SYLLABUS

AEN 321 COMMERCIAL PRODUCTION OF BIOCONTROL AGENTS 0+2

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PRACTICAL

Requirments of a biocontrol unit-rearing of host insects for parasitoids and pathogens-rearing of prey insects for predatorys-rearing of egg parasitoid Trichogramma- Rearing of egg-larval parasitoid *Chelonus*-Larval parasitoids, *Goniozus*, *Bracon* and *Eriborus*-pupal parasitoids Tetrastichus Israeli, *Trichospilus pupivora* and *Brachymeria*-rearing of predators-Coccinellid-*Cryptolaemus montrouzieri*-Rearing of *Chrysoperla carnea*-Mass production of entomopathogens-production of nuclear polyhedrosis virus of *Helicoverpa armigera* and *Spodoptera litura*-granulosis virus of sugarcane early shoot borer *Chilo infuscatellus*, fungus *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii*- Standardization of insect pathogens-Field utilization techniques of biocontrol agent-Improving the efficacy of biocontrol agents.

Cost analysis and preparation : Principles of enterprise management-preparation of agricultural project reports-project analysis and financial management-agricultural finance-source of finance-acquisition-ratio analysis-principles of costing-economics of farm enterprise.

PRACTICAL SCHEDULE

1. Establishment of a biocontrol unit.
2. Rearing of host insects – *Corcyra cephalonica*.
3. Rearing of host insects – *Corcyra cephalonica*.
4. Rearing of host insects – *Helicoverpa armigera*.
5. Rearing of host insects – *Helicoverpa armigera*.
6. Rearing of host insects – *Spodoptera litura*
7. Rearing of host insects – *Spodoptera litura*
8. Production of egg parasitoid – *Trichogramma chilonis*
9. Production of egg parasitoid – *Trichogramma chilonis*
10. Production of egg – larval, larval and pupal parasitoids
11. Production of egg – larval, larval and pupal parasitoids
12. Rearing of predators – *Chrysoperla carnea*
13. Rearing of predators – *Chrysoperla carnea*
14. Mass culturing of mealybugs
15. Mass culturing of mealybugs
16. Rearing of predators – *Cryptolaemus montrouzieri*

17. Mid semester examination
18. Production of nuclear polyhedrosis virus of *Helicoverpa armigera*
19. Production of nuclear polyhedrosis virus of *Helicoverpa armigera*
20. Production of nuclear polyhedrosis virus of *Spodoptera litura*
21. Production of nuclear polyhedrosis virus of *Spodoptera litura*
22. Production granulosis virus of sugarcane early shoot borer
23. Production granulosis virus of sugarcane early shoot borer
24. Standardization of insect pathogens
25. Standardization of insect pathogens
26. Mass production of fungal pathogens – *Metarhizium anisopliae, Beauveria bassiana and Verticillium lecanii*
27. Mass production of fungal pathogens – *Metarhizium anisopliae, Beauveria bassiana and Verticillium lecanii*
28. Mass production of fungal pathogens – *Metarhizium anisopliae, Beauveria bassiana and Verticillium lecanii*
29. Cost analysis and – Ratio analysis
30. Project preparation for large scale production of biocontrol agents – *Trichogramma chilonis*
31. Project preparation for large scale production of biocontrol agents – *Trichogramma chilonis*
32. Project preparation for large scale production of biocontrol agents – nuclear polyhedrosis virus of *Helicoverpa armigera* and *Spodoptera litura*.
33. Project preparation for large scale production of biocontrol agents – nuclear polyhedrosis virus of *Helicoverpa armigera* and *Spodoptera litura*.
34. Final examination.

REFERENCE BOOKS

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Ex.No. :

Date :

Establishment of Biocontrol Unit

1. Introduction

The ability to rear insects in large numbers successfully is the basic requisite for effective implementation of biological control programmes. In order to produce good quality biocontrol agents, a well-established laboratory with necessary infrastructure facilities, trained manpower and standard operating procedures are essential. The insect rearing and biocontrol agent production facilities vary widely in type and operation methods. The activities carried out in the production facility involve,

a. regulation of environment,

b. handling of different life stages of insects,

c. sanitation in the work place,

d. preparation of feed materials for the insects, and

e. preparation or processing of biocontrol agents.

Therefore, different kinds of equipment and rearing materials such as glassware, plastic ware, cages, storage racks and chemicals are required for successful management in production. The choice of the equipment will depend upon the categories of biocontrol agents produced. A general – purpose facility will need most of the materials and they can be used commonly for majority of the biocontrol agents.

2. Objective

1. To study types of facilities of biocontrol agents available and design requirements
2. To develop a conceptual floor plan for a biocontrol laboratory
3. To list the types of equipment and materials needed, their placement and safe handling procedures in biocontrol laboratory.

3. Types of facilities

Currently the facilities in biocontrol agents production are generally for Research and Development and Commercial Production. The former is aimed to explore and identify categories of biocontrol agents, study their biology, effectiveness against crop pests, develop cost effective methods of production, prescribe quality control parameters and demonstrate the effectiveness of the agents under field condition. A research and development centre may have a commercial unit to supply the biocontrol agents in limited quantity to the farmers and to experiment on commercial feasibility of the agents. The Tamil Nadu Agricultural University, Coimbatore and the Project Directorate of Biological Control, Bangalore is working on several biocontrol agents on the above lines. The commercial production facility is aimed to make available the biocontrol agents in large numbers to the farmers with an object to make profit with quality. A commercial facility may have an internal research and development unit to find out ways and means to solve immediate day-to-day problems and also to keep abreast of the changing needs of biocontrol agent production.

Depending upon the number of species of host insects reared and biocontrol agents produced the facilities are classified into two different types.

a. **General purpose facility:** When many species of insects are reared for production of different kinds of biocontrol agents and the facility concentrates on a very large clientele group then these facilities are called general purpose or multiple species facility.

b. **Specialized facility:** The numbers of biocontrol agents produced are narrowed down to few categories but in large numbers. In this type the clientele group services is also narrow.

A combination of procedures, equipment and space environment decides the scale of production of various categories of biocontrol agents. It may be a Cottage facility wherein the produce with simple techniques produces biocontrol agents in limited numbers for own-farm use and supplied

to few needy groups. This facility depends extensively on either family or hired labour in the farmstead. On the other hand, an entrepreneur may produce biocontrol agents and market them locally or widely. Due to constraints in capital investment, lack of suitable market channels and no-availability of professional input of techniques for scaling up production the entrepreneur attempts to produce less and caters a limited clientele. The service area covered could be few thousands of acres only. The production centers of such types are called Pilot Scale Production Facilities. Wherever possible and required essentially, labour is substituted by machines in production. A pilot scale production facility may not be dependent on machines in production. These units are called Traditional Pilot Scale Facilities. When they partially use machines in production the systems are called Semi-automated Pilot Scale Facilities. These types of systems function in a decentralized manner. Several such pilot scale production centers are needed to supply biocontrol agents if large areas of crops are to be covered in any season. The ideal form of existence are the Industrial Production Facilities that are in a position to supply in greater amounts of quality biocontrol agents and cover large acreage of crops. These systems have a very large clientele group and with excellent net work of market channel offer better services on the methodologies of how to use the biocontrol agents. These units effectively substitute automated methods in production and the dependence on labour is limited.

4. Design and Space Requirements

The broad objectives of the programme and the procedures involved are vital in designing a facility. However sophisticated a facility is unless designed properly, there will not be consistency in production. Not all the insect and their biocontrol agents require the same type of facility, equipment and procedures. A compact facility may be sufficient to produce certain species of entomophages like *Cryptolaemus montrouzieri*, or an elaborate structure is needed as in *Helicoverpa armigera*. Separate facilities are needed in entomopathogens multiplication. This is essentially required to prevent the contamination of host insects in the insect rearing area with the

entomopathogens. For e.g. at TNAU the nuclear polyhedrosis virus production and formulation is housed in a separate lab. Most structures currently available in India are designed originally for other purposes and do not meet the standard requirement. All the more, describing an ideal form of insectary or biocontrol agents production facility is difficult. The facilities have to be located well away from urban activities but easily accessible to city, free from direct radiation and with good drainage. The materials used in construction must provide permanence, cleanability, serviceability, economy and safety. The interior walls must be of smooth finish preferably epoxy coated and fungus resistant. The floors in general have to be ceramic tiled to facilitate easy sanitation. The critical areas the walls should have ceramic coat. The ceiling in rearing areas should be insulated with gypsum board. The entire area where insects are housed the doors, windows and ventilators should be made insect-escape-proof.

Depending upon the business objective and capital investment, new facilities can be created or older structures modified. The size requirements depend upon the target of production. However old or new, the facilities should be designed or modified to meet the primary activities *viz.*, maintenance of the different stages of the insects, their biocontrol agent production. In addition, associated activities that are very vital for maintenance of the culture *viz.,* feed/diet preparation, water and electricity supply, sanitation and facility maintenance, regulation of rearing environment, storage of rearing materials, drainage and waste disposal have to be given importance in designing the facility.

A production complex may be divided into several sections *viz.*, administration, quarantine, production, storage, wash and waste disposal. Areas where different stages of insects are maintained are often prone for contamination and prevention of entry of the pathogens and cent per cent control in the event of contamination are very critical. Therefore, in the production facilities, it is ideal to separate these critical holding areas from the rest. Moreover, if these holding areas are further subdivided into separate cubicles, the possibilities of human traffic and entry of pathogens are greatly

reduced. This holds good when different strains of species of parasitoids or predators are maintained in the laboratory. For e.g. *Trichogramma chilonis* and *T. japonicum* have to be maintained in separate enclosures or holding rooms if the purity of the product is to be maintained.

5. Equipment and materials in biocontrol laboratory

5. 1. Quarantine area

Whenever new specimens of insects or their biocontrol agents are brought to the laboratory, either from field or from another laboratory, the specimens should not be moved to the production area without examination for presence of biological contaminants. Therefore, the specimens have to be kept in quarantine area, which require the following.

1. Table or bench
2. Large sink with running water and draining racks
3. Drain with soil trap
4. Tools for cutting (saw, axe, secateurs, chisel and stout knife)
5. Buckets, sieves, plastic trays, plastic containers of different sizes
6. Bags (thick paper and plastic), specimen tubes or similar containers
7. Hand lens, scalpel and forceps, writing / marking materials.
8. Microscopes for preliminary diagnosis

5. 2. Main Laboratory

The basic requisites in the laboratory are furniture including a well-laminated bench, drawers, cupboards and shelves. The number and size depend upon the target of the biocontrol agents to be produced. A continuous electric power supply (mains or generator) is essential for smooth running of equipment.

5. 2. 1. Large items of equipment

a. Refrigerator, preferably with separate freezing compartment or separate freezer

b. Incubator – for culture growth

c. Water deionizer or still – supply of pure water

d. Hot air oven

e. Gas stove

f. Autoclave – a large domestic pressure cooker or industrial type

g. 'Top pan' balance – 0.05 – 500 g – for weighing chemicals and media

h. A pH meter for checking pH of media

i. Microbiological safety cabinet or Laminar flow chamber

j. Stereoscopic (Low-power – x20 – 200) dissecting microscope for examination of specimens and culture

k. Research (high-power – x100 – 1000) microscope for pathogen identification and some tissue examination procedures

l. Vacuum cleaner for regular cleaning of the laboratory

m. Personal Computer with printer, uninterrupted power supply system and voltage stabilizers for equipment

5. 2. 2. Instruments

a. Standard dissecting instruments such as forceps, scalpels, scissors, mounted needles are required in both fine and coarse sizes.

b. Holders and wire for inoculating loops are required for sub culturing.

c. Tripod and gauze, spirit/gas burner, saucepans and dishes, draining racks, buckets preferably plastic.

5. 2. 3. Glassware

a. Beakers 10, 25, 100, 250, 1000 ml.

b. Erlenmeyer flasks (conical flasks) 250, 500 ml.

c. Desiccators

d. Measuring cylinders 10, 25, 100, 250, 1000 ml.

e. Pipettes 2, 5, 10 ml graduated (with rubber filler)

f. Petri dishes 5, 9 cm diameter (glass and/or plastic)

g. Watch glasses or embryo dishes.

h. Medical flats (Medicine bottles), amber glass, wide neck, 300 ml (do not fill more than two-thirds full) for storing made-up culture media.

i. Screw cap bottles, universal style with wide neck, 25 ml sizes (glass or plastic) for cultures, specimens, etc.

j. 'Plain cap', wide neck, bottles 10, 20 ml for holding or treating specimens.

k. Large test tubes for heating specimens.

l. Microscope slides.

m. Cover slips.

n. Dropping bottles, amber glass, for holding stains.

o. Reagent bottles, amber glass, various sizes.

p. Boxes, clear plastic, for incubating specimens.

q. Bottles, various sizes.

r. Bags, various sizes, paper and plastic.

s. Trays.

t. Sieves, brushes and strainers.

In addition to the above items, specific items are required in the production of various biocontrol agents.

a. Adult emergence cages of different sizes to handle the adults of lepidopterans *viz*., *Helicoverpa armigera, Spodoptera litura, Plutella xylostella*, *Corcyra cephalonica* and in mass production of *Cryptolaemus montrouzieri*.

b. Plastic basins for the production of *Corcyra cephalonica*.

c. Open storage racks for *Corcyra cephalonica*.

d. Oviposition chambers for *Corcyra cephalonica*.

e. Moth collector and scale separator for *Corcyra cephalonica*.

f. UV Chamber for sterilizing the eggs.

g. Domestic mixer for preparation of semisynthetic diet.

h. Pipetting systems for preparation of viral suspensions.

i. Centrifuge for processing the viruses.

j. Haemocytometers for counting and standardization of virus.

k. Storage bins, dust bin type buckets, etc.

l. Air conditioners.

m. Humidifiers/air coolers.

n. Safety equipment like fire extinguishers.

o. First aid box.

6. Placement of equipment in the laboratory

The equipment, instruments and miscellaneous items have to be placed where potential hazards for the workers are non existent. Heat generating equipment like autoclave, hot air oven, etc., should be placed in sterilizing zones with good ventilation and should not be placed near the culture rooms. Temperature and humidity controllers should be used in culture areas. Laminar Flow Chambers should be placed in culture rooms or inoculating rooms. Diet preparation equipment, etc. should be placed separately in proximity to larval culture area.

7. Chemicals

The following are some general reagents needed for a number of routine operations in the Biocontrol Laboratory.

a. Distilled or deionized water – cleaning, preparation of other reagents and media.

b. Industrial alcohol or methylated spirit – cleaning, surface sterilizing.

c. Sodium hypochlorite solution – for surface sterilizing. A 10% dilution of fresh commercial bleaching solution is suitable but must be kept cool, in a dark bottle and replaced regularly.

d. Formaldehyde (commercial grade) for critical sterilization.

e. Potassium permanganate for fumigation of the laboratory.

Care is needed when handling or using reagents of all kinds and brief details of the substances referred to in the text are given in the section on Handling Notes in the labels.

8. Laboratory handling procedures

A clean and tidy laboratory shows a professional attitude towards the work in hand, apparatus, chemicals and the health and safety of colleagues. Equipment not in use should be returned to its proper storage place in a clean and working condition. Laboratory reagents and chemicals should be placed on the appropriate shelves immediately after use with their labels to the front. Culture material should be kept in trays or bags and not left loose on benches; they should normally be kept in incubators or fridges when not being examined. Laboratory benches and other surfaces should be kept clean. It is preferable to clean up after each stage of operation, overcrowding leads to accidents; personnel and equipment are valuable, make sure they are not damaged.

Contamination of cultures can be a problem, in laboratories where fungal cultures are prepared but not stored and not kept clean and dust-free. All sources of debris on which fungi might sporulate should be removed daily. Adequate ventilation is desirable in warm countries but this increases risk of contamination by air-borne spores. Culture work can be carried out in cabinets, the two most commonly used are, ‘clean air’ benches which provide a gentle flow of filtered air into a partly enclosed chamber or hood and can be used for tasks which will not create aerosols of microorganisms. Class I or Class II microbiological safety cabinets can be used for sub culturing sporulating microorganisms or when engaged in techniques which generate aerosols. If these are not available, an area of bench can be partly screened off

using a framework covered with polythene and the area kept very clean by swabbing with industrial alcohol, 70 per cent ethanol or 4 per cent sodium hypochlorite.

9. Operation of equipment

Equipments used in the Biocontrol Laboratory serve a distinct purpose. The equipments are either purchased from manufacturers or fabricated to suit the requirement. Acquaintance with the machines functioning is the primary need before operating them as the equipments are costly. Each of the equipment used in the laboratory is provided with instruction manuals for operation. The manuals have to be consulted for trouble free operation of the equipments and maintenance.

10. Practical Session

i. Study the TNAU Biocontrol Laboratory and discuss briefly the facility available

ii. What are the agents produced in the TNAU Biocontrol Laboratory and classify the type of facility.

iii. Give neat conceptual floor plans for the production of biocontrol agents

a. Facility for producing parasitoids, predators and insect pathogens

b. Facility for producing parasitoids and predators

c. Facility for producing obligate and facultative insect pathogens

iv. Study the different equipment available in the biocontrol laboratory. Record the following :

Name of the item	Manufacturer	Dimensions	Purpose	Price

v. Work out the cost of various items listed under different sections for preparation of cost investment on fixed and variable assets for biocontrol laboratory

Name of the item	**Manufacturer**	**Dimensions**	**Price**

vi. Practice the method of operation and write the steps involved in the operation of the following equipment in simple flow charts

1. Hot air oven

2. Autoclave

3. Laminar flow chamber

4. Vacuum pump

5. Electronic balance

6. Stereo zoom microscope

7. Phase contrast microscope

8. Centrifuge

9. Incubator

vii. What are the items sterilized in hot air ovens and autoclave? At what temperature the materials are sterilized? How long?

