DEPARTMENT OF PLANT PROTECTION

M.Sc. Ag. (Entomology) CBCS II Semester

Core Course- III: Biological Control of Crop Pests and Weeds

Basic Facilities Required For Biocontrol Laboratory

Insectary : The place where insects are housed or propagated is called as Insectary. The basic insectary facilities and equipment's are as follows :

A. Personnel :-

- The persons concerned should have interest , curiosity and enjoyment in working with insects**.**
- Formal training in entomology is desirable.
- Faithful performance of the personnel

B. Location of insectary and biocontrol laboratory :-

- Temperate or cool climate is preferable
- Easy access with roads, approaches and trading places
- Should be away from the vicinity of agricultural areas
- Avoid urban or industrial atmospheric contamination.
- Site of the insectary should be leveled.
- The insectary and lab should be arranged in East West direction to have maximum natural lighting
- The vicinity should include plant species which serve as host for the phytophagous insects propagated in the insectary.

C. Building facilities :

- The insectary quarantine area should be provided
- The corridors should be atleast 4 ft wide and the door ways atleast 3 ft wide for easy movements of racks, trays etc.,
- The ceiling height recommended is 7 ft. This facilitates easy collection of insects from the ceiling and this will also reduce the cost of air condition.
- The dust control in the floor is important
- The storage facilities for equipments and samples should be provided in the building.
- The paints used should be glossy and moisture resistant. The paints should be tested for their toxicity to beneficial insects. Normally blue or green is preferred.
- Good artificial lighting is essential
- Light traps to attract and capture escaped natural enemies from the host.
- Provision of adequate water supply, drainage with light covers.
- Proper air-conditioning is necessary.

D. Safety :-

- Provision of fire prevention kit
- Avoid electric shock hazards
- Provision of first aid kit

E. Furnishing :-

- The work tables, storage cabinets , chairs, cages, collection, packing and shipping equipments are to be provided.

F. Green House

G. Fumigation facilities

H. Workshop to make cages and other equipments

I. Well equipped photographic studios

J. Bio-climatic cabinets

K. Rearing and handling facilities :

- In rearing room adequate facility for water supply and washing should be provided. Rearing room should be isolated and used only for insect culturing.
- Movement of personnel should be restricted to minimize contamination.
- The laboratory furniture like work table, rearing racks should have non-porous surface to enable the use of disinfectants .
- Various cages and containers have been developed for insect rearing . If wooden material is used it should have a lamination to avoid the growth of fungus.

L. Disinfectants:

Many disinfectants are available for using in the rearing house. The purpose is to keep all the rearing materials and the room free from contamination of disease producing microorganisms. Formalin, sodium hypochlorite are used as disinfectants. .

A. Mass Culturing of Rice Moth – *Corcyra cephalonica*

Materials required for *Corcyra* rearing

1. Broken cumbu grains
2. Mosquito net (6'x6'x6')
3. Plastic basins (30 cm dia)
4. Specimen tubes glass (15 x 2.5 cm)
5. Khada cloth , Aspirator (TNAU Model)
6. Yeast tablets
7. Rubber bands and twines
8. Moth's scale separator
9. Groundnut kernel, sieves and filters (Plastic)
10. Sulphur- Wettable powder, Streptomycin sulphate (0.05 %)
11. Home milling machine, Vacuum pump
12. Exhaust fan /Ceiling fan
13. Measuring cylinder (10,50, 100 ml)
14. G. I. Mating drum (25 x 25 cm)
15. Formaldehyde 40 % , enamel tray , hand sprayer and hand atomizer
16. Blotting paper, sheets
17. Honey
18. Camel hair brush
19. Vitamin – E capsule
20. Shoe brush
21. Absorbent cotton

Steps for culturing *Corcyra*

1. Preparation of medium: The rice moth, ***Corcyra cepahlonica*** is cultured in broken grains of pearl millet. Heat sterilized broken grains is taken at the rate of 2.5 kg per plastic basin to which groundnut kernel powder is added @ 100 gm /basin and yeast powder @ 5g/basin. To prevent bacterial infection in the food medium streptomycin sulphate @ 0.05 % spray is given at 10—20 ml/basin using a hand-operated sprayer or atomizer. Sulphur WP was added @ 5 g/basin to prevent storage mite.

2. Inoculation of Corcyra eggs : Nucleus culture of ***Corcyra*** eggs were added @ 0.5 CC / basin containing 2.5 kg of grain medium. After uniform mixing of the contents of the basin it was covered with khada cloth and secured by twine. The young ***Corcyra*** larva that hatch out from the egg in 3-4 days feeds on the medium by constructing webs. The adult ***Corcyra*** starts emerging from the medium from 30-35 days onwards and continues to emerge upto 90 days after inoculation of the eggs due to staggered development of larvae in the medium.

