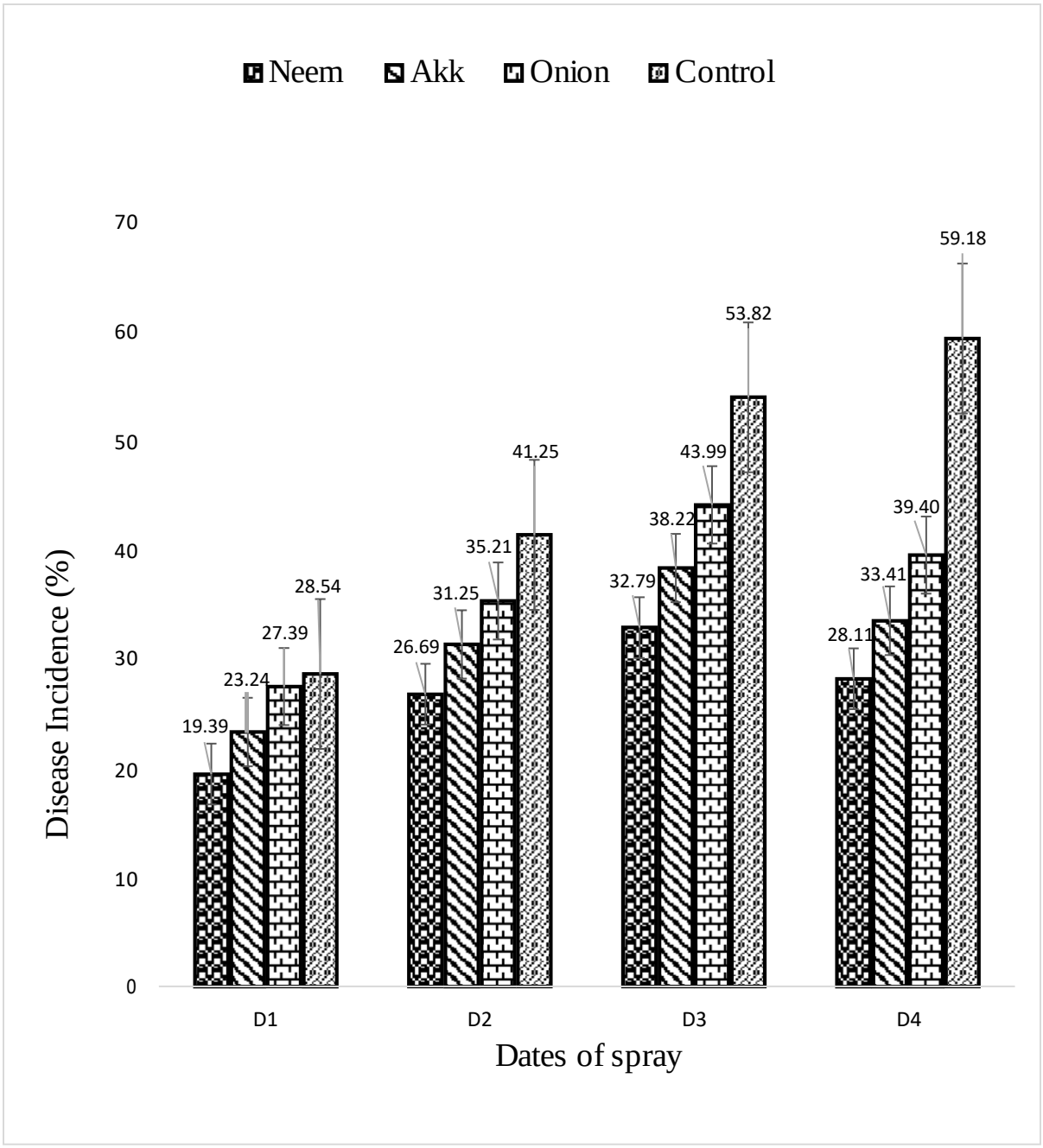


Figure: 4.7 Comparative efficacies of different treatments on ToMV disease incidence