Additional Notes

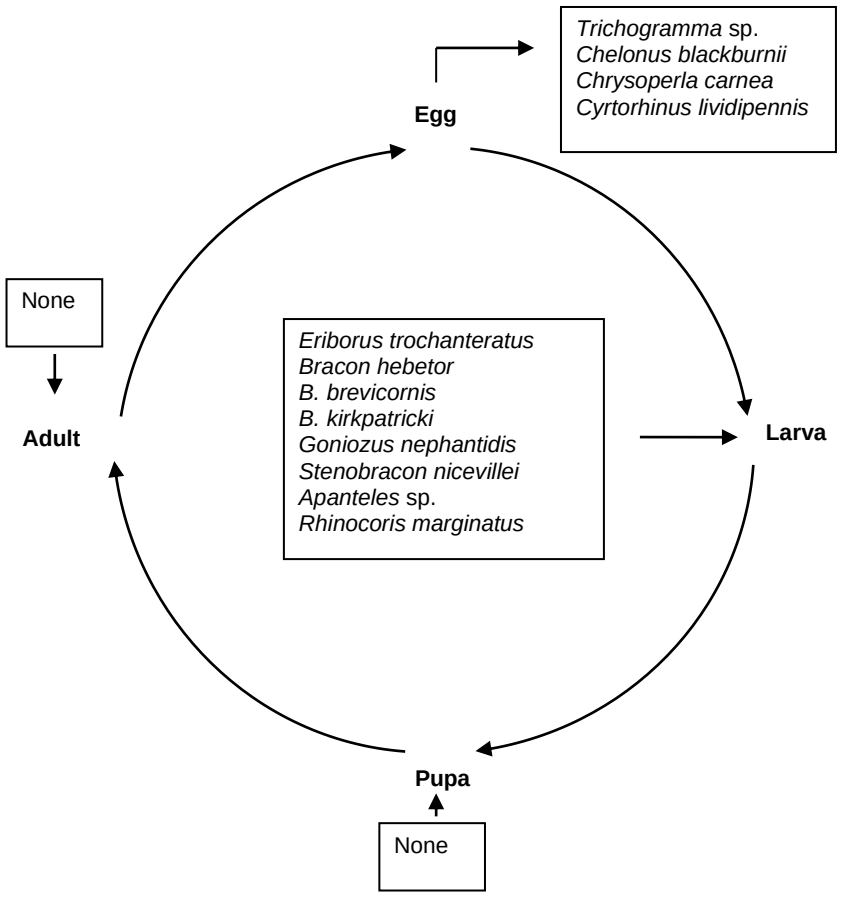
Ex.No. : **Mass Production of *Corcyra cephalonica***

Date : **(Order : Lepidoptera : Family : Pyralidae)**

1. Introduction

Corcyra cephalonica commonly called as rice meal moth or rice moth is a pest of stored foods, *viz.*, cereals, cereal products, oilseeds, pulses, dried fruits, nuts and spices. Many of the natural enemies mass-bred in the laboratory for use in field against crop pests are dependent on either egg or larval stages of *Corcyra* due to the simple reason that it is easier and cheaper to produce natural enemies on different stages of *Corcyra* (Fig.1) than on their original hosts.

Fig.1. Important natural enemies produced on *Corcyra cephalonica*



2. Objective

To study the mass culture procedures followed in *Corcyra* production.

To study the requirement of materials in *Corcyra* production

To study the management procedures in *Corcyra* production

3. Morphology and Biology of *Corcyra*

The eggs are oval and measure 0.5 x 0.3 mm. The white surface is sculptured and has a short nipple-like process at one end. The larvae are generally creamish – white except for the head capsule and the prothoracic tergite, which are brown. There are well-developed prolegs on abdominal segments 3-6 and 10. A fully matured larva measures 15 mm. The last-instar larva spins a closely woven, very tough, double-layered cocoon in which it develops into a dark-brown pupa. The anterior portion of the cocoon has a line of weakness through which the adult emerges. The adults are small. The hind-wings are pale-buff, and the fore-wings are mid-brown or greyish-brown with thin vague lines of darker brown colour along the wing veins. The males are smaller than the females.

Sexual activity usually begins shortly after adult emergence. There is a pre-oviposition period of about 2 days. Egg-laying mainly occurs during the night. The greatest numbers are laid on the second and third days after emergence, although oviposition may continue throughout life. Eggs take about 2-3 days to hatch. Optimum conditions for larval development of *C. cephalonica* are 30 – 32.5^{0}C and 70 per cent RH, at which, the period from egg hatch to adult emergence is only 26-27 days. There is considerable variation in the number of larval instars; however, males generally have 7 and females have 8. The last-instar larvae pupate within the food. The adults emerge through the anterior end of the cocoon, where there is a line of weakness. The sex ratio is 1:1. The adult moth is nocturnal and is most active at nightfall.

4. Mass production of *Corcyra* in the laboratory

4. 1. Materials required

Absorbent cotton	Storage racks
Blotting paper	Streptomycin sulphate
Broken cumbu grain	Rubber band
Camel hair brush	Measuring cylinder
Enamel Tray	Oven
Honey	Home milling machine
Khada cloth	Sieves
Mosquito net	Formaldehyde 40%
Moth aspirator (collector)	Filter paper
Oviposition drums	Moth scale egg separator
Plastic basin	Face masks
Shoe brush	Storing drums
Soap	Ground nut kernel
Specimen tube	Sulphur (WP)
Yeast	Coarse weighing balance

5. Procedure

5.1. Preparation of rearing basins

The basins (16” dia) used for *Corcyra* multiplication are thoroughly cleaned with 0.5% detergent wash and rinsing in tap water followed by wiping with dry, clean – used towel and shade drying. Whenever the trays are emptied after a cycle of rearing, they have to be cleaned preferably to 2 per cent formaldehyde and returned to storage until further use. On reuse the cleaning steps are repeated (Fig 2).

5. 2. Preparation of bajra medium for *Corcyra*

a. The required quantities of bajra grains are coarsely milled and broken into 2-3 pieces in a milling machine. The broken grains are heat sterilized at 100^0C for 1 hour to eliminate the residual population of stored product insects *viz., Rhizopertha dominica, Sitotroga cerealella, Tribolium castaneum* and fungal contaminants. Upon sterilization the grains are cooled under fan in a clean area. The grains are then transferred to plastic basins @ 2.5 kg/basin.

b. Groundnut kernel in required quantity is broken using a pounding machine or a mechanical blender (domestic mixer). Then 100 g of the broken kernel is transferred to each basin and the contents are hand mixed thoroughly.

c. Dry yeast (Bakers) and wettable sulfur is added @ 5g/ basin and the contents are mixed thoroughly. A spray of 10 ml of 0.01-0.05% streptomycin sulfate and mixing of the contents follows this. This medium is used for rearing *Corcyra* larvae (Fig. 2)

d. The number of basins required for egg infestation is calculated and the medium is prepared accordingly.

5. 3. Preparation of *Corcyra* eggs

The primary source of *Corcyra* eggs is reputed laboratories, commercial producers for bulk preparation. If it is intended to begin the production with nucleus colony, the adult moths can be collected from warehouses where the food materials are stored (Fig 3).

a. The eggs used for building up the colony of *Corcyra* have to be free from contaminants like the moth scales and broken limbs and not exposed to UV light.

b. The collections of overnight laid eggs are measured volumetrically to ascertain the number of trays that can be infested with eggs. A cc of eggs is known to contain approximately 16000 – 18000 eggs.

5. 4. Infestation of medium with eggs

The overall production scheme (Fig. 4) involves initial infestation of the cumbu medium with *Corcyra* eggs in desired quantities. This is accomplished by sprinkling the freely flowing eggs on the surface of the medium in individual basins. Per basin 0.5 cc eggs of *Corcyra* is infested. The basins are then covered with clean *khada* cloth and held tightly with rubber fasteners. The basins are carefully transferred to the racks. At a time 84 such basins are stacked in the rack designed at TNAU.

5. 4. Handling the trays during larval development

The larvae that hatch out in 3-4 days begin to feed the fortified Bajra medium. At this stage, light webbings are noticed on the surface. As the larvae grow up they move down. During this period the larvae are allowed to grow undisturbed in the trays.

5. 5. Handling of adults

The adults begin to emerge in 28-30 days after infestation of the eggs. The adults can be seen on the inner side of the *khada* cloth. They are either aspirated with mechanical moth collector or collected with specimen tubes. The whole operation is carried out in a tent of mosquito net. This prevents the large-scale escape of the moths, which if uncontrolled can migrate to the storage area and spoil the grains stored by laying eggs. Workers involved in the collection of moths should wear face moths continuously to avoid inhalation of scales. The moths collected are transferred to the oviposition drum @ 1000 pairs per drum at a time (Fig 4). The oviposition drums of size 30 x 20 cm are made of galvanized iron. The drums rest on tripod frames with legs of height 5cm. The bottoms of the drums are provided with wire meshes that enable collection of eggs. The walls of the drums have two vents (ventilation holes) opposed to each other. The vents are again covered with wire mesh. The lids of the drums have handles besides slots for introducing the moths and adult feed. The oviposition drums filled in a day are maintained

for four to seven successive days for egg collection after which are emptied and cleaned for next cycle of use.

The adults are provided feed containing honey solution. The adult feed is prepared by mixing 50 ml honey with 50 ml water and 5 capsules of vitamin E (Evion). The feed is stored in refrigerator and used as and when required. Piece of cotton wool tied with a thread is soaked in the solution and inserted into the drum through the slot at the top. From a basin, moths can be collected upto 90 days after which the number of moths emerging dwindles down and keeping the basins is not economical for the producer (Fig 4).

5. 6. Handling of eggs

The moths lay the eggs in large numbers loosely. The scales and broken limbs are also found in larger quantities along with the eggs. They cause potential hazard to the workers after years of working in *Corcyra* laboratory. To minimize the risk of scales freely floating in the air, the oviposition drums are placed on sheets of filter paper in enamel trays which trap effectively the scales. Sets of several oviposition drums are kept in ventilated place near an exhaust fan to enable the workers comfort. Daily morning the oviposition drums are lifted up and the wire-mesh bottoms are cleaned gently with a shoe brush so that the eggs and remnants of scales and limbs settled on the mesh are collected along with those on the filter paper. The collections are cleaned by gently rolling the eggs on filter paper to another container. Then they are passed to sieves in series and finally clean eggs are collected. The eggs are quantified in measuring cylinders and used for building up the stocks and natural enemy production.

About 100 pairs of adults produce 1.5 cc of eggs in 4 days laying period inside the oviposition drums. From each basin an average of 2500 moths are collected. Hence from each basin 18.00 – 20.00 cc of eggs can be obtained in 90 days.

5. 7. Maintenance of history sheet

Accurate information is needed on the history of individual basins. The following information is furnished.

1. Date of egg infestation
2. Date of preparation of feed

3. Source of egg
4. Expected date of adult emergence
5. Daily collection of moths
6. Problems encountered with the basin during production
7. Personnel handling the basin

6. Practical Session

i. Study the life stages of *Corcyra cephalonica* and draw neat sketches of the different stages.

Egg	Larva
Pupa	Adult

ii. Prepare individually the fortified medium, infest the eggs and record the observations date wise on larval growth, webbings in the tray, adult emergence.

Date of diet preparation :

Date of egg inoculation :

Date of first emergence of adult :

Days to emerge :

Moths emerged daily basis till 90th day :

Day of emergence														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Total No. of moths emerging =														

iii. When did you observe the peak adult emergence in the basins? Draw a graph plotting the cumulative values of moth emergence in Y ordinate and days after infestation in X ordinate. Draw your conclusions.

iv. Record the time taken to handle adults in different days as follows individually

Day of emergence and time taken to handle the adults in minutes														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Total time required to handle =														

v. Work out the mean time required to handle 100 pairs of adults during the peak period of emergence as follows.

Peak emergence period and time required to handle (Day of peak emergence)

Student	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2															
3															
4															
5															
6															
7															
Student	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1															
2															
3															
4															
5															
6															
7															
Total															
Mean															
Mean time required to handle 1000 pairs =															

vi. Calculate the requirement of feed material, plastic basins, racks, oviposition cages if it is planned to produce 500 cc of *Corcyra* eggs daily from 1st January to 31st December in a year. How will plan and execute the order?

vii. An entrepreneur plans to execute an order of 2500 cc of *Corcyra* eggs supply on 15th November on one time basis. He decides to utilize the excess eggs for other purposes. Work out the quantity of feed material required to produce the desired target.

On which day he should inoculate the trays with eggs to obtain 2500 cc eggs?

What will be the surplus quantity of eggs left out before and after 15th November?

What will be the quantity of eggs required to infest the trays?

What will be the requirement of the trays and racks?

viii. During which stages of *Corcyra* production, automation will be useful?

ix. What are the means by which the excess eggs of *Corcyra* are stored?

x. How will you test the quality of grains used for feed preparation?

Additional Notes

Ex.No. :

Date :

Mass Production of *Corcyra cephalonica:* Problems encountered in production

1.Introduction

The mass production of *Corcyra* involves materials and production management and assurance of quality. The rearing area in the *Corcyra* laboratory has to be maintained in clean condition and free from dust always. The larvae have to be confined within the basin and escape of larvae and adults compound the problems to the producer. Therefore, the producers must have prior knowledge on the problems encountered during culture and methods to manage (Fig 1).

2. Objective

To study the problems frequently encountered in *Corcyra* mass culture

To study the management methods

To study the materials management

To study the quality control procedures

3. Problems encountered

The bajra medium used for *Corcyra* rearing is attractive to other insects and they compete with *Corcyra* for the food and the yield of adults from individual basins gets reduced. In addition, the mite, *Pyemotus ventricosus* parasitic on the *Corcyra* multiply in large numbers and debilitate the larvae. Hence, there is potential danger of losing the culture.

3.1. *Tribolium castaneum* (Red flour beetle)

Family: Tenebrionidae; Order: Coleoptera

T. castaneum is cosmopolitan in occurrence. The eggs of *T. castaneum* are approximately 0.5 mm long, cylindrical and white. They are covered with a sticky secretion, which causes them to become covered in flour and stick to containers. The larvae are yellowish-white, cylindrical and covered with fine hairs. The head is pale-brown and the last segment of the abdomen has two

upturned dark, pointed structures. The pupa is naked (without a cocoon), yellowish-white, becoming brown later. The dorsum is hairy and the tip of the abdomen has two spine – like processes. The adult is 2.3 – 4.4 mm long, rather flat, oblong and chestnut – brown (reddish-brown). The head and upper part of the thorax are covered with minute punctures and the wing covers are ridged lengthwise. Adult females of *T. castaneum* lay up to 450 eggs in stored products. The incubation period of the eggs is between 5 and 12 days. The grubs and adult stages of *T. castaneum* infest the host. The fully grown grubs are 6 mm long and pupate in 27-29 days. Pupation occurs in the host medium. The pupa of *T. castaneum* is naked (without a cocoon). Adults emerge from the pupa within 7 days. The adults may live for as long as 18 months, depending on weather conditions. Adults fly in large numbers in the late afternoon.

i. Detection of infestation

At low levels of *T. castaneum* infestation, the medium may not show any sign of holes or tunnels. At high levels of infestation, holes and tunnels can be seen. Adults are present on the walls of the basins; larvae inside the grains; and eggs are covered with flour or dust and are found sticking to basins. In the event of outbreak, the medium becomes mouldy and emit a pungent odour. The adults can be seen during the afternoon hours congregating on the walls of *Corcyra* laboratories. More than the presence in the medium, the beetles are major sources of irritation to the workers. They get into the garments and cause inconvenience.

ii. Losses caused

Tribolium grubs effectively compete with the *Corcyra* larvae for feeding and displace them. In this process, there is potential loss in growth of the *Corcyra* larvae. When the infestation reaches alarming proportions there is a corresponding decrease in the moth yield per basin.

iii. Management

Preventative control of *Tribolium* is better. This is achieved by selection uninfested stock bajra grains from the market, good house keeping, sanitation in the rearing environment, avoidance of spills of grains in the rearing and storage areas, heat sterilization of the grains during media preparation etc. In addition, the area where the insects are housed should not have cracks and crevices as they act as good hiding places for the adult insects.

Timely management has to be taken when infestations are noticed. Though fumigation methods are available they can be practiced if only the basin with entire media and insects are to be disposed off. More over it requires care and supervisory control has to be followed. The detection of insects in bulk storage is difficult and conventional methods are insensitive to low population densities. All the techniques developed are so far aimed at management of the adults only. Trapping techniques appear to offer a more effective approach to pest detection in bulk media.

1. Paper boards of size 10x10 cm are placed on the surface of the media a week after infestation of *Corcyra* eggs in the basins. The paper strips are observed daily. The adults clinging to the paper strips are collected manually and killed in water containing 0.5 per cent teepol (surfactant).

2. The adults are attracted to near UV rays. After dusk 4 watts UV lamps are setup near the racks where the basins are kept. A basin containing water and 0.5 per cent teepol is placed below. The *Tribolium* adults are attracted towards the light and trapped in water. The traps are to be operated when all the activities are completed and no worker is allowed to enter the lab as the rays are harmful to workers.