3. Collection of moths for mating : The emerged adult ***Corcyra*** moth rest on the inner surface of the cloth cover. They are to be collected in the morning hours using glass specimen tubes (15x 2.5 cm) or a specially designed modified vacuum aspirator (TNAU, model) . The moth collection is effectively done by keeping the basin inside a mosquito-net so that the escape of the moth is prevented. The adult moths are transferred to a

specially designed mating drum made of G.I sheets with wire mesh at the bottom. Adult moths are provided with honey solution (50 %) added with vitamin E (1 capsule per 20 ml of 50% honey) to boost the vigour of the adult and to get higher quantity of healthy eggs. The adult food is given by dipping cotton swab and allowed to hang inside the drum with a thread. Daily fresh moths are collected and allowed into fresh mating drum which is cleaned and dried under sun.

4. Collection of *Corcyra* eggs : *Corcyra* eggs are loosely laid and they are collected through the wire mesh at the bottom on a receiving container with funnel setup on an enamel tray. Eggs are to be collected daily and continuously for 4 days from each drum.. On the fifth day it is to be vacated and cleaned. A sheet of blotting paper is spread on the tray or in the funnel set up. It retains most of the moths scales and body fragments while the eggs were easily rolled out during cleaning. The eggs are cleaned and separated from the moth's scales by using a new gadget namely ***Corcyra*** moth scales and egg separator developed by TNAU.

5. Cleaning of eggs: The eggs thus obtained are further cleaned with the help of plastic sieves of different meshes . One CC of ***Corcyra*** eggs contains approximately 18,000 eggs . About 100 pairs of ***Corcyra*** moth (50 % female) produce 1.5 cc of eggs during their egg laying period of 4 days. From each ***Corcyra*** rearing basin an average of 2,500 moths emerge. Hence from each basin 18-20 CC of eggs are obtained during the period of 90 days . After 90 days the contents of the basin are discarded and the basins are cleaned, washed, disinfected with 2 % formalin solution and dried thoroughly before reusing.

Problems encountered in *Corcyra* Culturing

1. Redflour beetle : ***Tribolium castaneum***

Management :

- Get cumbu grains free from stored grains pest
- Sterilize grains at 100 ^{0} C for one hour using hot-air oven
- Place a thick paper (file thickness) 12x 12cm and daily after collection of moths remove the paper gently and tap so that the adult beetles which congregate at the bottom surface can be easily killed by transferring them in a small trough containing water mixed with sticky substance.
- Set up a 4 watts UV lamp during night hours over a yellow pan with water. UV lamp attracts the beetles and destroys them regularly.
- Keep trays/basins at random containing 250 g of wheat flour with 5 % brewer's yeast to act as flour trap @ 1 trap for every 100 rearing trays.
- See the flour is cleaned alternate days and destroy the grubs and beetles attracted towards it.
- Avoid using food grains which have more of bran and husk.

2. ***Bracon hebetor*** : Larval parasitoid –(parasitises ***Corcyra*** larvae)

Management

- Cover windows and all other openings with wire mesh sieve

- If khada cloths were used to cover the trays, frequently change the older clothes which are with small holes.
- Setup light trap with 60 W bulb over a yellow pan with water. Switch on the light during night hours to attract and kill the parasitoid. In case of severe incidence, spray 0.1 % malathion over the trays, furnitures, racks and side walls inside the laboratory.
- Destroy the culture medium immediately whenever the severe incidence is noticed.

3. **Mites** :- ***Pyemotes ventricosus***

Mites suck the sap from ***Corcyra*** eggs laid on the mating cages, spreading fast in the rearing cage.

Management

Mix 5 g wettable sulphur per basin regularly as a prophylactic measure. Dust sulphur over the trays , furnitures, racks if the incidence is severe. The laboratory should be free from dust and scales

4. Microbial pathogens

Bacterial , fungal and NPV are the common microbial pathogens infecting ***Corcyra.*** Treating the culture trays with 0.05 % streptomycin sulphate helps to check bacterial contamination. Viral infected trays should be rejected immediately to check the spread.

5. Scales

Accumulation of moths scales leads to breathing problems to persons who are engaged in rearing. It also causes allergic reaction on eyes and skin when exposed repeatedly.

Management

- Wearing of face mask
- Provision of sufficient numbers of exhaust fan in the laboratory

B. Mass Culturing of *Trichogramma* Spp.

Materials required for *Trichogramma* rearing

1. Nucleus parasitised egg cards
2. Ceiling fan
3. Fresh ***Corcyra*** eggs cards
4. Polythene bags
5. Drawing boards (Charts)
6. Gum, brush, sieves
7. UV lamp
8. Honey
9. Air cooler
10. Gemclips
11. Refrigerator
12. Stapler and pins
13. Dissection microscope
14. Glass plate and
15. Hand lens.