Table: 4.6 Comparison of mean value of dates of spray, interaction of dates of spray and varieties

Varieties	Dates of spray				Means
	15-03-2015	30-03-2015	14-04-2015	29-04-2015	
SBS-292	22.18	30.28	37.75	35.26	31.36e
GHT-2	23.38	32.62	38.32	37.06	32.84d
NEMADOR	24.09	33.52	40.56	38.37	34.13c
GSL-198	27.83	35.99	42.73	39.60	36.53b
NAGINA	28.73	36.58	44.68	40.83	37.70a
Means	25.24d	34.39c	40.80b	38.22a	

LSD (Varieties) = 1.21

LSD (Dates of spray) = 2.35

It was observed that ToMV disease incidence (%) on all varieties / lines increased during second, third and fourth dates of spray. ToMV disease on SBS-292 increased that was 22.18% at first date of spray next on second and third dates of spray it reached to 30.28 and 37.75, respectively. Similarly ToMV disease incidence (%) on GHT-2 was 23.38% and it also increased to 32.62 and 38.32, during second and third dates of treatments, respectively. Disease incidence on Nemador was 24.09% at first date of spray and during second and third dates of spray increased in disease incidence (33.52 and 40.56 percent, respectively) was recorded. Similarly ToMV disease incidence on GSL-198 variety was 27.83% at first date of spray and next on second and third dates of spray increased in disease incidence 35.99 and 42.73, respectively was observed. Disease incidence on variety Nagina rose from 28.73 to 36.58 and 44.68 percent at second and third date of treatments, respectively. But the fourth date of treatment did not show increase in ToMV disease incidence as shown in Table: 4.6. ToMV disease incidence was less on all varieties at fourth date of spray.