3. Flour traps containing 250 g whole wheat flour and 5 g dry yeast is spread in basins and placed on the floor below the racks @ 1-2/rack. These flour traps are good oviposition attractants for the beetle. Alternate days the flour in the basins is sieved using a fine mesh sieve and the eggs and adults in the flour are collected and destroyed in 0.5% teepol.

4. The adults found on the walls are removed with vacuum cleaner regularly.

3.2. *Bracon hebetor*

Family: Braconidae; Order: Hymenoptera

They occur sporadically in the rearing laboratory. They are larval parasitoids and pupate in groups outside the body of the host insect. The adults can be seen hovering in the lab or seen settled on the top of *khada* cloth fastened onto the basin. These parasitoids complete developmental period in Corcyra larvae in 7-10 days. The oviposition lasts for 13 days. During its life a *Bracon* female can lay 100-150 eggs and a single adult can parasitize upto 32 *Corcyra* larvae.

i. Detection

The adults are small and have long antennae. The larvae that are parasitized become paralyzed and undergo discoloration. When the grubs emerge out for pupation, the host larvae die, turn black and become scaly. From the cocoon formed, next generation of adults emerge and continue the damage caused in previous generation.

ii. Losses caused

The *Bracon hebetor* is a contaminant in *Corcyra* culture. It can cause loss to the extent of 50 per cent in production. The loss will be all the more high if *Tribolium* infestation occurs together with *Bracon*.

iii. Management

1. Total management of the population of *Bracon* is needed to eliminate the infestation. The basins that are used for rearing *Corcyra* should be disposed after 90 days. After this period the food inside the basin is either exhausted or becomes unsuitable for the remaining larva inside. At this stage, migration of the larvae starts. This is made easier when the *khada* cloth used for covering either has holes or is too saggy and touches the surface of the medium. The migrant larva and those found inside or outside the *khada* cloth are the primary sources for parasitization. In a short period the attack becomes alarming. Therefore, enough care has to be

taken to maintain the basins. The migrant larva if any found on the walls have to be removed and killed. The basins should be examined periodically and they should not be kept open except during observation and adult handling. During observation discoloured larvae, white cocoons if found are collected and immersed in 0.5% formaldehyde to kill the parasitoids.

2. The adults of *Bracon* are highly phototropic and attracted to incandescent light. In the *Corcyra* laboratory, during night hours a table lamp with 60W – tungsten bulb is set up over a basin with water. The adults are attracted easily and killed. The bulbs can be operated regularly in the laboratory.

3. As a routine practice, all the materials in the *Corcyra* laboratory are given a spray of 0.1 per cent malathion. Care should be taken to apply the pesticide even on to the furniture and basins. When the problems of *Bracon* turn acute disinfection of all the basins are done one by one in separate room. The rearing area is completely sprayed with malathion and the doors, windows and exits are closed for thee days. The laboratory is cleaned thoroughly before transport of the rearing basins and other materials back to the rearing area.

3.3. *Pyemotes ventricosus*

Family: Pyemotidae; Order: Acarina; class: Arachnida

Their occurrence is occasional contaminating the culture and affects the egg laying and larval development. The mites are microscopic and parasitic on the body of the host insect. Whenever the infestations are noticed, all the rearing materials and rearing area have to be disinfected with sulfur dust. Additionally, sulfur is dusted on the medium surface so that a thin layer of the disinfectant is present. When the infestations assume severe proportions, *khada* cloth pretreated with acaricide, kelthane (0.5%) and shade dried are spread on top of the medium surface. The mites that come into contact with the acaricide are killed rapidly.

4. Disposal of spent medium

The media used for *Corcyra* multiplication have to be disposed after 90 days of use. If discarded in open near the rearing laboratory there are

possibilities of invasion of *Tribolium* and *Bracon* again in the laboratory. Therefore, they have to be disposed in deep pits and covered with sand.

5. Materials management

Bold bajra grains suitable for human consumption, free from insect infestation and disease attack, devoid of foreign impurities and pesticide contamination have to be procured from known source in required quantities. Winnowing before use is advisable. The gunny bags used for procurement, transport and storage in the laboratory have to be devoid of gaping holes and preferably fresh. The materials have to be stored under rat and insect proof conditions. The bajra grains should not be purchased in bulk on yearly basis as materials occupy lot of space and are prone for insect and disease infestation leading to spoilage in course of time. Also, additional labour is required for care of the materials under storage. Similarly, the recurring items like yeast, honey, groundnut etc., have to be procured in splits. The whole process will relieve the producer of financial burden and the risk of spoilage is minimized.

5. Practical Session

i. Practice the method of handling *Tribolium* and *Bracon* problem in the laboratory. Describe the steps you have followed.

ii. Prepare flour bait for *Tribolium* and assess the number of beetles collected per trap for15 weeks. Change the flour bait once every month.

Date observed	Trap 1		Trap 2	
	Adults	Grubs	Adults	Grubs

Total				
Mean				

iii. Setup a light trap for collection of *Bracon hebetor* and assess the number of adults collected per trap for 5 weeks.

iv. Transfer 10 g of wheat flour to 1 oz plastic cups and allow 10 pairs of *Tribolium* adults and allow them undisturbed for one week. Maintains atleast 10 sets for oviposition by *Tribolium*. Prepare cumbu medium freshly as per the procedure. Transfer @ 100 g to six different clear plastic containers (500 ml). Allow 400 eggs per container. One week later collect the *Tribolium* grubs from the wheat flour medium and transfer @ 0, 5, 10, 20, 40, 60 grubs and close firmly the mouth. Observe the number of adults emerging totally in each treatment and observe the condition of the diet at weekly intervals. Draw your inference.

v. Prepare a bait of wheat flour. Weigh 5 g and transfer to 1 oz polypots with perforated lid. Place the trap in *Corcyra* basins @ 1/basin one week after infestation. Watch out for the beetles trapped. Count the numbers trapped on weekly basis for five weeks.

Date observed	Trap No. and beetles collected						
	1	2	3	4	5	6	7

vi. Prepare cumbu medium freshly as per the procedure. Transfer @ 100 g to six different clear plastic containers (500 ml). Allow 5 medium sized *Corcyra* larva per container. Transfer @ 0, 5 and 10 mated females of *Bracon* adults to the container and close firmly the mouth. Maintain two replicates of the treatments. Observe the changes in larval growth and behaviour. Draw your inference.

vii. What are the means by which the quality of grains and eggs can be checked?

Additional Notes

Ex.No. : **Mass production of *Helicoverpa armigera***

Date : **Order: Lepidoptera; Family : Noctuidae**

1. Introduction

The cotton bollworm, *Helicoverpa armigera* is one of the serious pests of many crops. The most important crop hosts in which *H. armigera* is a major pest are cotton, pigeonpea, chickpea, tomato, sorghum and cowpea; other hosts include groundnut, okra, peas, filed beans, soybean, lucerne, *Phaseolus*, tobacco, potato, maize, a number of fruits (prunus, citrus), forest trees and a range of vegetable crops. A wide range of wild plant species are alternate host plants and support larval development: important species in India include *Acanthospermum* spp., *Datura* spp., *Gomphrena celosioides*, *Amaranthus* spp., *Cleome* sp. and *Acalypha* sp. The pest can be effectively controlled by the nuclear polyhedrosis virus, an obligate pathogen that requires the live *H. armigera* for multiplication. The insect is cannibalistic and hence in the laboratory the rearing procedures are elaborate involving high degree of control on quality of the insects and contaminant control.

2. Objective

To study the mass production methods for *H. armigera*

To understand the general requirements in mass production of the insect

3. Morphology and Biology

The eggs of *H. armigera* are creamish yellow to pista green colour, spherical in shape and deeply sculptured. The larvae upon hatching are very small and black coloured. Normally six instars are observed and the larvae pick up different colours during development. The colours range from plain green to dark brown or brownish black. The older larvae have bristles on the body that is sparsely distributed. The head capsule is clear brown, and visible as the larvae advances in age. The cepahalic shield is clearly visible during moulting. The larvae undergo prepupal stage prior to pupation. The pupae are brown coloured and obtect type. The adults are stout. The forewings are

brown coloured in females and straw-pista green coloured in males. The egg period is 2.75-4 days. The larval duration is 16-21 days. The larva at its maximum age weighs 450 – 550 mg. The pupal period lasts for 11-13 days. The preoviposition period is 1-4 days; the oviposition period is 7-11 days and the post oviposition period is 1-2 days under a temperature regime of 25-27^{0}C. The peak oviposition period is between 5-8 days after emergence. The fecundity ranges from 200-250 eggs per pair. The fertility and hatchability ranges from 80-100% depending upon the maintenance.

4. Mass production

4.1. Facility

The healthy insects are held in two rearing rooms *viz.,* one for holding the larval stages and the other for the rest of the stages of the insect. The environment conditions required for rearing the insects is 25 ± 1^{0}C, synchronized with natural photoperiod. The relative humidity in the holding room for pupae, adults and eggs is to be maintained at 75-85 per cent, whereas, it is not regulated in the larval holding room.

4.2. Materials required

Adult feed	Semisynthetic diet
Distilled water	Camel hair brush
Filter paper (hand made)	Oven
Gas stove	Gas lighter
Laminar Flow chamber	Muslin cloth
Mixie	Autoclave
Plastic trays for larval rearing upto second instar	Ethyl alcohol
Polythene bags (autoclavable)	Oviposition chambers
Racks	Saucepan and spoons
Rearing vials	Forceps (blunt end)
Sterile absorbent cotton	Trays for keeping vials
Coarse balance	Electronic balance

4.3. Establishment of colony

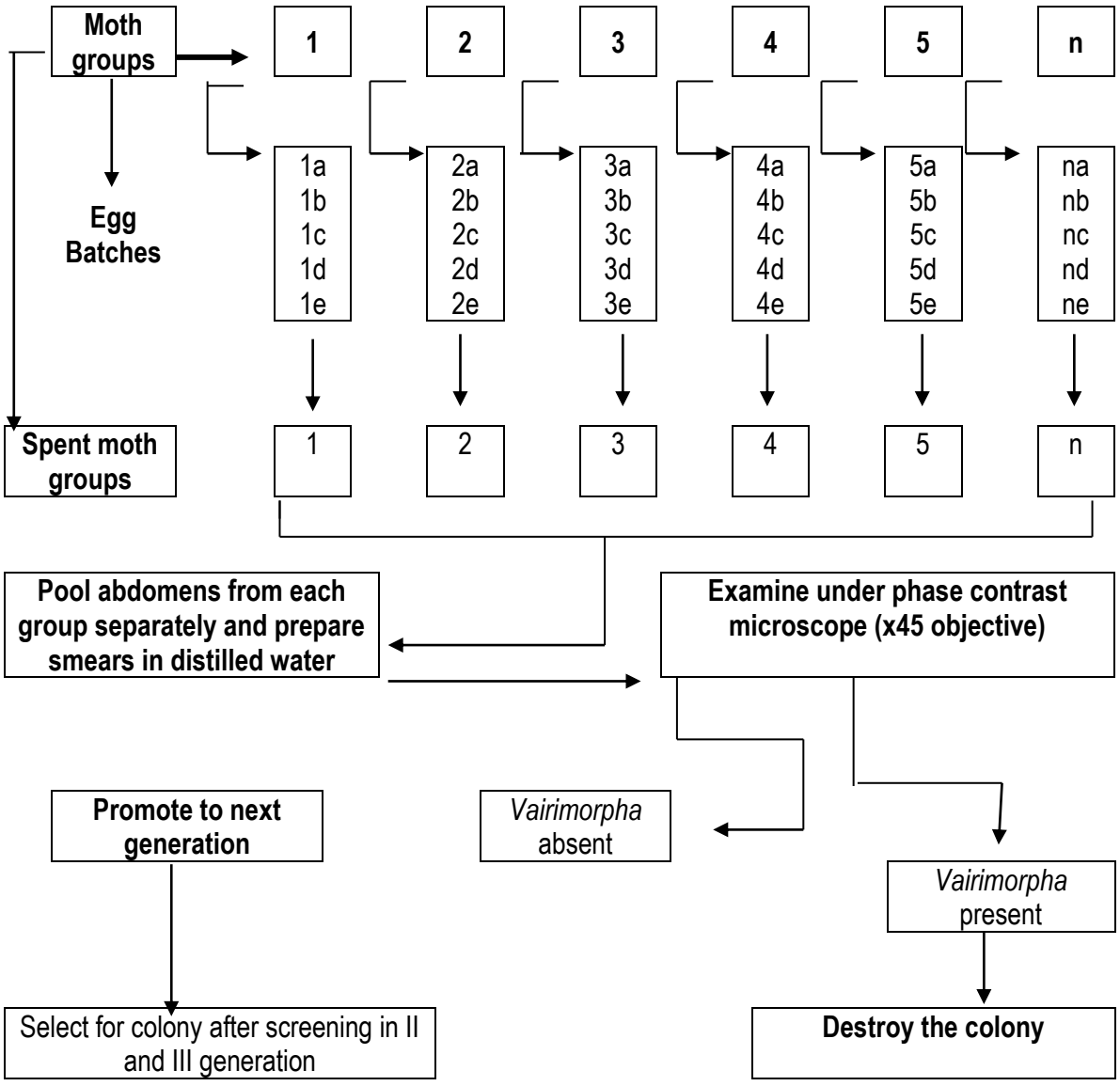
The success of the rearing programme depends upon obtaining a disease-free and a genetically efficient and uniform parental stock. The preferred stage from the field for starting the colony is gravid female, which can be collected at a light trap. This method offers a better chance of avoiding

diseases and parasitoids. However, in practice, it is difficult to collect too many numbers with uniformity. Therefore, *H. armigera* starter colony of can be established by field collection of grown-up larvae from chickpea, pigeonpea, cotton, groundnut, sunflower, bhendi, *etc*., so that a continuous supply of adult moths of both sexes will be available for the production.

The larvae are collected from different host crops during the cropping season in as many numbers as possible. The larvae are observed for any visible signs of weakness that could indicate disease or parasitization and such larvae have to be discarded. If collections are made from farmers' field the possibility of pesticide exposure is high. Many will die during transit. During collection, the larvae are not provided any feed. This ensures that the weakened insects do not survive and the fittest ones remain alive. The larvae have to be transferred individually to empty penicillin (5 ml sterile) vials directly. When collections are made from different crops, the batches from different crops are held separately with proper label on the crop, date of collection and numbers collected and transferred to the quarantine area in the production facility.

The larvae after few hours of starvation are provided semisynthetic diet and kept separately under observation without mixing them. The colonies established from collections made from different crops and areas have to be kept isolated and examined for the incidence of diseases continuously for three generations. The healthy pupae are separated from the dying ones. The batches of pupae serve as the primary source for colony development. If in subsequent generations the populations are free from any disease, the collections can be pooled and a single stable colony can be established. The colonies of the insects have to be examined for the presence of microsporidian pathogens especially, *Vairimorpha* sp. and *Nosema* sp. The adult moths are examined for the presence of the pathogens and viruses (Fig.1). Those colonies that are free from diseases alone are used for the culture.

Fig 1. Selection of microsporidian – free *Helicoverpa armigera* population



4.4. Maintenance of colony

4.4.1. Adult handling

The pupae from primary parental colony are kept in 30 x 30 cm adult emergence cages for eclosion. Upon emergence, ten pairs of healthy adults are transferred to oviposition chamber. The oviposition chamber consists of 30 x 20 cm plastic bucket. The mouth of the unit is covered with muslin cloth, which serves as oviposition substrate. Daily adult feed solution containing 10% sucrose fortified with vitamins (0.03%) is provided and the oviposition substrate is removed and replaced with fresh ones. To improve sanitation, the adults that are weak are removed.

4.4.2. Egg handling

The muslin cloths are collected daily and labeled properly. They are kept in an atmosphere of higher saturation inside a humidifying chamber. It consists of a plastic bucket with a lid. Sodium hypochlorite 0.25% (v/v) is poured inside the bucket upto 2-cm height level. The egg laden muslin cloths are kept inside a clear plastic container (20x10 cm) and allowed to float on the solution. The lid is firmly placed over the bucket. Twenty-four hours after the collection of egg-cloth, and further incubation, the eggs are watched for development of embryo. Eggs showing distinct germ band are suitable for surface sterilization. This is done to eliminate the contaminant microbes. Surface sterilization is achieved by treatment in formaldehyde. The egg-laden clothing is submerged in a 10% formaldehyde solution for 10 minutes. After the expiry of time, the cloths are washed in tap water for 20 min to eliminate the traces of formaldehyde. The cloths are shade dried and kept in sterile plastic cups inside a sterile humidifying chamber for eclosion.

4.4.3. Larval handling

The larvae are supported by a semi-synthetic diet. It is prepared by blending and cooling with the following ingredients:

Batch I	Chickpea seeds (White Kabuli)	:	100.00 g
	Distilled water	:	400.00 ml
Batch II	Agar agar	:	12.80 g
	Distilled water	:	400.00 ml
Batch III	Methyl *p* hydroxy benzoate	:	2.00 g
	Sorbic acid	:	1.00 g
	Wesson's salt	:	7.20 g
Batch IV	Carbendazim	:	0.50 g
	Formaldehyde 40%	:	1.00 ml
	Yeast (Brewer's)	:	30.00g
	Choline chloride 10%	:	7.20 ml
	Vitamin (ABDEC)	:	2.00 ml
	Ascorbic acid	:	3.20 g
	Streptomycin sulphate	:	0.04 g

Chickpea seeds of required quantity are cleaned, washed and soaked overnight in water at the ratio 1:5 (w/v). After decanting of water, the hydrated seeds are boiled in distilled water (Batch I). The contents along with water are transferred to a domestic blender and ground thoroughly. Simultaneously agar agar is melted in distilled water (Batch II). The molten liquid is transferred to the blender, and the contents are mixed for 2 min. The ingredients in Batch III are added one after another and blending is continued for 5 min. by which time, the diet temperature is reduced to less than 70^0C. Finally, the ingredients of Batch IV are added to the blender carefully and the contents are fully whirl mixed. The diet is poured into sterile squeeze bottles and dispensed into the rearing containers and 5 ml glass vials appropriately.