Preparation of egg cards : The ***Corcyra*** eggs collected from the mating drum are cleaned and finally taken in glass petri dishes (15-20 cm dia) . The eggs were sterilized by exposing to UV light (15 W) in a closed chamber for ½ hr duration., so that the embryo may be killed without damaging the egg contents. The UV sterilized eggs were sprinkled on drawing board cards (30 x 20 cm) smeared with thin layer of diluted gum, at 6.0 CC of egg/ card. The card was already drawn with lines to separate 30 rectangles (7 x 2 cm) to accommodate 6.0 CC eggs . The smaller cards cut along the line were used in the field.

Inoculation of parasitoids : The cards pasted with ***Corcyra*** eggs were dried under fan and taken into polythene bags (45x 30- cm) containing nucleus parasitised cards at 6 : 1 (fresh eggs to parasitised eggs) for exposure. The parasitoids emerging from the nucleus parasiotid eggs start parasitising the fresh **Corcyra** eggs. After 2 days exposure the cards were kept for another 1-2 days , during which the parasitised cards were then stored in refrigeration at 10^{0} C for 21 days. The parasitioids under normal room temperature emerge in 7 days where as they emerge in 2 days after removing from storage for field use. Accordingly the cards were taken to the field in time.

Field release : The parasitoids were released in the field as parasitised cards, atleast a day before emergence . It was pinned or stapled or tied on the ventral side of the leaves in the middle region of the plant. The small rectangular cards were used in the field @ 5 cards /acre for sugar cane and @ 12 cards / acre for cotton. The dose being 1.0 CC and 2.5 CC /acre respectively. The cards were tied in different spots avoiding border rows . For sugarcane 6 releases are to be made at fortnight intervals commencing from 3- 3 ½ months onwards and for cotton crop the parasitoids are released soon after noticing the adult moths of bollworms fling in the field or trapped in pheromone trap or light traps. Two or three releases at fortnightly intervals are needed for cotton.

C. Mass Culturing Of Granulosis Virus (GV) of Sugarcane Shoot Borer , *Chilo Infuscatellus*

Sugarcane is an important commercial crop in India . It is cultivated under diverse agro-climatic conditions . The crop is damaged by 5 important moth borers . Among these borers the shoot borer, ***Chilo infuscatellus*** is an important one and is widely distributed in all cane growing areas in India. The infestation reduces cane production, Parthasarathy ***et al*** (1953) observed a loss in weight of the infested clumps varying from 15.8 to 41.7 % A decrease in yield by 10 t /ha has been calculated by Ramachandrachari (1959) Avasthy (1968) correlated the incidence of shoot borer with cane yield and found 3.5 % loss in yield at every 5 % increase in borer infestation. High temperature , low humidity and scanty rainfall and poor irrigation facilitate high incidence of shoot borer.

Identification of shoot borer

The shoot borer infests the crop at the shoot stage killing the young plants till the 4 th month of the crop. The larva bores into the central young shoots and feeding results in drying up of central shoot called “dead-heart”. Fully grown larvae measures 20-25 mm in length with dark brown head. Body is cylindrical and exhibits a dirty white colour with 5 longitudinal violet stripes.

Granulosis Virus (GV) of *Chilo infuscatellus*

GV infected sugarcane shoot borer ***C. infuscatellus*** was reported from India in 1979. This virus is found widely distributed in the cane growing tracts of Tamil Nadu. The virus infects the host larvae all through the year. The GV persist in the ecosystem and cause high mortality under favourable conditions. The GV is highly pathogenic and all the larval instars are susceptible to infection. The virus application reduces the dead heart and enhances the cane yield. The virus is also able to tolerate high temperature and it can be stored for 3 or more years without apparent loss in infectivity The virus is found harmless to predators, parasites, pollinators and economic insects. In the sugarcane ecosystem especially honey bees and silkworms .

The GV application multiplies mainly in the cell nucleus and cytoplasm of fat body but in certain cases in epidermis and tracheal matrix also. The inclusion bodies (IB’s) are ellipsoidal and enclose only one or occasionally two virions. The IB’S are also known as capsule/granule. The virions are rod shaped, surrounded by two membranes, outer membrane and the envelope.

Mass production of GV

Materials required :

Plastic containers, pruning knife, hand made filter paper, conical flasks Plastic basins muslin cloth, rectified spirit , teepol / sandovit. GV for shoot borer can be multiplied only from the field collected larvae because sofar no artificial or semi-synthetic diet has been developed to culture this under laboratory conditions Collection of shoot borer affected plants can be done in 40 –90 days old sugarcane crop. The larvae will be collected from plants showing fresh dead hearts. The collected larvae will be

placed in plastic containers with shoot bits and reared.