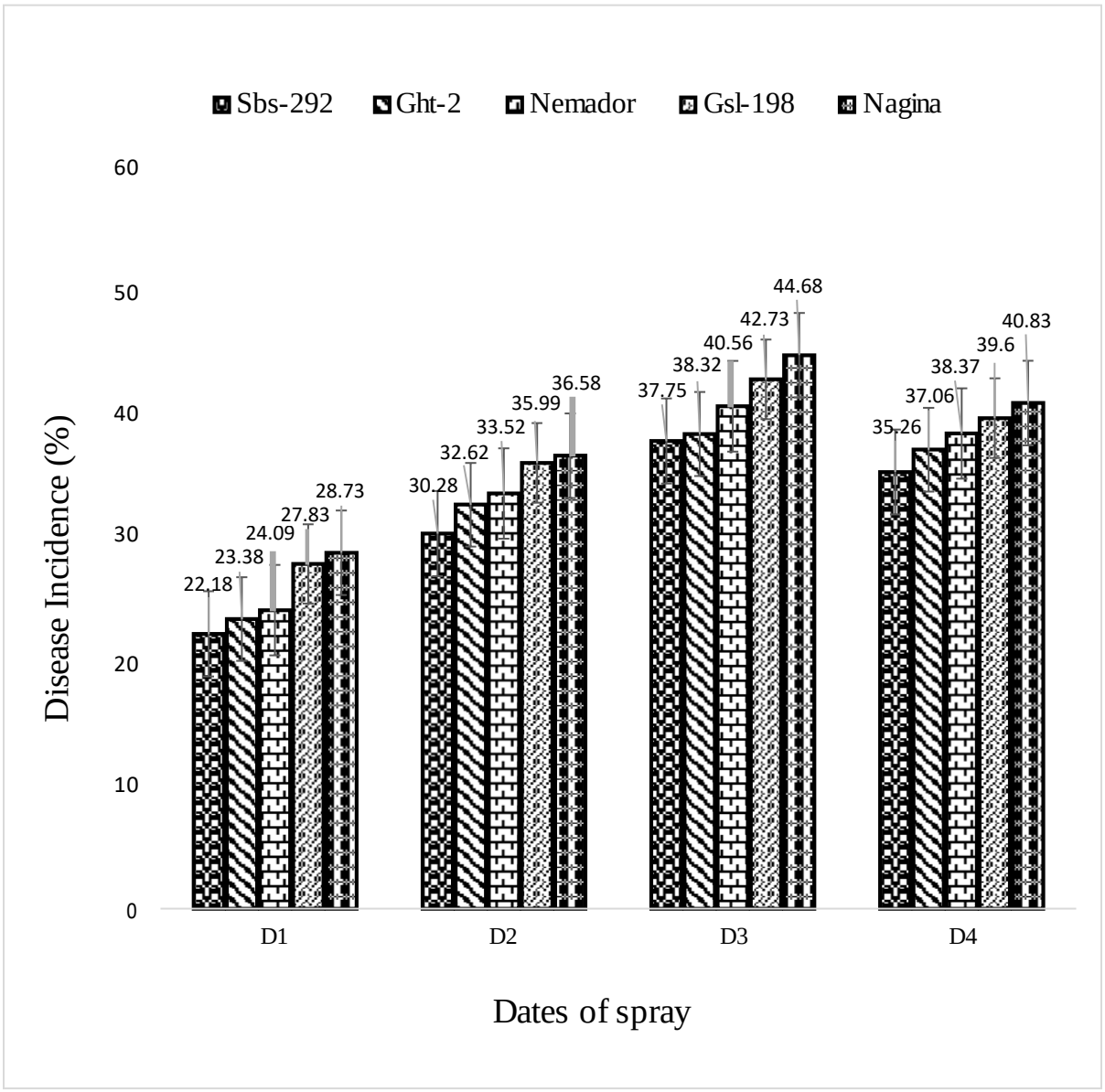


Figure: 4.8 Comparison of mean value of dates of spray, interaction of dates of spray and varieties

CHAPTER 5

DISCUSSION

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop of both tropics and sub tropics of the world, belonging to the family *Solanaceae* and ranks second in importance among vegetables. It is vulnerable to attack by a number of bacterial, fungal and viral diseases. Among the viral diseases, mechanically transmitted tomato mosaic virus is most common, widespread devastating and damaging. Tomato mosaic virus (ToMV) infection is appearing as a threat for tomato cultivation in Pakistan and causes severe yield losses in tomato crops. It was observed that so called resistant varieties in Pakistan all are susceptible to this virus. Different approaches are adopted for the management of viral diseases, however, use of resistant or tolerant varieties are considered to be the most desirable, economical and environmentally friendly approach. In pursuance of this strategy efforts were directed to screen some selected tomato lines/varieties under natural field conditions against tomato mosaic virus (ToMV) to find the source of resistance/tolerance, so that this may be incorporated into breeding programme to make them high yielding disease resistant cultivars of tomato.

Naqeeb appeared to be highly resistant to disease whereas line VRI-575 was found resistant. Riogrande and Savana these two varieties exhibited mild resistance against tomato mosaic virus. Lines SBS-292, GHT-2 and variety Baby Red were moderately susceptible. While one variety Nemador and one line GSL-198 were susceptible. Whereas the variety Nagina was highly susceptible to ToMV.

The result obtained from screening experiment matched the result of Hameed *et al.,* (1992) who screened 15 tomato lines against tomato viruses to find out the level of resistance. On the basis of symptom expression, biological essay and ELISA, line 442 and 838 appeared to be moderately resistant and line 943 was tolerant.

Ali *et al.,* (2002) who screened 12 commercial tomato varieties against ToMV, PVX, PVY and CMV. Two varieties (Florist and Forest) were resistant to four of the viruses including ToMV, for the highest incidence was recorded in nurseries and field. Two varieties

represent a previously described and potentially useful source of resistance to the four viruses under inoculated condition.

Similarly Ragupathi and Narayanaswamy (2000) screened out one hundred and sixty germplasm entries of tomato against tomato viruses during summer in Coimbatore, India. Under natural conditions only two wild species, namely *Lycopersiccon hirsutum* (LA 1353) and *Lycopersiccon hirsutum* f. sp. *glaboratum* (LA 1223) were free from virus infection. The remaining 157 cultivars had more than 50% infection.