The larvae are held upto third instar in groups in reusable plastic trays (30x15x9.5 cm). A batch of diet (1000 ml) is sufficient for 10 plastic trays for rearing early instar in groups or 225 numbers of 5 ml glass vials for rearing older instars individually.

In the rearing trays newly hatched larvae are transferred and confined properly with the help of orgami tissues reinforcement beneath the lid. The trays are kept inverted to facilitate the positively photropic and negatively geotaxic larvae to feed unhindered on the diet. When the larvae reach second instar premoult stage they are transferred to glass vials (5 ml) with diet and plugged with cotton.

4.4.4. Pupal handling

The prepupae of uniform size are collected from diet bottles and transferred to diet trays filled upto 1/3 rd level with cotton wool for pupation. Pupae are removed from the culture and washed in sodium hypochlorite 0.25% solution and kept in adult emergence cage. The production cycle is repeated.

5. Guidelines for production flow maintenance

The culturing is a labour intensive process and involves handling of live stages. Commonly we encounter development of fungus, bacteria in the diet used for mass and culture. NPV epizootics also occur. These can be overcome by proper sanitation, restriction of personnel inflow into the rearing area. The following important guidelines should be observed during the production process.

- Continuously examine each step in production for ways to reduce the time and labour input.
- Maintain good hygiene and cleanliness and monitor microbial contamination
- Keep biological records of the insects
- Check quality of the insect frequently

6. Preparation of schedules

It is often important to draw schedules for daily routine work. Without unifying a set of steps together in order, the return for current investment made cannot be harnessed. The following sequence of work has to be attended in order to maximise the productivity:

1. Prepare the facility for use
2. Sanitize the rearing areas

3. Prepare adult feed
4. Prepare semi-synthetic diet and cool
5. Release the freshly hatched larvae into the trays
6. Sanitize the implantation area
7. Move the rearing trays to holding rooms
8. Harvest, surface sterilize and incubate eggs
9. Provide adult feed
10. Collect adults, sex, pair and allow for oviposition
11. Harvest pupae, surface sterilize and keep in adult emergence cages
12. Sanitize the adult holding room
13. Sort out trays for host culture and subsequent recycling
14. Prepare materials for wash up operation
15. Surface sterilize the materials appropriately
16. Record production, quality etc.,
17. Close up operations

7. Constraints in production

In the mass production programme, the major constraints are availability of skilled labourers, flow of materials, sanitation, production and product quality control. Even in a pilot production scheme it requires a continuous supply of large number of insects that are uniform in size, physiological state and genetic traits.

Frequent replenishment of the colony from the field-collected larvae puts the insectary manager in a disadvantageous position. The characters of the wild stock are generally unknown. Along the wild colony many microbials both saprophytic and pathogenic forms are introduced inadvertently into the culture area. Contamination control exercise is often elaborate and needs specialists' attention and adds to the cost. Therefore, reliance on the wild colony has to be kept at the minimum possible level.

The work force is the single greatest rearing expense. Mismanagement leads to failure of the programme. If the workers do not carryout the stipulated duty, whatever be the technology developed, it will have little impact on production. Workers must realise that production responsibility

falls not on one person but on all personnel. A realistic workload plant is also important in making budget estimates.

Sanitation is essential for laboratory rearing. Yet it is often neglected. Unsanitary conditions cause disastrous effect on both quantity and quality of insects produced. Workers are the major source of spreading microbes in the rearing facility as well as important source of contamination. *Aspergillus niger*, *Rhizopus* spp., and a host of bacterial pathogens inflict heavy damage to cultures. Their occurrence interferes with the biological fitness of the host insects. The unsanitary conditions result in added cost per insect produced. Although use of sanitation measures may be expensive, the lack of sanitation can be more expensive.

8. Practical session

i. Study the mass production methods followed in different stages of culture of *H. armigera*. Record the observations you have made.

ii. Prepare individually the semisynthetic medium, sterilize and infest the eggs and record the observations date wise on larval growth in the tray and bottles and adult emergence.

Date of diet preparation :

Date of egg collection :

Date of hatching of larva :

Egg period :

No. of larva allowed in the tray :

No. of larva recovered as II instar :

No. of days to enter into pupation :

Pupal period :

First date of emergence of the adult :

When did you observe the peak adult emergence in the basins? Draw a graph plotting the cumulative values of moth emergence in Y ordinate and days after infestation in X ordinate. Draw your conclusions.

iii. Prepare a flow chart for the various activities carried out in the laboratory

iv. What are the sterilization methods followed for maintenance of cleanliness in the laboratory?

v. How the plastic trays and glassware are sterilized in the laboratory?

vi. What are the colour forms of larvae you have observed?

vii. Record the head capsule width, duration and the number of larval instars in *H. armigera*.

viii. What is the weight of larvae in different stages?

Insect No.	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Instar 6
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
Total						
Mean						

Draw a neat graph showing the rate of increase in weight of different larval stages.

ix. What is the weight of pupae? Measure the weight of 50 pupa and classify them into five class interval. What per cent of pupae fall in the normal range?

Class interval	1	2	3	4	5
Weight range					
Pupa No. and Wt					
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
Total					
Mean					

x. What are the diseases of *H. armigera* observed in the laboratory?

xi. What are the organisms associated but did not cause disease in the insects?

xii. Justify if you can keep the field collection made for colony maintenance?

xiii. Prepare semisynthetic diet and record your observations on

a. Time taken to prepare one set of diet

b. Time taken to assemble bottles sufficient enough to dispense one batch of diet

c. How long it takes for the diet to solidify

xiv. How do you sterilize the trays and bottles before diet dispensation?

xv. What are the different purposes of diet ingredients?

xvi. Classify the different items used in rearing into categories recurring and non recurring.

xvii. An entrepreneur plans to produce 2500 *Helicoverpa armigera* larva daily. He gives 10% cumulative allowances for mortality of different stages. He plans to use 10% of the production for colony recycling. How many insects (larvae) he should multiply to attain 100% target production. Work out the quantity of diet material required to produce the desired target. Work out the cost.

Additional Notes

Ex.No. : **Mass production of *Spodoptera litura***

Date : **Order : Lepidoptera ; Family : Noctuidae**

1. Introduction

S. litura a polyphagous pest is also known as tobacco caterpillar. It is a serious pest of tobacco nurseries. *S. litura* is also a sporadic pest of cauliflower, cabbage, castor, cotton, groundnut, potato and lucerne. In years of its outbreak it causes serious losses to several crops. The eggs are laid in masses on the under surface of the leaves and are covered by the hairs from female body. On hatching, larvae initially feed in groups and later-on, spread out and make holes and eat away the entire leaf. If suitable measures are not adopted, the pest is capable of defoliating entire crop.

2. Objective

To study the mass production methods for *Spodoptera litura*.

3. Morphology and Biology

Female moths on an average mate 3-4 times and males mate 10 times in a life span. The fecundity of the female moth varies from 1000 to 1600. eggs laid in clusters covered by scales and oviposition period spread over 6 to 8 days. Eggs hatch in about 3-4 days at 26^0C and feed gregariously. As they grow older, the larvae tend to disperse. The colour changes from light green to dark grey or black. Early instar larvae are tiny and blackish green with a distinct black band on the first abdominal segment. Mature caterpillars are 40-50 mm long, stout, smooth but having scattered short setae and have dull blackish and greenish body with yellow dorsal and lateral strips.The lateral yellow strips are bordered dorsally with a series of semi lunar black marks. The grownup caterpillars possess a ‘V’ shaped white mark on the front portion of the black head. The egg, larval and pupal period is 3, 14-16 and 9 to 11 days respectively. After completing the pupal period, during early hours of the night the adult moth emerge. The pupa is dark brownish black in colour. The males have short and pointed abdomen, measuring about 6mm, while

female measures 8mm with additional tuft of hair measuring 3mm in length. The fore wing of both male and female have similar pattern of coloration *i.e.* back patches with yellow marking from the apical margin up to anal angle.

4. Mass production

4.1. Facility and material requirement

The facility and materials required for rearing the insect are as in *H. armigera*.

4.2. Colony Establishment

The culture of *S. litura* is started by collecting eggs and larvae from the fields of castor, cauliflower, lucerne, tobacco etc. The different stages collected are first kept in quarantine for three successive generations and then moved to the actual production unit so that the parasitoids emerging in the first generation and the diseases occurring through the three generations are eliminated. Once the pure culture is established by following the procedures as in *H. armigera*, the mass production is commenced from the third laboratory generation.

4.3. Colony maintenance

Ten pairs of newly emerged moths of *S. litura* are placed in oviposition chamber (10 lit.). The inner wall of the container is lined with paper to obtain eggs. The bottom of the container is lined with sponge covered over by blotting paper. The moths are provided with 10% sucrose in 5 ml vials as in *H. armigera*. The eggs which are generally laid in batches on the paper provided are cut out with the help of scissors. Freshly laid egg masses are sterilized by dipping in 10% formalin for 10 minutes, washed in running water for 20 minutes, dried on blotting paper and kept for hatching in sterilized 250 ml containers.

The eggs can also be surface sterilized in 0.05 per cent solution of sodium hypochlorite (NaOCl) by placing the freshly laid egg masses for 5 minutes. These eggs are washed several times in the running tap water to remove the traces of NaOCl. The traces of NaOCl could be neutralized by

dipping the eggs in 10% sodium thiosulphate solution and again the eggs are washed thoroughly under running tap water. The surface sterilized eggs are kept in plastic tubes (7.5 x 25 cm) on moist tissue paper for continuing the stock culture.

After 2-3 days the newly hatched larvae are transferred to semisynthetic chickpea based diet used for *H. armigera*. It is prepared by blending and cooling with the following ingredients.

Chickpea seeds	:	100 g	Wesson salt mix	:	7.2 g
Agar agar	:	12.8 g	Streptomycin sulphate	:	40 mg
Yeast	:	30 g	Vitamin supplement	:	2 ml
Methyl-para-hydroxy benzoate	:	2 g	Formaldehyde 40%	:	1 ml
Sorbic acid	:	1 g	Carbendazin	:	500 mg
Ascorbic acid	:	3.2 g	Water	:	720 ml
Casein	:	16 g			

The larvae are held upto third instar in groups in reusable plastic trays (30 x 15 x 9.5 cm). A batch of diet (1000 ml) is sufficient for 10 plastic trays for rearing early instar in groups or 180-190 numbers of 10 ml glass vials for rearing older instars individually. In the rearing trays, newly hatched larvae are transferred and confined properly with the help of orgami tissues reinforcement beneath the lid. The trays are kept inverted to facilitate the positively photropic and negatively geotaxic larvae to feed unhindered on the diet. The eggs hatch within 24-48 h and neonate larvae crawl and spread on the diet. When the larvae reach third instar premoult stage they are transferred to glass vials (5 ml) and plugged with cotton. The larvae are fully fed pupate in 14-17 days at the bottom of the vials. The pupae collected again are surface sterilized with NaOCl as described for eggs. The extra moisture is removed by a blotting sheet on which the pupae are gently rolled. The pupae are sexed and kept on a lid over a wet sponge in adult – emergence cage (22 x 15 cm). After 10 days freshly emerged males and females are collected daily from their respective emergence cages, and used for obtaining the eggs for the subsequent generation.

4.4. Precautions

In mass production on semi-synthetic diet if utmost hygienic conditions, optimum temperature and humidity is maintained, the chances of getting any disease are meagre. But if NPV appears, segregate the lot and destroy. The sluggish larvae showing pinkish colour on the ventral parts of the body should be removed immediately as and when they are noticed. The texture and quality of the semisynthetic diet should be good.

5. Practical session

i. Study the mass production methods followed in different stages of culture of *S. litura*. Record the observations you have made.

ii. Prepare a flow chart for the various activities carried out in the laboratory.

iii. Handle the different stages of insects and describe how you have performed each activity with dates as noted below.

Date	Activity performed

iv. How did you collect the egg cloths

v. How did you prepare the adult feed

vi. Which stage of the eggs were sterilized

vii. How did you sterilize the eggs

viii. What precautions were observed during sterilization?

ix. What stage of the larvae was transferred to the rearing tray?

x. What stages of the larvae were transferred to the vials. How did you identify the instar?

xi. What are the precautions observed during transfer?

xii. What is the time taken to transfer 100 larvae to vials?

Additional Notes

Ex.No.	**:**	**Mass production of *Maconellicoccus hirsutus***
Date	**:**	**(Order : Hemiptera ; Family : Pseudococcidae)**

1. Introduction

The pink mealy bug (hibiscus mealy bug = grapevine mealy bug) is a serious pest of grapevine and citrus in India. These bugs are hard to kill in the plantations and gardens. Heavily infested clusters of the fruits give weak appearance, shrivel and droop down. Because of this the marketability is lost. The Australian Ladybird beetle, *Cryptolaemus montrouzieri* is an effective predator of the mealy bug. These mealy bugs are used as natural hosts for the mass production of the predatory beetle.

2. Objective

To study the mass culture procedures followed in *M. hirsutus* production.

3. Bioecology

The grapevine mealy bugs occur throughout the year on grapevine. The bugs are found the leaves, shoots, nodes, bunch and loose bark in the vine. The adult females are pinkish and covered with a mealy coat. The fecundity per adult is 350-500 eggs. The orange coloured eggs hatch in 5-10 days and the crawlers migrate and settle on places that support their food requirement. The crawlers become sedentary as they advance in age. The multiplication rate of the bugs is higher in summer months and life cycle gets prolonged during cooler months.

4. Production procedure

4.1. Colony establishment

The colonies of the mealy bugs are established from field collection initially. Guava plantations, vineyards, croton plants, citrus and pomegranate gardens are good reservoirs of the mealy bug populations. From them a

primary colony under quarantine is established separately in the lab utilizing the quarantine facility. During this period the colony is purified to obtain mealy bug population free of attacks by parasitoids and scavenging ants.

4.2. Culture maintenance

The mealy bugs are cultured on pumpkin (red) in the laboratory. It is very difficult to maintain the colony on the natural host plants. The selection of pumpkin is critical for successful development of mealy bugs. Fleshy pumpkins with intact peduncle and deep ridges and furrows of weight 2.5 kg devoid of wounds and mouldy patches are used for multiplication of the bugs. The pumpkins are soaked in carbendazim 0.5% for 1 min. and shade dried. The cut ends and wounds are plugged with molten wax. Along the furrows burlap is provided to facilitate settling of the crawlers. The pumpkins are placed in large sized cages over stainless steel stands. The cages are set up in ant proof conditions as the mealy bugs secrete honey dews which attract ants invariably.

Ovisacs of healthy adults are collected and placed on fresh pumpkin in the laboratory individually. From them, the eggs are allowed to hatch and multiply. The crawlers move along the burlap and settle. In a month time, the mealy bugs begin to smother the entire surface of the pumpkin. From this stock, subsequent colonies are established. When the colony is in active growth period with breeding females, the ovisacs are collected with the help of camel hair brush and transferred to fresh pumpkins prepared as above. During the mass production care is taken to avoid fungal invasion. The cages, steel ware and burlap used are sterilized using common bleach. Used pumpkin fruits with symptoms of mould invasion are disposed of immediately.

5. Practical Session

1. Prepare pumpkin fruits for mass production of the mealy bugs. Describe the procedure you have followed. Record the items required for the preparation of the pumpkins with respective quantity.

2. Release ovisacs at the rates 5, 10, 15, 20, 25, 30 and 35 on individual pumpkin of uniform size prepared as per the procedure. Observe twice a week the growth of mealy bugs on the pumpkin. Record the extent of coverage of the pumpkins on percent area basis.

Week	Per cent area covered	Remarks

3. When did you discard the pumpkin after the mealy bugs have completely smothered.

4. Work out the rate of success in terms of the number of Ovisacs released and colonization by the mealy bugs.

Additional Notes

Ex.No. : **Mass production of *Trichogramma* sp.**

Date : **Order : Hymenoptera ; Family : Trichogrammatidae**

1. Introduction

The genus *Trichogramma* is cosmopolitan in distribution and present in all terrestrial habitats and is one of 80 genera in the family *Trichogrammatidae*. *Trichogramma* are primary parasitoids eggs of lepidoptera, but parasitism also occurs in eggs of other orders such as coleoptera, diptera, hemiptera, hymenoptera and neuroptera. It is important for plant protection because of its wide spread natural occurrence and its success as biological control agent by mass releasing. Since this parasitoid kills the pest in the egg stage itself before the pest could cause any damage to the crop and also that it is quite amenable to mass production in the laboratories, it has the distinction of being the highest produced and most utilized biological control agent in the world *Trichogrammatidae* includes the smallest of insects, ranging in size from 0.2 to 1.5 mm. *Trichogramma* are difficult to identify because they are so small and have generally uniform morphological characters. Also, certain physical characteristics such as body color and the number and length of body hairs can vary with body size, season, rearing temperature and the host on which the adult was reared. A major advance in the systematics of *Trichogramma* was the discovery that characteristics of male genitalia can be used to identify species. This is the primary means of identification today, but body color, wing venation and features of the antennae serve as supporting characteristics. Females can not be identified with the same level of confidence, so collections submitted for identification must include males in addition to physical characteristics, studies of reproductive compatibility and mode of reproduction also have been especially valuable in identifying species.