Propagation of Virus :-

1. Third or fourth instar field collected larvae are suitable for multiplication of the virus.
2. Prepare a virus suspension containing 10^7 - 10^8 inclusion bodies /ml (IB's/ml) of water.
3. Feed the larvae with a drop of virus suspension through a pin head or dip the larvae head into the virus suspension.
4. Rear the virus infested larvae on sugarcane shoot bits @ 3-5 /plastic container (7.7 x 6.4 cm) . The plastic boxes are provided with filter paper for absorption of excess moisture and three pieces of sugarcane shoot bits split open at one end . The shoot bits and filter paper are changed on alternate days.
5. The infected larvae begin to show symptoms in about 5-8 days . The main symptoms are loss of appetite, sluggishness and appearance of milky white colour on the ventral surface.
6. The larvae start dying from the 8^{th} day upto 22 days
7. Collect the dead larvae due to virus infection in distilled water and store them in refrigerator at $5\ ^0$C.

Purification :

i. Macerate the infected larvae in distilled water .
ii. Filter through muslin cloth and remove the grass debris
iii. Centrifuge at 500 rpm for 2 minutes and discard the sediment
iv. Centrifuge the supernatant to remove the lipid soluble material and other contaminants at 10,000 rpm for 30 minutes
v. Discard the supernatant and obtain the suspension. This will be in pellet form. Resuspend the pellet in small volume of distilled water and this gives fairly pure preparation of virus.

Storage :

Store the virus suspended preferably in distilled water and in amber coloured bottles in a cool dark place . If possible the virus can be stored in a refrigerator at $4\text{-}5^0$ C.

Field use : Dosage : 100 LE/acre = 300 virosed larvae / acre / spray
Number of rounds and time interval : Two rounds i.e., on 35 and 50 days after planting
Spray fluid required : 200 l of water / acre
Sticker : Teepol / sandovit 0.5 ml/l
High volume sprayer Knapsack / Rocker sprayer is preferred for spraying

Standardization of Virus :

The potency of a microbial preparation is generally assessed by counting the particles microscopically/ electronically. In all the methods , the organism should be diluted to a suitable level. A sample of the stock solution is generally diluted serially so that a dilution factor of 10 or its multiplication are obtained.

Counts of Particles :

GV particles are counted using Petrof- Hauser and Helser counting chambers (depth 0.02 mm).

i. The counting chamber should be clean and dry.
ii. Introduce only just sufficient quantity of the liquid to fill up the calibrated area in the counting chamber
iii. Allow the granulosis virus particles to settle down for 5 minutes after adjusting the magnification
iv. Count the particles in about 25 of the 1/400 sq.mm at random. Take care to avoid duplication of counts of particles on the lines of calibrations
v. Calculate the number of particles per ml of original stock solution as per the example given below.

If number of GV inclusion bodies counted from 25 of 1/400 sq. mm squares of A Petrof – Hauser and Helser counting chambers is 318.

The dilution made is 100 times.

Calculate IB's /ml of stock solution
Area of 1 small square = $1/400\ mm^2$
Depth factor = 0.02mm

Volume of the fluid
Standing of each small sq = $1/400 \times 1/50\ mm^2$
$= 1/20000\ mm^3$
The number of IB 's / cubic mm . = 318/ 25 x 20, 000

The number of IB /ml of diluted sol. -= 318/25 x 20,000 x 1000

$= 12.4 \times 20{,}000 \times 1000$
$= 24.8 \times 10^7$
$= 2.48 \times 10^8$

The number of IB's /ml of original stock solution = $2.48 \times 10^8 \times 100$

2.48×10^{10} IB's /ml

D. Mass Culturing of Fungal Pathogen

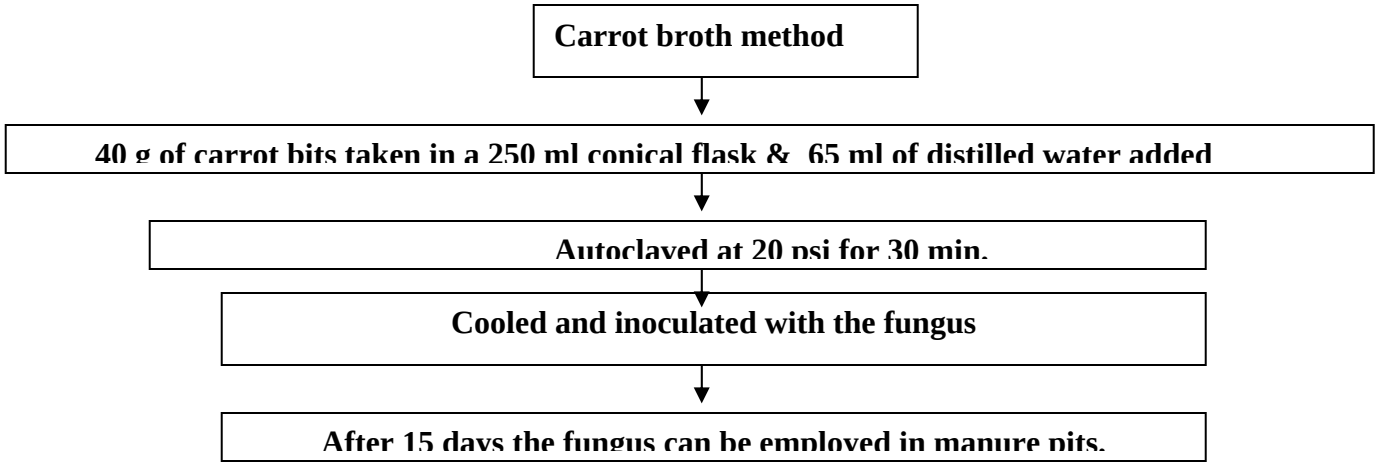
1. Green Muscardine fungus – *Metarrhizium ansopliae*