Micznski *et al.,* (2004) screened 28 accessions of wild tomato belonging to the species *Lycopersiccon peruvianum, L. glandulosum, L. chilens, L. pimpinellifolium, L. hirsutum* and *L. pennellii* for resistance to infection following mechanical inoculation with tomato mosaic tobamovirus (ToMV), cucumber mosaic cucumovirus (CMV) and tomato spotted wilt tospovirus (TSWV). All plants of *L. hirsutum* showed complete resistance to ToMV, and one accession of *L. peruvianum* remained uninfected by TSWV. Two other accessions of *L. peruvianum* exhibited a high level of resistance (97%) to TSWV. Resistance in the other accessions was completely resistant to all 3 viruses, but some showed relatively high resistance to combinations of two viruses.

Boukema (1980) reported that plants of the *Capsicum* spp. have resistance against tobamoviruses such as tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV), which are manifested by the appearance of necrotic local lesions at the infection site. The resistance is conferred by four different genes at L-locus known as L, L2, L3 and L4 respectively.

Smith and Murakishi (1987) conducted experiments for screening of tomato somaclones, for resistance to tobacco mosaic virus and tomato mosaic virus. Out of 370 soma clones found plants were observed to develop resistance to TMV/ToMV.

Epidemiology plays an important role in the spread and development of disease. Therefore, disease incidence data of ToMV was taken on weekly basis and the averages were correlated with environmental factors (maximum temperature, minimum temperature, relative humidity, rainfall and wind speed). The result revealed that there was significant

effect of some environmental parameters on disease developement. The effect of maximum temperature and relative humidity were highly significant on disease incidence.

Correlation of maximum temperature with disease incidence of ToMV on all five lines/the varieties (Nagina, GSL-198, Nemador, SBS-292, GHT-2) was positive indicating that disease increased with increase in maximum temperature. The correlation of minimum temperature with disease incidence was also positive showing that disease incidence increased with increase in minimum temperature. On the other hand correlation of the relative humidity with disease incidence of tomato mosaic virus was negative showing that disease increased with the decrease in relative humidity. Rainfall was also negatively correlated because disease incidence decrease with the increase in rainfall. While on the other hand wind speed was positively correlated because as the wind speed increases diseases incidence also increases. It was concluded that maximum and minimum temperature play an important role in disease development. These results were in accordance with the finding of Hassan *et al.,* (1993) who studied the epidemiology of tomato viruses and found that nursery plants were the initial and primary source of tomato mosaic virus (ToMV) infection which was attributed due to high percentage of the infected seeds. Tomlinson (2008) studied epidemiological factors which determined virus infection of vegetable crops. Similarly Broadbent *et al.,* (2008) studied the epidemiology of tomato mosaic. Tomato plants were easily infected with tomato mosaic virus by contact. ToMV persisted for over three years in a dark enclosed space, but was inactivated within a few weeks in daylight.

In the management experiment three plant of *A. indica* (Neem), *C. procera* (Akk) and *A. cepa* (Onion) extracts were tested for their efficacy against ToMV. All the extracts were applied at one percent concentration at four different dates. All the treatments caused significant reduction in disease incidence as compared to control where only distilled water was spray. The lower mean values of treatment *A. indica*, *C. procera* and *A. cepa* were compared with the treatment where no extract was applied. Low value by *A. indica* extract suggested that this plant extract was more effective than *C. procera* and *A. cepa*. The extract of *A. cepa* was less effective as compared to *A. indica*, and *C. procera*.