2. Objective

To study the mass production methods for *Trichogramma* sp.

3. Biology of *Trichogramma*

The development of all *Trichogramma* spp. is very similar. Being an egg parasite, the female drills a hole through the chorion and deposits its eggs within the egg of the host. The internal pressure of the egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk, which increases their longevity and under laboratory conditions a female parasitizes from one to ten eggs per day or from ten to 190 during her life. Large females parasitize more eggs than smaller females. The number of eggs laid per host egg may vary from 1 to 20 or more depending upon the size of the host egg. However in sugarcane, in which moth borer eggs are small, generally 1 or 2 parasites develop per egg. A female parasitoid can distinguish already parasitised eggs, thereby avoiding superparasitism or multiple-parasitism under natural conditions. Fecundity varies from 20 to 200 eggs per female according to the species, the host, and the longevity of the adult. Eggs in the early stages of development are more suitable for parasite development. Older eggs, especially those in which the head capsule of the larva is visible, are not usually parasitized and if they are, parasite survival is much lower. Venom injected by the female at the time of oviposition is believed to cause this predigestion of the egg's contents. During the 3^{rd} instar (3 to 4 days after the host egg was parasitized) dark melanin granules are deposited on the inner surface of the egg chorion, causing the host egg to turn black. This is an invaluable diagnostic character for distinguishing them from unparasitised eggs. Larvae then transform to the inactive pupal stage. The adult wasps emerge from the pupae and escape the host egg by chewing a circular hole in the egg shell. The black layer inside the chorion and the exit hole are evidence of parasitism by *Trichogramma*. The egg, larval and pupal stages of *Trichogramma* at 28 ± 2^0C are completed in about 1 day, 3 to 4 days, and 4 to 5 days respectively. Thus, the life cycle is completed in 8 to 10 days, but it may be prolonged at lower temperatures or hampered at very high temperatures. The adults are short lived (6-8 days). Mating and oviposition take place immediately after emergence. The sex ratio is generally 1:1.

4. Mass production

Different species and strains of *Trichogramma* typically prefer different host eggs and crop habitats and have different searching abilities and tolerance to weather conditions. Efficacy is improved by selecting the most effective and adapted species or strain for the specific crop / pest situation.

***Trichogramma* species used in India**

Species	Target pests
T. chilonis	Sugarcane internode borer *Chilo sacchariphagus indicus* American bollworm *Helicoverpa armigera* Pinkbollworm *Pectinophora gossypiella* Spotted bollworm *Earias* spp. Citrus caterpillar *Papilio demoleus* Pomegranate fruit borer *Virachola isocrates* Rice leaffolder *Cnaphalocrocis medinalis*
T. japonicum	Sugarcane top borer *Scirpophaga nivella* Rice yellow stemborer *S. incertulas*
T. brasiliensis	Tomato fruitborer *H. armigera* Cotton bollworms
T. achaeae	Cotton bollworms

In the laboratory the parasitoids are multiplied on *Corcyra* eggs. The eggs laid by the *Corcyra* moths are collected and sieved to remove the moth scales etc. The pure eggs thus obtained are exposed to ultra-violet light in UV chamber to kill the host embryo but at the same time permit parasitization. The quantity of the sterilized eggs is assessed in a measuring cylinder volumetrically. The eggs in volume of six cc are then sprinkled uniformly over a 144 gsm (chart paper) card of 30 x 18 cm size (Fig.1). The card is divided

into two halves of 30 x 9 cm (LxB). Lengthwise it is subdivided into 15 grids (G1 to G15) of size 2cm. The dimension of each grid is 7x2 cm. Each grid can accommodate 0.2 cc of the eggs. Label information on the manufacturer, species of the parasitoid, date of parasitization and expected date of emergence are given in the left over spaces of size 30 x 1 cm on the top and bottom of each half of the card.

Fig.1. Tricho Card

1 cm	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU
G1 *7x2 cm*	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
1 cm	*Trichogramma chilonis*	Date of parasitisation :								Date of emergence expected :				
1 cm	*Trichogramma chilonis*	Date of parasitisation :								Date of emergence expected :				
G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30
1 cm	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU	TNAU

A coat of 10% gum arabic is applied on the grids (G1-30) and the eggs are sprinkled uniformly in a single layer with the aid of a tea strainer. The excess eggs pasted are removed by gently passing a shoe brush over the card after sufficient air drying under fan. The egg cards are placed into polythene bags of suitable size and the nucleus card of *Trichogramma* are introduced in it. The easiest way to accomplish this is to place a piece of '*Tricho* egg card' containing parasitized eggs (i.e. pharate adults) that are ready to yield the adults and to hold them in subdued light for 2 to 3 days. The emerging parasites readily parasitize the fresh eggs. The parasitoid - host ratio is adjusted accordingly to 1:6 get effective parasitism. The parasitized eggs in the *Tricho Card* turn back in 3 or 4 days and the adult parasitoids emerge in 8 to 10 days from the date of parasitization. The parasitized eggs in which the parasitoids in the larval or pupal stage (i.e. before or after turning black) can be stored in the refrigerator (at 5^0C) for about 3 weeks without any loss in emergence.

5. Precautions

Poor quality of mass reared *Trichogramma* can result in control failures. The artificial conditions of mass rearing can select for genetic changes that reduce the effectiveness of the *Trichogramma* in the field. Such rearing conditions include rearing multiple generations on unnatural host eggs, the absence of plants, crowding and interference, rapid generation time, and failure to rejuvenate genetic stock. Except for obvious problems such as lack of adult emergence or wing deformities, growers and pest consultants cannot detect poor quality *Trichogramma* prior to release. Commercial suppliers are responsible for maintaining desirable characteristics necessary for good performance in the field. Production colonies should be periodically replaced with individuals from a stock culture maintained on the natural or target host. Suppliers also should assess the per cent host egg parasitization, adult emergence, and the sex ratio of emerged adults to be sure they are within acceptable standards. Standards for established cultures on *Corcyra* are 95±5 per cent egg parasitization, 90±5 per cent adult emergence, and a sex ratio of 1 to 1.5 females per male.

6. Delivery

Tricho cards are delivered for use in the field. The cards in volumes of 6 cc as per TNAU method of production are assembled in aerated polythene bags and packed in paper cartons for transport. The cards have to be transported by the most rapid method of transport to reach the destination. During transport and holding the cartons should not be exposed to extreme conditions like toxic fumes, open sunlight, high temperature areas as the consignment could be damaged leading to mortality of the *Trichogramma* stages.

7. Field release

The parasitoids are released in the pharate stage or when few adults begin to emerge from the host egg during the evening hours. The cards are cut into bits neatly along the grids with least damage to the eggs and stapled beneath the foliage in the upper canopy level. To maximize the field

parasitization it is recommended to release the parasitoids is as many locations as possible. Recently scientists are beginning to advocate the release of cards @ 1/5m row length.

8. Recommendation

The dose of the parasitoid and its species vary with the pests to be controlled. The following are the TNAU recommendations.

Crop and Pest	*Trichogramma* species recommended	Dose/ha
Rice		
Scirpophaga incertulas	*T. japonicum*	
Cnaphalocrocis medinalis	*T. chilonis*	
Cotton		
Helicoverpa armigera	*T. chilonis*	
Pectinophora gossyipiella	*T. chilonis*	
Sunflower		
Helicoverpa armigera	*T. chilonis*	
Groundnut		
Helicoverpa armigera	*T. chilonis*	
Tomato		
Helicoverpa armigera	*T. chilonis*	
Brinjal		
Leucinodes orbonalis	*T. chilonis*	

6. Practical Session

i. Prepare a tricho card of 6cc volume. Describe the method you have followed.

ii. What are the difficulties you have faced in the preparation of the card and suggest measures to overcome them.

iii. Explore the possibilities of alternative methods of Tricho card preparation for easy delivery in the field.

iv. How will you distinguish the unparasitized and parasitized egg cards?

v. Observe critically under stereo zoom microscope three day old parasitized egg card and nucleus card. Record the observations with neat diagrams.

vi. What are the possible reasons for low levels of parasitism in laboratory culture. How will you overcome them?

vii. Calculate the quantity of nucleus card of *Trichogramma* required for 365 days of work if it is intended to produce 2500 *Corcyra eggs* daily and allocate the eggs for the production of colony, *Trichogramma* and other natural enemies @ 10: 75:15.

viii. If the entrepreneur decided to produce the target given in the above problem within 240 days what will be the revised schedule of production and allocation on daily and annual basis?

Additional notes

Ex.No.	**:**	**Mass production of *Chelonus blackburnii***
Date	**:**	**Order : Hymenoptera ; Family : Braconidae**

1. Introduction

C. blackburnii is introduced from Hawaii. A parthenogentic egg-larval parasitoid, *C. blackburnii* has a fairly wide host range but in India the common meal moth *Corcyra cephalonica* and potato tuber moth *Phthorimaea operculella* have often been used for multiplication of this parasitoid. It could also be multiplied successfully on *Spodoptera exigua*. *C. blackburnii* has been used for the biological suppression of *P. operculella, Earias vitella, Pectinophora gossypiella* and *Helicoverpa armigera* on cotton and other host plants in many states. It is becoming an important component of IPM systems on potato, cotton, etc.,

2.Objective

To study the mass production methods for *Chelonus blackburnii*.

3.Production procedure

A set of 100, 0-24 hr old eggs of *Corcyra* (not exposed to UV) are pasted to 5 x 5 cm card. This card containing eggs is exposed to 30 *C. blackburnii* adults in a 1.5 l container. The plastic container has windows with plastic mesh for aeration. Two cotton swabs, one soaked in 10% honey solution and the other in drinking water are also placed inside from the side opening which is closed tightly with a cloth covered cotton plug. The egg card after exposing to *C. blackburnii* for 24 hrs is removed and placed on 500 g sterilized cumbu medium. In 30 days time, adults start emerging from the cocoons formed in the cumbu medium after completing development on *Corcyra* larvae. The adults live for 25 days and their fecundity is about 400 eggs.

The parasitoid could also be reared on potato tuber moth (PTM). A set of 1500 egg of laid on a cloth are stapled to a card. This card containing 0-24

hr old eggs is exposed to 30 *C. blackburnii* adults. The plastic container (14 cm x 11 cm) is converted into *C. blackburnii* rearing unit by cutting windows and fixing plastic mesh aeration. Two cotton swabs, one soaked in 50% honey solution and the other in drinking water are also placed inside from the side opening which is closed tightly with a cloth covered cotton plug. The PTM egg card after exposing to *C. blackburnii* for 24 hrs is removed and placed on punctured potatoes. This provides more entry points for PTM larvae and kept in a similar plastic container as described for exposure to *C. blackburnii*. The bottom of this container is lined with sterilized sand. In 25-27 days time, adults start emerging from the cocoons formed in sand at the bottom of the cage or sometimes inside potatoes after completing development on potatoes. The adults live for 23-31 days and their fecundity is about 288-390 Parasitoid host ratio of 1:50 should be maintained and the fresh lot of eggs provided every day.

4.Practical Session

i. Practice the mass production method for the parasitoid individually

ii. Prepare *Corcyra* egg cards with 100 eggs individually, allow 4-5 *Chelonus* adults in a plastic container. After parasitiztion, provide cumbu medium and watch continuously the development of *Chelonus*. Record your observations on the number of adults emerged and period of emergence. Workout the ratio of eggs and emergence.

iii. Draw neat sketch of the parasitoid.

Additional notes

Ex.No. : **Mass production of *Bracon hebetor***

Date : **Hymenoptera : Braconidae**

1. Introduction

B. hebetor is a common gregarious ecto – larval parasitoid. There appears to be two strains of the parasitoid, one attacking field pests and the other stored product pests. Among the hosts recorded are *Corcyra cephalanica, Dichocracis punctiferalis, Eublemma olivacea, Hellula undalis, Ostriniaicius kashmirica, Helicoverpa armigera* and *Opisina arenosella* of which the *O. arenosella* is the important host. Parasitism is observed throughout the year, it ranged from 26.2 to 26.7% during the peak period of *O. arenosella* infestation.

2.Objective

To study the method of mass production methods for *Bracon hebetor*.

3.Production procedure

B. hebetor is reared on *C. cephalonica* at wide ranging temperature and fluctuating relative humidity. Pairs sorted out from freshly emerged brood are caged in specimen tubes (8 x 2.5 cm). Under laboratory conditions it is easy to rear. Fifth instar caterpillars of *C. cephalonica* of similar size are provided for oviposition and the parasitised larva removed daily from the tubes and maintained separately. The parasitoids are fed with 50% honey provided as tiny droplets on wax-coated paper strips.

B. hebetor completes its total developmental period in 7-12 days. The egg, larval and pupal period are completed in 1-2, 2-4 and 3-7 days respectively. The females start laying eggs 2-5 days after emergence on partially paralised host caterpillars. The paralysed larvae (not receiving the eggs) die within 3-5 days. Female parasitoids feed on hosts haemolymph.

The eggs are laid singly or in groups of two to eight. A female is capable of laying 229 eggs (142-345) on 14-32 hosts caterpillars during its life span of 20-63 days, the daily average being 2-27, and laid maximum number of eggs during the first ten days of oviposition period. A single host larva supports the development of 11 eggs and 9 larvae of the parasitoid. The preoviposition period is 3 days, oviposition period 22-55 days and post oviposition period 1-8 days. The fertile female lives for 20-63 days. *B. hebetor* complete its developmental period on larvae of *Corcyra cephalonica* in 8.6 (7-10) days. Oviposition period lasts for 13 (5-22) days. The mean fecundity is 72 (13 – 148). Females live for 24-53 days. Egg viability is 92% and adult emergence 81%. Male to female ratio is 5:1.

4.Precautions

To avoid wastage, the surplus parasitoids could be stored in the cocoon stage for one month in the refrigerator. The coccons are kept in a clear plastic jar (19x9 cm) for emergence and provided with raisins as food. Segregate the mite (*Pediculoides ventricosus*) infested cultures of the host insect immediately.

5.Practical Session

i. Study the mass production methods of *Bracon hebetor* under laboratory conditions.

ii. Allow *Corcyra* larvae and *Bracon* adults in differing ratios in a test tube. Provide

adult feed. Record the observations on the change in the colour of parasitized larvae, per cent parasitization, number of parasitoids emerging out and days to emerge.

Ex. No.:

Date :

Mass production of *Bracon brevicornis*

Order: Hymenoptera ; Braconidae

1. Introduction

B. brrvicornis is an important gregarious larval ecto-parasitoid of many lepidopterans. It is widely distributed and recorded from *Antigastra catalaunalis, Adisura atkinsoni, Earias* sp., *Helicoverpa armigera, Noorda moringae*, *Opisina arenosella, Pectinophora gossypiella*, *Chilo partellus* etc., Its natural parasitism is not very high. However, it paralyses many lepidopteran larvae and some of them never revive. This contributes to indirect control.

2.Objective

To study the mass production methods for *Bracon brevicornis*.

3.Prodcuction procedure

B. brevicornis is amenable for mass rearing in the laboratory on the alternate host, *Corcyra cephalonica*. For small scale culture, glass chimney and the 'Sandwich' technique are adequate. About 20 mated females are confined in a glass chimney, covering both sides of the chimney with muslin sheet held in place with rubber bands. A cotton swab soaked in 50% honey water solution is stuck to the side of the chimney to serve as food. With many hymenoptera, adult nutrition is of great importance as it influences sex-ratio. High protein diet at times improves the sex ratio so that more female progeny are produced. 'Proteinex' can be used to produce the desired results. Replacing honey with laevulose or fructose also is beneficial in some cases. Exposure to sunlight frequently stimulates mating, oogenesis and fertilization of eggs.

About 10 full grown larvae of *Corcyra* are placed between two sheets of facial tissue paper and placed over the muslin sheet covering the wider

mouth of the chimney. The tissue is again covered with a sheet of muslin and fastened with a pair of rubber bands. The chimney is then placed with the host larvae facing a window or light source. Females of *B. brevicornis* are attracted to the host larvae, probe through the muslin and paralyse the larvae on each of which they lay about 25 eggs per day. At the end of 24 hours, the tissue sheets bearing parasitized larvae are removed and held in flat plastic containers until the parasitoid grubs hatch, complete development and spin cocoons. The egg, larval pupal and adult stages are completed in 28-36 hours, 4-7, 3-6 and 15-40 days respectively. The female parasitoid is capable of depositing 150-200 eggs in its life time. Emerging adults are again collected for mating and egg laying. Adults survive upto 15-40 days but egg laying usually tapers off after the first ten days. Two day old adults of *B. brevicornis* could be stored for 30 days at 5^0C and 50-60% RH.

4. Practical Session

i. Prepare a set of 1.5 l plastic container assembly instead of the glass chimney for the mass production and study the usefulness of the same.

ii. Work out the productivity of the parasitoid if it is estimated to produce 1000 adults per day.

Additional notes

Ex. No.:

Date :

Mass production of *Goniozus nephantidis*

Order: Hymenoptera, Braconidae

1. Introduction

Goniozus nephantidis is the most widely used parasitoid of *Opisina arenosella*. It is a sturdy gregarious larval or prepupal ectoparasitoid. The female practices maternal care of eggs and larvae. The host larvae are parasitised and the parasitoid even feeds on host body fluid. The parasitoid is also capable of suppressing the population by merely stinging and paralyzing 1^{st} – 2^{nd} instar larvae. *G. nephantidis* is the most common and effective parasitoid of late instars caterpillars of *O. arenosella* in several parts of the country. The parasitoid is being mass multiplied and released in Karnataka, Kerala and several other states.