The Rhinoceros beetle , ***Oryctus rhinoceros*** is one of the serious pests of coconut , which has wide distribution and persistent occurrence in all coconut growing areas in India. This fungus infects the grubs and adults of Rhinoceros beetle . The adult beetles cause severe damage to coconut palms by feeding on tender fronds and crown resulting in the stunting of trees. The damage due to spathe results in loss of nuts. Young seedlings are sometimes killed outright. Since the insect breeds in the farm yard manure and fallen coconut trees, the control measures have to be directed at the breeding site as well as on the trees. The control measures adopted against grubs and adults in coconut grooves are costly, tedious and have to be repeated. Hence, an easy alternate method utilising a safe and specific fungus namely, ***Metarhizium anisopliae*** (Deuteromycetes : Moniliales) for the management of coconut rhinoceros beetle is aimed .

Symptoms :

1. Body is mummified , shrunken and becomes hard and dry
2. Body is covered with dark olive green powdery mass with hardy spores

1. Mass multiplication of GM Fungus



a) Methods of inoculation and optimum dosage of inoculum time required for penetration and symptoms of infection.

Among the methods tried for inoculation, smearing or spraying of spores of the fungal pathogen on grubs produced 100 per cent mortality due to mycosis. All the instars of grubs of ***O.rhinoceros*** are susceptible to fungal infection.

The first instar grubs are, however, more susceptible. Irrespective of the instars, the loss in weight due to infection by the fungal pathogen increases as the days after incubation progressed. The time required for the penetration of the fungal pathogen in the final instar host grub is 96 hours. The LC_{50} of the fungal pathogen in the final instar

grub of ***O.rhinoceros*** is 416 spores, and LT_{50} at 10^6 spores per g of farm yard manure is 21.22.days . The effective inouculum dose for field trial is 34.4×10^4 spores per g of farm yard manure. Two to three inoculations of the fungal pathogen at monthly intervals are needed for the manifestation of mortality of the grubs due to mycosis.

At the dosage of 8×10^4 spores per g of farm yard manure, the infection prolonged for 49 days culminating in 40 per cent mortality of grubs, thereby enabling the pathogen to persist over a long time. Mycosis of the egg is reported for the first time . Mycosed eggs were olive green , flat, shriveled, scaly and invisible. In mycosed grubs , loss of appetite is often an early behavioural symptom. The infected grubs are inactive and sluggish with decreased irritability to external stimuli. External signs comprise of colour changes, the earliest one observed is greyish white spots. The cadavers are soft and flat with shrunken body wall in certain cases. A profuse live green fungal mat and spores cover the entire body surface except the head capsule. Later, in certain individuals . the body either shrinks and hardens to a small olive green lump or mummified. In other cases, the body becomes brittle and breaks into small pieces even at the slightest disturbance. In adult beetles, the initial visible symptom is the appearance of olive green patch in the pygidial region. The connecting muscles of the neck and legs are the next to show the fungal growth. Due to the destruction of these muscles the head and legs are severed from the body. Slowly the fungus can be seen to spread on the entire surface with profuse growth at the inter segmental portion. The dead beetles are light in weight.

b) Influence of abiotic and biotic factors of the environments on the pathogenicity of fungus

The mortality of the grubs, either inoculated with the fungal pathogen or introduced in the fungus – inoculated farm yard manure takes place when the moisture regimes of the farm yard manure are from 10-70 per cent ; however , the time taken for 100 per cent mortality varies. The temperature of fresh dung declines from 23.6 to 24^o C in a period of one year. At 37.4^o C temperature only mycosis starts and develops to an extent of 40 per cent. It is 100 per cent when the temperature decreases to 29^0 C and below. The spores of the fungal pathogen added to the farm yard manure survive in the medium only upto two months irrespective of the dosage tried. Addition of host grubs to farm yard manure enable the spores to survive up to one year of observation.