These findings are in accordance with the result of Aslam and Naqvi (2000) that tested the efficacy of *A. indica* product and stated that it is safer and environment friendly. Similarly

Dohroo and Gupta (1995) reported that Azadirachtin and other limonids were quite effective in controlling plant diseases of diverse nature. Neem extract and neem oil were the most potent in reducing incidence of virus. Baranwal and Ahmad (1997) studied the effect of *Clerodanrum aculentum* which was applied as a powder to the soil or in liquid form as a foliar spray. Tomato plants who received CA as a soil application, spray or both showed delayed incidence of virus infection and comparatively higher yields of tomato. Kumar (2004) studied the response of pre inoculation spray of different fraction of plant extracts from *Mirabilis jalapa* (roots), *Asparagus adscendens* (roots), Vitex negundo (leaves), *Symphytum perigrinum* (roots) in inhibition of TMV. Extract of *vitex negundo* (leaves) proved to be effective by inhibiting virus up to 67.12%. Madhusudhan *et al.,* (2005) studied the effect of inducers against tobamovirus infection in tomato and bell peppers. When the seedling were sprayed with salicylic acid (50mM) and neem oil (5%), the concentration of tomato mosaic tobamovirus (ToMV) and tobacco mosaic tobamovirus (TMV) was assessed based on the number of local lesions on *Nicotiana glutinosa.* The results showed that the seed or seedling treatment with inducers reduced the number of local lesions when compared to untreated control.

The result of transmission studied are in conformity with the result of previous researchers such as Van Regenmortel (1981) and Jan *et al.,* (2005). These results proved that mechanical means such as wounds, contacts etc caused by the agronomic practices and human activities play important role in the transmission of ToMV under natural field conditions. Therefore, disease management strategies must start by raising healthy seedling of tomato in the nursery, elimination of infected seedling, disinfection of hands of workers and all possible phytosanitary conditions in the field, agro techniques, transplanting and picking.

In present study, only four line/varieties of tomato (Naqeeb, VRI-575, Riogrande, and Savana) were found to be resistant against ToMV. So, there is need for further screening of tomato germplasm to find out resistant/tolerant lines or varieties against ToMV.

CHAPTER 6

SUMMARY

Tomato mosaic virus (ToMV) is a severe disease of tomato (*Lycopersicon esculentum* Mill.) throughout the world, including Pakistan. The virus is not transmitted through insect vector but transmitted by mechanical means. ToMV is responsible for great loss, several factors which are responsible for the development of ToMV in the country that include environmental factors, vulnerable host and inoculums potential. Environmental factors play important role in the spread and development of ToMV.

The resistant varieties are the only economical source of managing the viral disease. Therefore for identifying the source of resistance against ToMV ten different lines / varieties (Nagina, Naqeeb, Riogrande, VRI-575, SBS-292, GHT-2, GSL-198, Savana. Nemador, Baby Red) were screened under field conditions. Out of ten lines / varieties, one variety Naqeeb was resistant. While one line VRI-575 was resistant, two varieties/lines (Riogrande, Savana) were moderately resistant, three varieties/lines (SBS-292, Baby Red, GHT-2) were moderately susceptible, two varieties/lines (Nemador, GSL-198) were susceptible and one variety Nagina was highly susceptible to ToMV.

The correlation of maximum temperature with ToMV disease incidence for all the varieties/ lines (Nagina, GSL-198, Rio GHT-2, Nemador and SBS-292) was positive, showing that disease incidence increased as maximum temperature increased. The correlation of minimum temperature with disease incidence was also positive i.e. disease incidence increased with the increase in minimum temperature. On the other hand, the correlation of relative humidity with disease incidence was negative, indicating that disease incidence decreased as the mean relative humidity increased. There was no correlation of rainfall with ToMV disease incidence, i.e. disease incidence decreased with increase in rainfall. ToMV disease incidence was positively correlated with wind speed.

In the management experiment extract from *A. indica* (Neem), *C. procera* (Akk) and *A. cepa* (Onion) were applied at one percent concentration. All the treatments gave

significant reduction in disease.The most effective treatment was *A. indica* extract at 1% concentration gave significant reduction in disease as compared to untreated control.

It is concluded that incidence of ToMV can be significantly reduced by sowing resistant varieties, timely and appropriate application of plant extracts and adaptation of phytosanitary condition.

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