2.Objective

To study the method of mass production of *Goniozus nephantidis*.

3. Production procedure

The parasitoid is multiplied on *Corcyra cephalonica* larvae in diffused light. A pair of parasitoid is introduced in tube (7.5 x 2.5 cm). The adults are provided honey in the form of small droplets on wax coated paper. After a pre-oviposition period of six days one healthy last instar larva is provided in a vial. The larvae parasitized and containing eggs of *G. nephantidis* are removed regularly from the vials till the death of the female. Such larvae are kept in accordion type strips of paper in plastic boxes which are covered by muslin cloth.

Considering the fecundity as 20-50, the female is capable of parasitising 6-7 larvae in three oviposition spells each separated by 4-5 days. The life cycle of the parasitoid is completed in 10-14 days (incubation 24-36

hrs, larval feeding 36-48 hrs, prepupal stage 48-60 hrs and cocoon period 48 to 56 hrs + resting adult inside the cocoon 108-128 hrs).

4. Practical session

1. Study the mass production method for *Goniozus nephantidis*.

Additional notes

Ex. No.:

Date :

Mass production of *Brachymeria* spp.
(Hymenoptera, Chalcididae)

1. Introduction

Brachymeria are important parasitoids of coconut black headed caterpillar, *Opisina arenosella.* Out of the six important species of *Brachymeria, B. nosatoi*, *B.nephantidis B. lasus, B. excarinata, B. hime attevae* and *B. euploeae* recorded on *O.arenosella* recorded on *O. arensella, B. nosatoi* is the dominant species followed by *B. nephantidis*, *B. nephantidis* is widely distributed, but it is more effective in southern districts of Kerala where the parasitism reaches 25 per cent. *B. excarinata* is a dominant species in Mahua; Gujarat. Significant parasitism by *B. hime attevae* has been observed in Salem, Tamil Nadu. *B. nosatoi* is a solitary endoparasitoid, posses the essential attributes and great potential in suppression of *O. arenosella*. It adheres to rigid selection of masses and elaborate courtship, provides higher percentage of parasitism, breeds well in summer months and prolonged drought conditions and disperse uniformly in pest-infested coconut gardens. The pupae parasitised by *Brachymeria* show one or more black dots, which are the characteristic oviposition punctures made by the females.

2. Objective

To study the method of mass production of *Brahymeria*.

3. Production procedure

About 50 adults of *B. nosatoi* comprising both sexes are released in a clean, dry cylindrical jar of 17.5x6.75 cm A. 12 cm long and 6.25 cm wide cardboard piece is inserted to facilitate the parasitoids to move and rest. The mouth of the jar is secured with a piece of muslin cloth tightened with rubber bands. The jar is kept horizontally. The parasitoids are transferred to fresh

clean jar every 4 to 5 days. For adult parasitoids, undiluted honey is provided daily in minute droplets on wax coated paper.

The jar containing parasitoid is kept in diffused sunlight for 10-15 minutes daily for about 3-4 days after which only the host pupae are to be offered for parasitisation. Exposure to sunlight stimulates mating.

Pupae of *O. arenosella* reared in the laboratory are carefully removed with cocoons and silken galleries intact or leaf-bits containing pupae within cocoons and silken galleries and placed on a piece of card board, 12 x 6 cm in such a way that they are accessible to the parasitoid from all the three sides. The card board piece containing several pupae is inserted into the horizontally placed glass jar containing the mated parasitoids for parasitisation.

The parasitoids partially disorganize the pupal tissues standing on the galleries with their ovipositors by repeated thrusts and oviposit in the pupae. The pupae without cocoons and silken galleries are placed on the card board and covered with silken galleries as the parasitoid will not parasitise naked pupae. Depending on the activity of female parasitoids, the host pupae can be exposed for a period of 4-6 hours for parasitisation. To avoid superparasitism the parasitised host pupae are to be removed immediately after oviposition by the parasitoids (holes chewed in the cocoons indicate oviposition). The card board piece containing parasitised pupae is transferred to a similar glass jar or the parasitised pupae alone to a conical flask and kept for emergence of the parasitoid. Normally the emergence of parasitoid commences 12 days after oviposition and continues upto 20 days in the laboratory at temperature and RH ranging between 22-30^{o}C and 45-80% respectively. The parasitoids are aspirated into field release container.

4. Practical session

1. Study the mass production method for *Brachymeria nastoi*.

Additional notes

Ex. No.:

Date :

Mass production of *Chrysoperla carnea* (Neuroptera, Chrysopidae)

1. Introduction

In India, 65 species of chrysopids belonging to 21 genera have been recorded from various crop ecosystems. Some species are distributed widely and are important natural enemies for aphids and other soft bodied insects. Amongst them, *Chrysoperla carnea* is the most common. It has been used in cotton ecosystem for protection from aphids and other soft bodied insects. *C. carnea* is now used extensively all over the country.

2. Objective

To study the method of mass production of *Chrysoperla carnea*.

3. Morphology and Biology

The eggs are stalked and green in colour. The length of the eggs in various species range between 0.7 to 2.3 mm and that of the stalk between 2 to 26 mm. The eggs are laid singly or in clusters. Eggs turn pale whitish and then black before hatching. Egg period lasts 3-4 days. The larva is white in colour on hatching. The larva has 3 instars which are completed in 8-10 days. The larva spins a cocoon from which the adult emerges in 5-7 days. Adults on emergence mate repeatedly. Generally, pre-oviposition period lasts for 3-7 days. Adult females start laying eggs from 5^{th} day onwards and peak egg-laying period is between 9-23 days after emergence. The male longevity is 30-35 days and female can even live upto 60 days. Fecundity is 600-800 eggs/female. The sex ratio Male : Female is 1 : 0.85. The adult males and females live 41 and 53 days, respectively.

4. Production procedure

In mass production, the adults are fed on various types of diets. The larvae are either reared in plastic tubes or empty injection vials or in groups in large containers or in individual cells. The most common method for the production of chrysopids is detailed below.

The adults are collected daily and transferred to pneumatic glass troughs or G.I. round troughs (30 cm x 12 cm). Before allowing the adults, the rearing troughs are wrapped inside with brown sheet which act as egg receiving card. About 250 adults (60% females) are allowed into each trough and covered with white nylon or georgette cloth secured by rubber band. On the cloth outside three bits of foam sponge (2 sq.in) dripped in water is kept. Besides an artificial protein rich diet is provided in semisolid paste form in three spots on the cloth outside. This diet consists of one part of yeast, fructose, honey, Proteinex ^{R} and water in the ratio 1:1:1:1. The adults lay eggs on the brown sheet. The adults are collected daily and allowed into fresh rearing troughs with fresh food. From the old troughs, the brown paper sheets along with *Chrysopa* eggs are removed.

Storage and destalking of eggs

The brown paper sheet kept inside the adult rearing troughs contain large number of eggs each laid on a stalk or pedicel. As such the sheets are stored at 10^{o}C in B.O.D. incubator or refrigerator for about 21 days. When the eggs are required for culturing or for field release the egg sheets are kept at room temperature for a day and the eggs during this period turn brown and hatch on 3 days later. The first larvae are either taken for culture or for recycling or for field release.

Group rearing of grubs

It is done in GI round basins (28 cm dia) at 250 larvae/basin covered with *khada* cloth. The eggs of *Corcyra cephalonica* is given as feeding material for the larvae in the laboratory. For rearing 500 *Chrysopa* larvae the total quantity of *Corcyra* eggs required is 22 cc @ 5 cc/feeding for five

feedings in alternate days. The *Chrysopa* larvae pupate into round white coloured silken cocoons in 10 days. The cocoons are collected with fine brush and transferred into 1 lit plastic containers with wire mesh window for emergence of adults. From the cocoons, pale green coloured adults with transparent lace like wings emerge in 9-10 days.

Individual rearing of grubs

In the first step of larval rearing, 120 three day old chrysopid eggs are mixed with 0.75 ml of *Corcyra* eggs (the embryo of *Corcyra* eggs are inactivated by keeping them at 2 feet distance from 15 watt ultraviolet tube light for 45 minutes) in a plastic container (27x18x6 cms). On hatching, the larvae start feeding. On 3rd day the larvae are transferred to 2.5 cm cubical cells of plastic louvers @ one per cell. Each louver can hold 192 larvae. *Corcyra* eggs are provided in all the cells of each louver by sprinkling through the modified salt shaker. Feeding is provided in two doses. First feeding of 1.5 ml *Corcyra* eggs for 100 larvae and second feeding of 2 ml for 100 larvae with a gap of 3-4 days is done. Total quantity of *Corcyra* eggs required for rearing 100 chrysopid larvae is 4.25 ml. The louvers are secured on one side by orgami or brown paper sheet and after transfer of larvae covered with acrylic sheet and clamped. Orgami or brown paper is used for facilitating pupation and clear visibility of eggs. The louvers are stacked in racks. One 2m x 1m x 45 cms angle iron rack can hold 100 louvers containing 19,200 larvae.

Cocoons are collected after 24 hours of formation (when they get hardened) by removing orgami or paper from one side. The cocoons are placed in adult oviposition cages for emergence (Adults are sometimes allowed to emerge in louvers and released on glass window panes from where they are collected using suction pumps).

5. Practical session

1. Study the mass production of the predator

2. Study the biology of the predator

3. How the grubs are provided food? At what rate? How long? At what intervals?

Additional notes

Ex. No.:

Date :

Mass production of *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae)

1. Introduction

C. montrouzieri has been introduced from Australia for the control of *Coccus viridis* on coffee. But the predator has established on many species of mealybugs and green shield scale. In the field its practical use for the suppression of mealybugs *viz*., pink mealy bug, *Maconellicoccus hirsutus,* citrus mealy bug *Planococcus citri*, tailed mealy bug *Ferrisia virgata* and mealy scale *Pulvinaria maxima* on citrus, coffee, grapes and several other fruit crops and ornamentals has been demonstrated. Use of *C. montrouzieri* is the break through in applied classical biological control. The coccinellid predator is native of Australia. In 1892, it was introduced into California by Albert Koebele for the control of citrus mealybugs. Following the success, the beetle was introduced into India in 1898 by New Port. It has given effective control of mealybugs in fruit crops like citrus, grapes, guava, etc. *C. montrouzieri* is one of the outstanding example in the biological control history. Its importance is also evident by its growing commercialisation in India.

2. Objective

To study the method of mass production of *Cryptolaemus montrouzieri*.

3. Production procedures of predator

In the laboratory, the life cycle is completed in approximately 30 days. The premating and preoviposition periods are about 5 and 10 days respectively. The oviposition is about 10 days. Eggs are laid from late evening to early morning. They are pale yellowish white, the surface being smooth and shiny. It is oval to cylindrical, both the ends beings smoothly rounded. Incubation period ranges from 5 to 6 days but extended in winter months. Viability of eggs is 90 to 100 per cent.

The newly hatched grub is sluggish but becomes active after 3 to 4 hours. The tiny grub is pale greyish with white lines across the body along intra segmental regions. These white lines become prominent after few hours and white wax strands develop after a day. The grub has four larval instars, and the larval stage occupies about 20 days. They feed on al stages of mealybugs. Duration of first, second, third and fourth instar grubs are 3-4, 4, 4-5-7-8 days respectively. Grownup grubs are entirely covered with white wax strands. When the grub is disturbed, it exudes a yellow fluid from the dorsal surface of the body for defensive purpose. The prepupal period is 2 to 4 days when it suspends feeding activities. The pupal period varies from 7 to 9 days.

The adult spends about one day in the pupal case before it emerges. It is covered with a white powder like substance for a day. The male could be distinguished from the female by the colouration of first pair of legs. The first pair of legs in the case of male is brown and the latter two pairs being black, whereas in the female all the three pairs are black. Male to female ratio is 1 : 1. Adults are also known to attack and feed the mealybugs. Longevity of adults ranges from 50 to 60 days and the fecundity is about 200-220 eggs.

Feeding behaviour

Both adults and grubs are predating almost all stages of the mealybug. However the grubs are voracious feeders. The coccinellid grub consumes a total of 900 to 1500 mealybug eggs in its development. A single grub can eat as many as 30 nymphs or 30 adult mealybugs. Fourth instar grub is the most voracious feeder of the mealybugs.

After 15 days of infestation of pumpkins with bugs they are exposed to a set of 100 beetles for 24 hrs. After exposing, the pumpkin is kept back in a cage as described for under production of *M. hirsutus*. The beetle during the period of exposure feed on mealybugs as well as deposit their eggs singly or in groups of 4-12. The grubs are visible in such cages within a week of exposure to beetles. The young grubs feed on eggs and small mealybugs but as they grow they become voracious and feed on all stages of mealybugs. For

facilitating the pupation of grubs dried guava leaves or pieces of papers are kept at the base of each of the cages. The first beetle from the cages start emerging on 30th day of exposure to *C. montrouzieri* adults. The beetles are collected daily and kept in separate cages for about 10-15 days to facilitate completion of mating and pre-oviposition. The beetles are also fed on diet containing agar powder (1 g), sugar (20 g), honey (40 cc) and water (100 cc). The adult diet is prepared by boiling sugar in 70 cc of water, adding 1 g agar, diluting 40 cc honey in 30 cc of water and adding to the sugar and agar mixture when it comes to boiling point. The hot liquid diet is kept on small white plastic cards in the form of droplets which get solidified on cooling. Such cards containing adult diet can be fed not only to *C. montrouzieri* but also to many other species of coccinellids. From each cage about 175 beetles are obtained. The emergence of the beetles is completed within 10 days.

Beetles can also be reared on *Corcyra cephalonica* eggs but empty ovisacs of *Planococcus citri* are to be kept for inducing egg laying by the beetles. The beetles are also multiplied on semi synthetic diet which is still in the process of further refinement.

4. Precautions

All due precautions should be taken to avoid scarcity of food for the grubs to avoid cannibalism by grubs. All the pumpkins showing signs of rotting should be properly incinerated.

5. Practical session

1. Study in detail the biology and morphology of the predator.

2. Draw neat sketch of the various stages of the insect you have observed.

3. How do you provide the adult feed for the predator?

Additional notes

Ex. No.:

Production of the nuclear polyhedrosis virus of *Helicoverpa armigera*

Date :

1. Introduction

In India, *Helicoverpa armigera* is of major importance damaging a wide variety of food, fibre, oilseed, fodder and horticultural crops. The nuclear polyhedrosis virus of *H. armigera* (HaNPV) is currently used for the management of *H. armigera* on chickpea, cotton, pigeonpea, tomato and sunflower. Mass production of Nuclear Polyhedrosis Virus (NPV) on commercial scale is restricted to *in vivo* procedures in host larvae which are obtained by

a) Field collection from cotton, pigeonpea and chickpea – *H. armigera*

b) Mass culturing in the laboratory in semisynthetic diet – *H. armigera*

Some small scale producers use field – collected larvae for mass production of NPV in spite of the following constraints.

(i) Collection of a large number of larvae in optimum stage (late IV / early V instars) is time-consuming and can be expensive in terms of labour and transportation costs.

(ii) Wild populations of insects may carry disease causing organisms like microsporidians, cytoplasmic polyhedrosis virus, stunt virus and fungal pathogens which will affect both virus production and quality.

(iii) Introduction of wild strains of NPV resulting in quality control problems.

(iv) Transportation of a large number of larvae with cannibalistic behaviour will be a difficult task.

(v) Parasitized larvae collected from the field will die prematurely yielding little virus.

Rearing of larvae in the natural host plant will involve frequent change of food at least once a day during the incubation period of 5-9 days increasing the handling time and hence the cost. In order to reduce the cost, field collected larvae are released into semi synthetic diet treated with virus inoculum. Mass culturing of insects in semi synthetic diet involves high level of expertise, hygiene and cleanliness.

2. Objective

To study the mass production of nuclear polyhedrosis virus of *H. armigera*.

3. Production procedure

The NPV of *H. armigera* is propagated in early fifth instar larvae. The virus is multiplied in a facility away from the host culture laboratory. The dose of the inoculum used is 5×10^5 polyhedral occlusion bodies (POB) in 10 µl suspension. The virus is applied on to the semisynthetic diet (lacking formaldehyde) dispensed previously in 5 ml glass vials. A blunt end polished glass rod (6 mm) is used to distribute the suspension containing the virus uniformly over the diet surface. Early fifth instar stage of larvae are released singly into the glass vials after inoculation and plugged with cotton and incubated at a constant temperature of 25°C in a laboratory incubator. When the larvae exhausted the feed, fresh untreated diet is provided. The larvae are observed for the development of virosis and the cadavers collected carefully from individual bottles starting from fifth day. Approximately, 200 cadavers are collected per sterile cheese cup (300 ml) and the contents are frozen immediately. Depending upon need, cadavers are removed from the refrigerator and thawed very rapidly by agitation in water.