The recovery of spores of the fungal pathogen layered over the surface of the farm yard manure column is maximum from the top most 2.5 cm depth. The fungal spores were found absorbed in the farm yard manure substrata to an extent of 25.5 per cent. Carbaryl and hexa-chloro-cyclohexane inhibit the germination of the viable spores of the fungal pathogen to an extent of 86 and 82 per cent respectively and thus are found incompatible . However, aldrin, DDT, dieldrin, lindane and toxaphene inhibit the spore germination tro a very low extent ranging from 0.38 – 8.89 per cent only. The streptomycetes and the bacteria of the farm yard manure cause 43.60 and 14.67 per cent inhibition of ***M. anisopliae*** respectively.

c) Delimitation of the fungus and determination of optimum conditions for its culturing and storage.

Among the different media , carrot agar medium is the most suitable for culturing of the fungal pathogen. Maximum bio-mass, radial growth and spore production are obtained in this medium at the incubation periods of 25- 30 days. Temperature regimes of 30^oC and 25 ^{o}C are ideal for the effective culturing of the fungal pathogen. The thermal death point is 56 ^{o} C. The pH levels of 6.5 and 7.0 of the medium are

suitable for the maximum production of biomass, radial growth and spores of the fungal pathogen. The sporulation and germination of spores of the fungal pathogen commence at 92 per cent RH and increases as the RH increases to 100 per cent. However, the maximum germination is only 60 per cent at 100 per cent RH. The fungus can be sub-cultured in the artificial medium successively four times beyond which the growth, sporulation and mortality of the treated grubs are affected. Hence after every 4 sub-culturing attenuated fungal spores and fungus have to be passed through the host grubs which restore the virulence in them. The hot insect body is the best substrate for storing the spores of the fungal pathogen. The different storage containers tried namely, glass conical flasks, butter paper bags and gelatin capsules had no influence on viability of spores.

Test verification of the dosage level of inoculum in the field :

The effective inoculum dose of the fungal spores fixed earlier was test verified in six randomised , replicated trials with 100 grubs per replication by applying the spore inoculum to the farm yard manure. The results clearly indicated the effectiveness of M.anisopliae in causing 100 per cent mortality of the grubs of ***O.rhinoceros*** at 34.4×10^4 spores per g of farm yard manure.

The studies made showed that M. anisopliae can be utilised for pest management programme for ***O.rhinoceros.***

2. White muscardine fungus : *Beaveria bassaiana*

This fungus infects a wide group of insects including leafhoppers, plant hoppers and caterpillars

Mass multiplication

The fungus can be mass multiplied in carrot broth medium as described under green muscardine fungus. This fungus can be mass cultured in nutrient "Agar Medium" , nutrient broth " Potato dextrose Agar Medium " or an other fungal culture medium.

3. Verticillium fungus :

*Vereticillium lecanii (*Zimmerman*)* Vieges (Moniliales , Moniliacae*)*

V. lecannii is the most common entomofungal pathogen in mandarin growing areas of Karnataka, Kerala and Tamil Nadu. It is a key mortality factor not only for ***Coccus viridis*** but also for ***Saissettia coffeae.***

Mass production

The pathogen can be cultured on crushed moist sorghum,/rice/maize grains or carrot broth. The pure culture of the fungus is maintained on potato dextrose agar medium/ standards maltose agar + 1 % yeast slants in tubes.

Two methods of production of this fungus are followed, one crushed grains and the and the other on coconut water.

Ist Method :

After 10 days of suitable growth and sporulation at 26 ^{0}C the fungus is suspended in sterile water + 0.1 % Tween – 80 under aseptic conditions and the conidial load is counted in the suspension (using modified Neauberg's chamber) and adjusted to 10 ^{6} / ml by adding sterile distilled water .

▼

Grains are crushed in mixer for 30 seconds to make small pieces , which can pass through 12 mesh sieve.

▼

200 gms crushed grains are placed in 0.2 mm thick 250 gms capacity high density polythene bags and 190 ml water is added. The bags are heat sealed and autoclaved at 120 ^{0} C for 45 minutes.

▼

After cooling 5 ml fungus spore suspension is added by cutting open one corner of bag under aseptic conditions (in a laminar flow chamber) . The opened area is released.

▼

The bags are incubated at 26 ^{0} C . for 20 days.

The fungal mass along with grain carrier is harvested and dried at 40 ^{0} C for 24 h and ground in a mixer to get a fine powder.

II Method :

Coconut water (40ml) obtained in 375 ml side –wise flat bottles plugged with cotton wool are sterilized in batches of 9-10 bottles in 12 litre pressure cooker for 15 minutes. The bottles inoculated with 1 ml spore suspension with the help of a previously boiled (for 30 min) injection syringe. Before inserting the needle within the sterile bottles for drawing spore suspension for inoculation. The needle of the syringe and the collar region of the bottles are flamed (over candle or glass burner or any lamp). The bottles are incubated resting on flat surface for 20 days or till the surface of the medium is fully covered by the olive green sporulated fungus. The whole culture is crushed thoroughly in a ordinary mixer and used in the field. From a single average sized coconut, 5 to 6 bottles of cultures can be made.