Processing of NPV

The method of processing of NPV requires greater care to avoid losses during processing. The cadavers are brought to normal room temperature by repeatedly thawing the container with cadaver under running tap water. The cadavers are homogenised in sterile ice cold distilled water at the ratio 1 : 2.5 (w/v) in a blender or precooled all glass pestle and mortar. The homogenate is filtered through double layered muslin and repeatedly washed with distilled water. The ratio of water to be used for this purpose is 1: 7.5-12.5 (w/v) for the original weight of the cadaver processed. The left over mat on the muslin is discarded and the filtrate can be semi-purified by differential centrifugation. The filtrate is centrifuged for 30-60 sec. at 500 rpm to remove debris. The supernatant is next centrifuged for 20 min at 5,000 rpm. Then the pellet containing the polyhedral occlusion bodies (POB) is suspended in sterile distilled water and washed three times by centrifuging the pellet in distilled water at low rpm followed by centrifugation at high rpm. The pellet finally collected is suspended in distilled water and made upto a known volume, which is necessary to calculate the strength of the POB in the purified suspension.

4. Practical session

1. Study the mass production method of HaNPV with 500 fifth instar *H. armigera* and record the observations.

   Per cent mortality =

   Yield of POB/larva =

   Yield of POB/100 inoculated larvae

2. Weigh randomly 10% of the cadavers. Classify them into different age groups and weight groups at death and comment on the importance of these parameters in the production of the virus.

3. Work out the cumulative mortality. What is the importance of total mortality on the production efficiency?

Additional notes

Ex. No.:

Production of the nuclear polyhedrosis virus *Spodoptera litura*

Date :

1. Introduction

The virus is effective against the pest on several crops. The virus is generally propagated in early fifth instar by surface contamination of semi-synthetic diet.

2. Objective

To study the mass production of the nuclear polyhedrosis virus of *S. litura*.

3. Production procedure

The mass production of the NPV is carried out in early fifth instar stage of *S. litura*, which yields maximum amount of the NPV. Therefore, in the host culture laboratory a continuous culture of the insects is maintained with proper handling procedures. The larvae are grown in diet held in 5 ml glass vials. When the larvae reach the appropriate stage they are transferred to the virus production facility. The NPV is multiplied by feeding the semiynthetic diet coated with a clean inoculum of the NPV that has previously been standardized. This is accomplished by placing aliquots of 10 µl of the viral suspension of concentration 1 x 10^8 Polyhedral Occlusion Bodies (POB) in the centre over the diet surface either in glass vials and spreading the suspension uniformly all over the surface with a polished glass rod. Larvae are released singly after 15 min. into each glass vial/cell and incubated at 25^{o}C for 10 days. The larvae begin to die from 5th day onward. The cadavers are collected individually and transferred to 500 ml plastic containers and frozen immediately until processing. The processing method is similar to that of *H. armigera*.

4. Practical session

1. Inoculate 100 larvae of *S. litura*, harvest the cadavers and process the NPV.

2. Spray diet filled trays with 1 ml of suspension containing $1x10^8$POB using a vacuum pump and atomizer. Transfer 25 larvae after the moisture has been absorbed by diet. Allow the larvae to feed for 24 h and transfer them to vials with diet. Record the observations on the production.

Additional notes

Ex. No.

Date:

Production of the Granulosis Virus of *Chilo infuscatellus*

(Order : Lepidoptera; Family: Pyralidae)

1. Objective

To study the mass culture procedures followed in *Chilo infuscatellus* Granulosis Virus production.

2. Introduction

Chilo infuscatellus is a devastating pest of sugarcane. Management of the pest with insecticides has become extremely difficult and the search for alternatives has yielded a granulosis virus. The virus is effective when applied at $1.5x10^{13}$ OB/ha repeatedly three or four times on early cane under field condtion.

3. Production procedure

The granulosis virus is produced *in vivo*. The method employed in production is by oral feeding treatment. Fresh bits of sugarcane shoots from field are used. The cut splits are washed and and placed on moist filter paper in 6.6x7.4 cm containers @ 3 bits per container. A dose of $1x10^{8}$OB/ml in 0.01% Teepol is prepared and larvae in 4^{th} instar collected from field are treated by dipping the head into the suspension containing the virus in a watch glass. The larvae @ 1-2/shoot bit are transferred to the container and the jar is closed. The feed is replaced with fresh ones every alternate day. The larvae begin to die after 6 days and are collected in sterile containers and stored in a freezer. The processing methods are similar to that of the nuclear polyhedrosis virus. In differential centrifugation, the cadavers are centrifuged at 700rpm initially and at 8000 rpm for 30 min finally to collect the pellet.

4. Practical session

1. Practice the method of inoculation of the virus individually.

2. After inoculation observe the changes in the colour of the body and differentiate the morphological changes.

Additional notes

Ex. No.:

Mass production of green muscardine fungus *Metarhizium anisopliae*

Date :

1. Introduction

Fungi represent a diverse group of insect pathogens. The insects attacked by the fungus die shortly after the fungus begins to develop in the haemocoel. The rhinoceros beetle, *Oryctes rhinoceros* is one of the serious and important pests of coconut and has a wide distribution and persistent occurrence in all the coconut growing areas in the country. The adult beetle cause sever damage to the growing palms by feeding on the tender fronds and crowns and resulting in stunting of the trees. The damage to the spathe results in the loss of nuts. Young seedlings are sometimes killed outright. Since the insect breeds in the farmyard manure and fallen coconut trees, the control measures have to be directed at the breeding sites as well as on the trees. The chemical control measures adopted against this pest are always costly, tedious and have to be repeated. Hence an easy method utilizing the safe and specific fungus, *Metarhizium anisopliae* for the management of this pest is aimed at.

The colony of *M. anisopliae* appears white when young, but as the conidia mature, the colour turns to dark green. The conidiophores are branched, and the initial conidium is produced at the distal end of the conidiophores. A chain of conidia is formed on each conidiophore with the youngest conidium being adjacent to the conidiophore. The mass of spore chains becomes so dense and coheres with each other to produce prismatic masses of columns of spore chains.

2. Objective

To study the mass production of *Metarhizium anisopliae*.

3. Production procedure

The fungus can be mass produced in conventional laboratory media as well as on crushed maize grains, etc. The cheapest media till date known are (1) Cassava chips mixed with rice bran supplemented with urea or fish meal extract, and (2) Coconut water wasted from copra making industry or (3) carrot broth.

3.1. On coconut water

Coconut water (40 ml) contained in 375 ml side wise flat liquor bottles plugged with cotton plug are sterilized in batches of 9-10 bottles in 12 litre pressure cooker for 20 minutes at 15 psi. The bottles are inoculated with 1 ml suspension containing spores of the fungus with the help of a sterile injection syringe. Before inserting the needle within the sterile bottles for drawing spore suspension for inoculation, the needle of the syringes and collar region of the bottles are flamed (Bunsen burner). The bottles are inoculated in a laminar flow chamber and rested on flat surface for 2 days or till the surface of medium is fully covered by the olive green sporulated fungus. The whole culture is crushed thoroughly in an ordinary mixer and used in the field. From a single average size coconut 5-6 bottles of cultures can be made.

3.2. In Carrot broth

Carrot cut into small pieces (40 g) is washed in potable water and transferred to conical flask (250 ml) and 15 ml of distilled water is added. The conical flasks are plugged with cotton and autoclaved for 20 min at 15 psi. The flasks are allowed to cool and taken to laminar flow chamber for inoculation. From a clean uncontaminated mother culture in slant loopful quantities of *M. anisopliae* spores are transferred aseptically. The flasks are incubated at room temperature. The spores can be harvested in a fortnight.

4. Practical Session

1. Practice the method of production of the fungus on different media.

2. Observe the characteristics of the spores produced on different media.

3. Work out the cost of production of the spores @ 500 units per day on different media.

Additional notes

Ex. No.:

Mass production of the fungus, *Verticillium lecanii*

Date :

1. Introduction

Vertilcillium lecani commonly called as white halo fungus is found sprouting on the body of coffee green scale *Coccus viridis.* It has been extensively studied against the pest in Lower Pulney Hills in Tamil Nadu. The fungus is known to cause epizootic when the environmental conditions are favourable.

2. Objective

To study the techniques in mass multiplication of the fungus *V. lecani*.

3. Production procedure

The fungus is multiplied on cheap media for large scale production. Sorghum grains devoid of pesticide residues (40 g) is washed in potable water and transferred to conical flask (250 ml) and 15 ml of distilled water is added. The conical flasks are plugged with cotton and autoclaved for 20 min at 15 psi. The flasks are allowed to cool and taken to laminar flow chamber for inoculation. From a clean uncontaminated mother culture in slant loopful quantities of *V. lecani* spores are transferred aseptically. The flasks are incubated at room temperature. The spores are obtained in a fortnight.

4. Practical Session

1. Practice the mass production of the fungus on sorghum grains

2. Record the observations on the time taken to sporulate and the colour of the spores.

3. Work out the cost of production of the spores @ 500 bottles per day

Additional notes

Ex. No.:

Mass production of the fungus, *Beauvaria bassiana*

Date :

1. Introduction

The fungus is otherwise called as white muscardine fungus. The fungus spores and mycelia are milky white and found sprouting on the body of lepidopterous insects like *Helicoverpa armigera*, *Spodoptera litura* and *Anadevidia peponis*.

2. Objective

To study the techniques in mass multiplication of the fungus *B. bassiana*.

3. Production procedure

Carrot cut into small pieces (40 g) is washed in potable water and transferred to conical flask (250 ml) and 15 ml of distilled water is added. The conical flasks are plugged with cotton and autoclaved for 20 min at 15 psi. The flasks are allowed to cool and taken to laminar flow chamber for inoculation. From a clean uncontaminated mother culture in slant loopful quantities of *B. bassiana* spores are transferred aseptically. The flasks are incubated at room temperature. The spores are obtained in a fortnight.

4. Practical session

1. Prepare the medium for the mass culture of the fungus.

2. Observe the development of the fungus and record your observations on the growth pattern of the fungus.

3. Work out the cost of production of the spores @ 500 units per day

Additional notes

Ex. No.:

Standardization of insect pathogens

Date :

Part 1: Enumeration of Insect pathogens

1.Introduction

Successful control of pests with insect athogens depends on the use of appropriate dose of agent. It is therefore, very essential to ascertain the strength of polyhedral occlusion bodies (POB) in the virus suspension or spores in fungal suspensions before they are applied on the crop.

2. Objective

To study the procedure involved in counting and standardisation of insec pathogens.

3.Procedure for counting of insect pathogens

3.1. Nuclear polyhedrosis virus

The concentration of the polyhedra can be estimated with the help of a haemocytometer under light microscopy. The suspension has to be made to a standard volume. A rapid observation at low power in the microscope will indicate the level to which the stock suspension is to be diluted. If clumps are still present during examination sonication has to be done.

3.1.1. Dilutions

Transfer 10µl of the suspension to a sterile microfuge tube and make up the volume to 100µl with distilled water. Label the solution as **A**. From **A** transfer 10µl to another microfuge and make up the volume to 100µl with distilled water. Label this working standard as **B**, which will be used for further enumeration. Use a digital micropipette for preparation of dilutions.

3.1.2. Enumeration

- Clean the coverslip and the haemocytometer by rinsing in ethanol (70%) and wiping with clean tissue.
- Place the haemocytometer over a clean and flat surface.
- Place the coverslip on top of the slide exactly over the depression in the counting chamber and press down firmly to ensure that the chamber is of the correct depth.
- Withdraw 10µl of the isolate suspension and expel into the chamber directly so that the chamber is filled completely. Avoid over flooding of the suspension.
- Leave the haemocytometer undisturbed for 2 min. So that the polyhedra in the suspension settle down and Brownian movement is reduced. If the work area is warm the suspension in the chamber will evaporate rapidly and the counting will become erroneous. Therefore, the enumerations have to be done under ambient conditions or preferably in an air-conditioned room. If such facilities are not available the haemocytometer can be kept under saturated atmosphere before measurements are made.
- Place the haemocytometer over the stage of a phase contrast microscope and fix the counting area at low power with appropriate settings. Focus the objective to the polyhedra dispersed in the centrally located squares ; increase the magnification and make finer adjustments.
- The central squares are divided into 5x5 squares equally. Each of these 1/25 squares is further subdivided into 16 smaller squares. Totally there are 400 smaller squars which occupy a surface area of $1mm^2$. The polyhedra have to be counted systematically in sequence across the grid. By doing so one will be able to count the polyhedra in 80 smaller squares. The polyhedra within each smaller square and those touching the top and left-hand sides alone are counted. If there are more than 5 polyhedra per smaller square counting will be difficult and interpretation becomes flawed. Under such conditions dilute the working standard **B** by another 10 fold and enuemrate.

- During enumeration, count the polyhedra present on a single place only. Don't attempt to use the fine adjustment of the objective, as the inadvertent inclusion of polyhedra present in deeper layers will yield incorrect strength.
- The counts have to be taken in three replicates and average has to be worked out.

3.1.3 Calculation

The number of polyhedra/ml is calculated by the formula

$$\frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where,

X = Number of polyhedra counted totally

Y = Number of smaller (1/400) squares checked

10 = Depth factor

1000 = Conversion factor for mm^3 to cm^3 to ml

D = Dilution factor

Alternatively number of polyhedra / ml is calculated by

$$\frac{D \times X}{N \times K}$$

Where,

D = Dilution factor

X = Number of polyhedra counted totally

N = Number of smaller (1/400) squares checked

K = Volume of suspension above one smaller (1/400) square

Explanation of K

- Area of each smaller (1/400) square is 0.0025 mm^2
- Depth of the chamber is 0.1mm
- The volume of the liquid above the smaller (1/400) square is 0.0025mm^2 x 0.1 m = 0.00025 mm^3
- To convert to cm^3 multiply by 1/1000

$\Rightarrow$ The volume of suspension (K) will be 2.5 x 10^7

3.2. Counting fungal pathogens

Unlike the nuclear polyhedrosis viruses, the fungal pathogens can be counted very easily as the size of the spores is bigger. More over, the counting procedure is as in the case of enumeration of the NPV.

4. Practical exercise on the counting NPV

- It is important that the sample is counted accurately. If it is too concentrated it will be difficult to count and the result may be incorrect. It should be diluted carefully by 10, 100 or even 1000 times as appropriate to give a count of about 5-10 polyhedra / small square.
- Remember to include the dilution factor in the calculations after counting.
- Do not reduce the concentration too much. A very diluted sample is also difficult to count accurately.

5. Example

Suppose in a sample diluted by a factor of 1000 we count 535 polyhedra in 160 small squares, then :

D = 1000

X = 535

N = 160

K = 2.5×10^{-7} cm^3

Thus

$$\frac{1000 \times 535}{160 \times 2.5 \times 19^{-7}} = 1.34 \times 10^{10}$$ polyhedra / ml of undiluted sample

It is important that the sample is counted accurately. If it is too concentrated it will be difficult to count and the result may be incorrect. It should be diluted carefully bu 10,100 or 1000 times as appropriate to give a count of about 5-10 POB/small square. Remember to include the dilution factor in the calculations after counting. Do not reduce the concentration too much. A very diluted sample is also diccifult to count accurately. To enable you to familiarise with the counting and standardisation of POB here are some problems which you may solve.

6. Worked examples

Problem 1. Calculate the strength of POB in the stock suspension from the following information :

a. Dilution: 1 ml of stock made up to 100 ml

b. POB counted = 425

c. No. of small squares counted = 80

Problem 2. What is the quantity of virus stock required to spray one hectare of chickpea for the countrol of *Helicoverpa armigera* using 1.5×10^{12} POB/ha if the strength of the suspension is 1.5×10^{8}?

Answers to problems :

1. 2.125×10^{9} POB/ml
2. 10 lit.

Part II. Quality control in the production of entomopathogens

1. Introduction

The use of biopesticides especially the entomopathogens is increasing in view of the difficulties faced with other methods of management. Quality control is prerequisite for the ultimate success of the biopesticides usage. Defective manufacture, contamination during production, poor storage conditions, use of ineffective strains improper production methods etc., affect the quality of the biopesticides. Of the biopesticides, the nuclear polyhedrosis virus is used extensively other than the bacteria. Standards are being developed for the use of the nuclear polyedoris virus. This virus have been included under the Insecticides Act recently.

2.Objective

To study the quality control parameters ivolved in production of entomopathogens.

3.Quality control standards

i. Virus concentration per gram / ml

In order to reduce the cost of packing and transportation, it is advisable to have a concentration of 6×10^9 POB / ml which would result in volume of 250 ml pack to be used for one/ha. In India, the minimum concentration prescribed is 1×10^9 POB/ml.

ii. Activity of the virus

For good product performance, the activity of the virus should be maintained over a reasonable period of shelf life. A good indicator of the shelf life is the LC_{50} established through appropriate bioassays. The LC_{50} would vary with the stage of the insect pest as well as the viral isolate. Basic information on the relative virulence of different isolates of NPV to early instar larvae like II instar is rquired. Based on this, a maximum permissible value of LC_{50} can be proposed. In India, the value proposed for HaNPV based

on the diet – surface contamination method is 0.5 POB/mm^2 of diet surface for II instar larvae of *H. armigera*.

iii.Total load of contaminant bacteria

Human pathogens like *Salmonella*, *Shigella* or *Vibrio* should not be present. The total non-pathogenic bacteria present should not exceed $1x10^8$/ml or g of the formulated product.

b.Establishment of a regulatory mechanism

In India NPV has been included under the schedule of insecticide act making it mandatory for commercial producers to register their products with the Central Insecticides Board of the Government of India by submitting all the required data. Under the act, the officers of the state department of Agriculture are empowered to collect market samples of the product and send them to accredited quality control laboratories for analysis. If the samples do not meet the prescribed standards, legal action is pursued.

c.Accreditation of quality control laboratories

Quality control work can be done in an insect pathology laboratory under the control of the federal / state Government or the state agricultural university. The laboratory should have trained personnel and should be accredited by the appropriate authority.

Additional notes

Ex. No. **Preparing cost estimates for *Trichogramma* Production**

Date:

1. Objective

To prepare cost estimates for *Trichogramma* production

2. Procedure for preparation of production plan of *Trichogramma* for 1000ha

Area required : 4,000 sq. feet

Target : 1,00,000 cc/yr

Production process : 25000 cc/batch

MATERIALS REQUIRED

- Broken cumbu grain
- Mosquito net
- Plastic basin
- Specimen tube
- Khada cloth
- Aspirator
- Yeast
- Blotting paper
- Honey
- Camel hair brush
- Shoe brush
- Rubber band
- Turners
- Moth scale egg separator
- Ground nut kernel
- Sieves
- Filter
- Sulphur (WP)
- Streptomycin sulphate
- Home milling machine
- Measuring cylinder
- *Mating drum*

- Absorbing cotton Formaldehyde 40%
- Enamel Tray

COST OF EQUIPMENT

Sl.No.	Items	Physical Unit	Price / Unit	Total
1.	*Corcyra* rearing equipment (trays)	1310	65	85,150
2.	Racks (15'x5')	20	7,000	1,40,000
3.	Mating drum	50	500	25,000
4.	Enamel drum (1 feet square)	50	90	5,000
5.	Moth scale - egg separator	10	1,500	15,000
6.	Mosquito net	10	140	1,400
7.	UV light	1	4,000	4,000
8.	Scissor	3	20	60
9.	Filter	2	15	30
10.	Camel hair brush	10	20	100
11.	Shoe brush	5	20	100
12.	Stapler	1	50	50
13.	Microscope	5	1,000	5,000
14.	Refrigerator	5	10,000	50,000
15.	Rent		2,000	2,000
16.	Building (land value)	4000 sq.ft	x 80	3,20,000

Total		6,52,860

WORKING CAPITAL

S.No.	Items	Physical Unit	Price /Unit Rs.	Total
1.	Broken cumbu grains	3275	10.00/kg	32,750.00
2.	Groundnut kernel	131	50.00/kg	6,550.00
3.	Milling charge	-	0.250/kg	9,000.00
4.	Yeast	6.55	250/-	1,637.50
5.	Streptomycin sulphate	2.5	1,000/-	2,500.00
6.	Sulphur	6.5	600/-	593.50
7.	*Corcyra* nucleus eggs	655 CC	20 / CC	13,100.00
8.	Kada cloth	500m	22.00m	11,000.00
9.	Rubber band	5kg	12.00/kg	60.00
10.	Twines	5kg	20.00/kg	100.00
11.	Labour	4a+6b	A 50, B 35	1,60,000.00
12.	Chart	500 m^2	5 /m^2	2,500.00
13.	Gum	2.5 lit	40 / lit	100.00
14.	Polythene cover	100	90/kg	9,000.00
15.	Electricity + Water charge			5,000.00
16.	Transporting charge			5,000.00
17.	Glass tube	20	30/ tube	600.00

18.	Measuring cylinder	2	90/-	180.00

TOTAL FIXED COST (TFC)

Depreciation on equipment	=	13%
Depreciation per year	=	Rs.56,798.82
Interest on fixed cost (@ 18%)	=	1,17,514.80
Annual rent	=	24,000/-
Insurance	=	10,000/-
Total fixed cost per year	**=**	**1,51,514.80**

TOTAL VARIABLE COST (TVC)

Working Capital	=	2,66,271/-
16% interest on working capital	=	41,643.36
Total variable cost per year	**=**	**3,07,914/-**

Total cost = TFC + TVC

1,51,514 + 3,07,914 = 4,59,428/-

Cost of production / CC = 4,59,428 / 1,00,000	=	Rs.4.60 / CC
Break even quantity	=	1,51,514 / 15 - 4.6

3. Practical Session

Analyze the above project and comment on the feasibility

Additional notes

Ex. No.

Date:

Economics of *Helicoverpa armigera* nuclear polyhedrosis virus production

1. Introduction

The production methodologies envisaged here are as per the procedures given in different chapters in the manual. The programme is labour oriented with substitution of machinery at appropriate stages. The risk involved in production include:

- Insufficient training and lack of skilled personnel,
- Incidence of disease in healthy colony during culture,
- Batch variation in larvae during production,
- Improper sanitary conditions during production,
- Fluctuations in demand for the product, and
- Other extraneous situations beyond the control of operators

2. Objective

To study the economics of *H. armigera* NPV production

To prepare an inventory of the materials required for production of the NPV

3. Cost of production

The raw material, labour plant and machinery requirement is described hereunder.

A. Host Culture

I. Adult handling

No. of oviposition cages / person / day = 50

Operations involved are :

a. Prepare adult feed

b. Prepare oviposition cages

c. Collections, transfer of adults and labelling

d. Collect egg masses and stored in humidifying chambers

e. Surface sterilize, shade dry and store eggs for transfer to diet

Calculation

No. of pairs that can be housed per oviposition./chamber = 10

Fecundity/pair during a holding period of 8 day = 400

Percentage of fertile pairs @ 80 per cent = 8

Production / oviposition unit = 3200 eggs

Mean per cent fertility = 90 per cent

No. of fertile eggs / oviposition unit = 2880 eggs

Production of fertile eggs / day =

No. of eggs/unit x Total no. of units/holding period

= 14400

Labout cost/yr (@Rs. 40/day) = 14,600

II Larval handling

Operations involved are:

1. Handling of early instars

(a) Prepare group rearing trays for dispensation of semisynthetic diet. The trays

have to be surface sterilised with 70 per cent ethanol.

(b) Meter the diet ingredients

(c) Prepare the diet, dispense in trays, cool the diet

(d) Transfer freshly hatched larvae to the trays cover with sterile lid and pack in

polythene bags. Label individual trays with details of production

(e) Incubate till the larvae reach third instar premoult stage.

2 Handling older instars

(a) Transfer the normally growing larvae to trays again when the larvae reached

third instar premoult

(b) When the larvae reach fifth instar, separate the culture for subsequent colony

and virus production.

Calculation

Output of eggs from adults = 14,400

Allowance for escapism and handling mortality

during neonate stage = 5 %

No. of larvae available / day = 13,680

Proportion of larvae used for virus production = 90%

Allowance of larvae for natural cause of

mortality, slow growth, escapism etc. = 10%

=12,312

No. of pupae required daily for sustained maintenance of 50 oviposition units continuously.

This is based on the assumtpion that after establishment of colony in 5-6 cycles the production will stabilise at 50 oviposition units/day. Each day, 10% of the spent adult oviposition chambers are removed from the holding room and this 10% loss is made good by addition of 5 fresh oviposition chambers.

No. of adults added / day = 5x2x10 = 100

To select 100 vigorous and healthy adults, the number of pupae required after giving the allowance for

(a) malformed adults - 10%

(b) malformed pupae 10%

(c) Under weight pupae and pupal mortality - 5%

No. of pupa required/day = 125

Therefore, percentage of larvae to be taken for pupation = 1%

Summary

Stage	Output
Adult	= 50 pairs / day
Eggs	= 14,400
Larvae (host culture)	= 100
Virus production	= 12,312

Materials Requirement

a. Adult handling

1. Ovipostion chambers

No. of oviposition chamber / day = 50

Add 10% for maintenance of the peak production

of 50 buckets/day, i.e., 5 buckets are discarded/day

and 5 buckets are added/day = 10

Total = 60

Allowance for breakage (10%) = 6

Total = 66

Rounded off to = 70

2. Adult emergence cage

Required No. of cages @ 5 days holding period = 5

3. Adult feed requirement @ 5 ml / day / bucket

Oviposition buckets = 50

Adult emergence = 5

Total = 55

Total vol. / year = 55x5x365 = 100.375 l

Quantity of sugar needed = 10.03 kg

Vitamin solution @ 0.1% = 1 lit.

Cotton requirement @ 400 g / 1000 vial = No. of vials/year

= 365 x 55 = 20,075

Therefore, quantity of cotton = 20075/1000 = 20.5 kg

Cost of adult maintenance

1. Cost of labour

Total cost per year @ 1/day (Rs. 40/day) = Rs. 14600

2. Cost of inputs

Cost of sugar @ Rs. 20.00/kg = 400.60

Cost of Vitamin solution (1 lit) = 1466.67

b Egg handling

1 Cloth requirement

A metre of muslin cloth is sufficient for 8 buckets.

Therefore, requirement of cloth	= 50/8 = 6.25 m.
Total quantity required f@ 4 changes/year	= 6.25x4 =25m
Holding period/cycle	= 5 days
Allowance for spoilage	= 10%
Total	= 137.5 m
Value @ Rs. 40 / m	= Rs.5500
2 Requirement of formaledhyde	
Quantity of formaldehyde(10%) / day	= 200 ml
Therefore, quantity required at 40%	= 50 ml
Requirement / year	= 50 x 365
	= 18.25 lit/year
Cost	= Rs.1,314.00
3. Egg holding cups	= 50
Holding time	= 3 days
Total number	= 150
Allowance for breakage @ 10%	= 165
Cost of the cups @ Rs. 5/cup	= Rs. 825
c. Larval Handling	
1. Cost of larval diet for 1 set of 1000g	Rs. p.
1. Bengal gram - 100 g	- 4.20
2. Agar Agar - 12.8 g	- 5.38
3. Yeast - 30 g	- 4.05
4. Methyl paraben - 2 g	- 2.00
5. Sorbic acid - 1 g	- 2.00
6. Ascorbic acid - 3.2 g	- 6.14
7. Wesson’s salt - 7.2 g	- 0.50

8. ABDEC - 2.0 ml		- 1.36
9. Streptomycin Sulphate - 40 mg		- 0.50
10. Formaldehyde 40% - 1 ml		- 0.20
11.Carbendazim- 500 mg		- 0.45
12. Distilled water - 720 ml		- 0.25
	Total	-27.03

Cost of diet per year

1.1. Early stages

A set of diet of 1 kg is sufficient for 10 trays in group culture from I-III instar.Therefore, number of trays / day @ 250larvae / tray = 55 trays

Quantity of diet / year@ 10trays/kg	= (55 x 365) / 10
	= 2007.5kg
Cost of diet	= 2007.5 x 27.03
	= Rs. 54262.73

1.2. Older stages

A set of diet of 1 kg is sufficient for 350 glass bottles(5ml) for III - VIinstar.

Quantity of diet/year @ (35.18 kg/day)	=12840.7 kg
Additional quantity of diet needed during virus replication/year and host culture	=12840.7 kg
Total quantity of diet / year	= 27688.90kg
Giving an allowance for food spoilage @ 2% of the total quantity of diet needed	= 28242.68 kg
Cost of the diet	= Rs. 763399.58
Add fuel cost @ 1000/month	= Rs. 12,000.00
Total cost	= Rs. 775400.00

1.3. Labour requirement during diet preparation

No. of sets of diets to be prepared/day	= 77.34
Productivity/labour/day	= 18
Cost of labour/year @ Rs. 40/day	= Rs. 73000

2. Transfer of neonate larvae

Productivity/hr @ 250 larvae /tray	= 20 trays
Labour requirement/day	= 0.34 units

Cost of labour/year @ Rs. 40/day	= Rs. 5000
3. Transfer of grown up larvae	
Productivity/day/labour	= 1200 larvae
Labour required/day for the transfer	= 11.4
Cost of labour/year @ Rs. 40/day	= Rs. 166440.00
4. Cost of cotton @ 1000 glass vials/400g	
Total quantity required/day	= 5.472 kg
Cost of cotton/year@ Rs. 175/kg with 2 cycles	= Rs. 174762
(4) Labour requirement for cleaning	
(a) For washing the glass vials/day capacity of 1000/labour	
Labour requirement	= 13.6
(b) Washing upkeep and sterilization	= 2
Cost of labour/year	= Rs. 2333600
VI TOTAL RECURRING COST	=Rs. 1454996.67
Add contingencies for glassware, chemicals	
for upkeep and lab apparels, etc. @ 10%	= 1,45499.67
SubTotal	= 1600496.67
Add elecricity and water charges	
@ 5000/month	= 60,000.00
Sub Total	= 1660498.00
Add overhead expenditure @ 15%	
(labour welfare, communication, etc.)	= 249074.45
Salary for scientist-in-charge @ Rs.1,2000	
per month + 10% for welfare	= 15,84,000.00
TOTAL MANUFACTURING COST	**= Rs. 2067970**

VII DETAILS OF PLANT AND MACHINERY

I. Adult handling		Qty	Rate
	Value		
1.	Oviposition units	70	30
	2100.00		
2.	Adult emergence cage	5	1000
	5000.00		
3.	Egg holding cups	165	5
825.00			
4.	Humidigying chamber	3	100
300.00			
II. Diet preparation			
1.	Mixi	5	2500
12,500.00			
2.	Gas stove	5	2500
12,500.00			
3.	Utensils	5 sets	500
2500.00			
4.	Balance (Ordinary)	1	2000
2000.00			
5.	Electronic balance	1	25000
25000.00			
6.	Distillation apparatus		
	(100 lit/day)	1	15000
15000.00			
III Larval handling			
1.	Group rearing trays	550	30
	16500		
2.	Penicillin vials	415800	80/1000
	33264		

3.	Trays for vials	746	20	14925.00
4.	Laminar flow chamber	1	80,000	80000.00
IV	Sterilization equipments			
1.	Hot air oven	2	7000	14000.00
2.	Autoclave (small)	1	2000	2000.00
3.	Plastic tanks	20	500	10000.00
V	Lab Furniture Assortment			150000.00
VI	Temperature control			
1.	A/c (with voltage stabilizer)	3	30000	90000.00
2.	Air Cooler	2	2500	5000.00
3.	Refrigerator + voltage stabilizer (365 lit.)	1	17000	17000.00
VII	Washing and waste disposal			
1.	Plastic crates	20	500	10000.00
VII	Installation Cost			20000.00
			Total	

5,58,924.00

VIII Fixed expenditure

Rent for the building = Rs.36,000.00

Depriciation @ 16% = Rs. 89427.84

Total production cost

(manufacturing cost +fixed expenditure) = Rs. 2193397.84

Cost of production of one larvae = Re.0.439

B. Virus production

a. For virus inoculation

@ 5 min/1900 vials = 6.48 laboures/day

Cost of production = Rs. 94608

b. Transfer of larvae = 1000 larvae/individual

= 12.312 = 13 labour

Total cost of labour/year = 16 x 365 x 40

= 2,33,600.00

c. Harvesting of cadavers @ 3000 vials/labour/day (with 90% recovery)

No. of labourers required = 4

Cost of labour Rs. 58400

d. Processing of cadavers

Expected cadaver weight @ 350 mg/larva = 3.878 kg/day

Dilution factor for processing = 10

Total volume of the cadaver homogenate to be processed = 38.78 l

No. of processing cycles in the centrifuge/day = 16

Quantity to be processed/cycle = 2.42 l

Labour required for processing the cadaver = 1

Labour requiredd for purification of the cadaver = 2

Cost of the labour/yr =Rs. 43800

Cloth required during processing

@ 1mx Rs.40/m/day per year = Rs. 14600

e. Quantification and packing

To get 100 LE virus, larvae to be collected =125

(60% of larvae will give one LE/larva and 40% larvae, will yield 0.5 LE/larva)

Virus production from 11080.8 larvae = 8864.6 LE

Total production/year = 3235579 LE

Cost of packing @ 250 ml containing 1.5x 10^{12} POB = Rs 5. 00

Cost of the containers = Rs. 65700

Total Recurring Cost during virus production

1. Labour cost for inoculation and transferring = 386608.00

2. Labour cost for harvesting and washing = 14600.00

3. Packaging cost = 65700.00

Subtotal = 466908.00

4. Add contingencies for glasswares, chemicals,

upkeep, etc. @ 10% = 466908.00

Sub Total = 513598.80

IV Details of plant and machinery

1. Atomizer	1	10000	10000.00
2. Blender (250 g capacity)	2	6000	12000.00
3. Centrifuge (2 lit. capacity)	2	60000	120000.00
4. Air Conditioner	3	30000	90000.00
5. Freezer	1	30000	30000.00
6. Refrigerator	1	17000	17000.00
7. Microscope	1	60000	60000.00

8. Auto clave	1	20000	20000.00
9. Hot air oven	1	7000	7000.00
Total			Rs. 3,66,000.00

Fixed expenditure

Rent for building = Rs.36,000.00

Depriciation @ 16% = Rs.58,560.00

Total = Rs.94,560.00

Total production cost (virus multiplication & purification alone) = 1206774.40

C. Total Virus production cost = larval production cost + virus production cost

= 2048062.60 + 1206774.40

= 3554837.00

Total fixed cost = 5,69,500 + 3,66,000

= 9,35,000

Total variable cost = 1920942.60 + 1112214.40

= 3033157.00

Annual fixed cost = 1,27,120 + 94,560

= 2,21,680

Amount of virus produced

Sale price = Rs.200/100 LE

Total returns = 3235579 x 200/100

= Rs.6471158

Total production cost = 3554837.00

Total profit/year = 9387479.00

Benefit cost ratio = Total return / total cost

= 6471158/3554837

= 1.82

Price / LE = Rs.2/-

Variable cost / LE = 3033157/3235579

= 0.94

Break even point =

Annual fixed cost/(price per unit - variable cost/unit)

= 221680/(2-0.94)

= 209132.08 LE

5. Practical Session

1. Based on the cost estimates suggest if the project will be viable.

2. Prepare a project for NPV production with a daily target of 25000 LE.