FIELD APPLICATION

The fungus is applied in water suspension. The required quantity of spores are mixed with 0.05% sandovit.

E. Technique For Mass Production of *Spodoptera litura* , Nuclear Poly Hedrosis Virus (Sl NPV)

Identification of larvae :

The tobacco cutworm , ***Spodoptera litura*** attacks cultivated crops like cotton, tobacco, groundnut, tomato, chillies, sunflower and castor. Young larva feeds on the leaves by scraping and causes skeletonisation of leaves. Grownup larvae defoliates the leaves and also damages fruiting parts especially during the day time. The larva is pale greenish with dark markings. It damages the leaves by reducing them to papery structures initially which later becomes reddish brown patches. Then riddled with large irregular holes.

To control the ***Spodoptera litura*** in cotton the NPV of ***S. litura*** is used. The NPV is host specific and safe for predators and parasitoids.

Mass culturing of *S. litura* larva

Materials required :

- Aluminium tray (50x 50x 4 cm)
- Working table
- Plastic tub (38 cm dia x 21 cm ht)
- Beaker 250 ml
- Measuring cylinder
- Scissors
- Forceps
- Water pans
- Honey
- Virtamin E
- Castor leaves
- Black cloth
- Cotton wool
- Neerium leaves
- Black chart paper
- Cello tape
- Rubber band

Procedure

- Collect the adult moths from light traps . Allow 5 pairs inside a plastic container close with muslin cloth . Provide sugar 10 % solution in a cotton swab as adult feed. . Provide a castor leaf as an egg laying substrate.

- Collect the egg masses from the fields every day and place it over another leaf in a plastic bucket.

- For 3 days larvae will feed by scraping the leaves.

- Insert 4-5 fresh castor leaves along with cotton wool in 150 ml conical flask having water and then place a egg mass on leaves and keep the conical flask inside the plastic jar (16 cm dia x 20 cm ht) covering its mouth with black cloth.

- Allow 300 first instar larvae in a conical flask containing castor leaves . If it is second instar allow 200 larvae and if third instar 100 larvae are allowed

- Place a newspaper in the bottom of the plastic tub (38 cm dia x 21 cm ht) , keep 10-15 castor leaves with wet cotton , allow 50 fourth instar larvae and cover with black cloth. Do this till pre – pupal stage.

- Spread the heat sterilized sand (at 100 ^{0} C temperature for 30 minutes in hot air-oven in a plastic jar upto 2.5 cm height.

- Allow 25 pre –pupae for pupation . For pupation provide sand inside the bucket , when the larvae were 12-15 days old . Within 3-5 days adult will emerge

- Dissolve 100 ml honey in 300 ml distilled water and add 400 mg vitamin E capsule and provide diet for adult.

- Insert neerium leaves along with cotton wool in conical flask having water and keep it in a plastic jar, allow 10 pairs of adults and cover with black cloth, provide diet through cotton swabs.

- Cut neerium leaves containing the egg masses , keep on fresh castor leaves inserted in a conical flask for another cycle.

Spodoptera litura NPV

NPV : - In the initial stage of infection, the larvae show symptoms like cessation of feeding , sluggishness and colour change. In advanced stage, they become flaccid, white fluid exudes from the ruptured skin and larva dies. Some of the diseased larvae found hanging downwards from the plants.

The virus consists of polyhedral inclusion bodies inside which the virions or virus rods are embedded. Soon after the entry of the larval gut , the liberated virions pass through midgut cells, enters the haemocoel and infect the nuclei of cells of different parts i.e., fat bodies, brain, gonads etc.,

Mass culturing of S. litura NPV
Materials required :
- Plastic tub
- Conical flask
- Nucleus of Sl NPV solution

- Distilled water
- Disinfectant
- Brush
- Black cloth
- Blender
- Centrifuge
- Beaker
- Funnel
- Sieves
- Filter paper
- Castor leaves
- Wash bottle
- Cotton wool
- Compound microscope
- Haemocytomter
- Staining chemicals (Eosine)
- Sticker

Procedure :-

- Collect 3 rd instar larvae
- Prepare 1x 10^{8} POB /ml NPV suspension or dilute solution in plastic tub and add sticking agent. Dry the leaves in shade
- Provide this leaves to starved larvae
- Repeat twice after 24 hours , 4 days after inoculation , disease symptoms start to appear and larvae will die within a week
- Take 250 ml conical flask, add 150 ml distilled water, 100 diseased larvae in it and tighten the mouth with cotton and keep the flask for 15 days for putrefication
- Blend the solution for homogenization and filter through muslin cloth
- Centrifuge the filtrate for 5 minutes at 500 rpm
- Collect the supernatant and again centrifuge at 4000 rpm for 30 minutes
- Collect sediment with distilled water and keep in glass bottle
- Store in refrigerator until use
-

Field Use : Dosage : 450 LE /ha two to three times at 10-15 days interval. Use 0.05 % Teepol as the sticking agent . Use high volume sprayer and spray in the evening hours.

F. Technique For Mass Production of *Helicoverpa armigera* Poly hedrosis Virus , *(Ha NPV)*

Identification larva : The larva is green with dark brown grey lines and with dark and pale bands. It shows colour variation - greenish to brown colour . The larva feeds on the boll by thrusting their heads alone inside. Bolls show irregular , circular bore holes and presence of granular faecal pellets outside the bore-hole.

Mass culturing
Materials required :

-Plastic jars
-Plastic tubs
-Pencillin vials with cotton plugs
-Bhendi vegetables

-Black cloth,
-Khada cloth
-Saw dust
-0.025 % Sodium hypochlorite
-Chick pea / groundnut seedlings
-Bleaching powder
-Coffee powder
-Multivitamin syrup
-10 % sucrose
-Beaker 500 ml
-Measuring cylinder
-Egg laying chamber

Procedure :

1. Release 10 males and 5 females at 2: 1 ratio in plastic containers and cover with thin black cloth . (Female require multiple mating to lay fertile eggs) .
2. To induce the moths to lay more eggs multivitamin syrup 2 drops + 10 % sucrose is given through cotton swabs
3. Daily collect the egg cloth after 3 rd day of copulation . Provide 25- 28 ^{o} C , 80- 90 % R.H during egg laying. A female lays 300 –700 eggs
4. Sterilize the egg cloth in 0.025 % sodium hypochlorite for ten seconds and immediately dip the egg cloth in distilled water in 3 different buckets having distilled water one by one and then dry it in shade.
5. Raise chickpea or groundnut seedlings in a week interval and provide for feeding
6. Place newly hatched larvae on chickpea/groundnut seedlings along with egg cloth for one day or place 3-4 eggs in vials containing artificial diet
7. Pick young larvae and rear on bhendi vegetable individually in penicillin vials to avoid cannibalism.
8. Daily change diet till pre pupal stage
9. Collect pre –pupae and allow for pupation in plastic container having saw dust
10. Pupae sterilization is done with the help of coffee filter by dip method
11. Transfer the pupae inside the egg lying chamber by keeping them on a separate petri dish without lid.

Mass Production Of *Ha NPV*

Materials required

- Blender
- Nucleus Ha- NPVsolution
- Distilled water
- Centrifuge
- Beaker
- Measuring cylinder
- Bengal gram / Bhendi
- Haemocytometer
- Sticker

Procedure :

- Starve the 3 rd instar larvae for 3-5 hours

- Prepare bhendi vegetable for larval feed
- Pour 2-3 drops of Ha NPV suspension in the pencillin vial and feed to the larvae
- Repeat the inoculation process twice after 24 hours
- Within 4 days disease symptoms appear and larvae die within a week
- Take the diseased larvae in distilled water
- Keep the flask for 15 days for putrefaction. Filter the solution through muslin cloth
- Centrifuge the filtrate for 5 minutes at 500 rpm
- Collect the supernatant and again centrifuge for 30 minutes at 4000 rpm
- Collect the sediment with distilled water and keep it in glass container
- Store the pure Ha- NPV in refrigerator until use

Field Use : Dosage : 450 LE / ha two to three times at 10 –15 days interval with 0.05 % teepol as sticking agent .

Standardization Of NPV

***Spodoptera litura* - NPV**

Haemocytometer is used for counting POB's of ***Spodoptera litura*** , NPV .

Virus suspension is preaprd by adding 0.1 ml in 9.9 ml of water , which is 100 times dilution.

One ml of diluted viral suspension is to be released in the "H" shaped constriction without over flowing. Before letting the solution the, "H " shaped constriction must be covered with cover slip. Then this should be focussed under microscope. The number of virus particles in each square is to be counted. The counting can be done in 25 small squares. There may be viral particles on the lines, the particle on top and left side can be taken for counting. Then the strength of the viral suspension is to be found using the formula

$$\frac{\text{No. of viral particles}}{\text{No. of squares}} \text{ X No of total sqares x dilution factor x depth factor x 1000}$$

Helicoverpa armigera :-

Counting of POB is done with the help of haemocytometer and a microscope . The concentration of POB is assessed as follows :

1) The POB in 80 randomly selected small sqares of 1/400 m^2 are counted.

2) If this number is 400 then POB/ml
= 400 / 80 x 400 x 10 x 1000 = 2×10^{7}

Where, 10 is the depth factor and 1000 is the factor for conversion /ml.

3) If the stock suspension is diluted prior to counting , the dilution factor should be taken into account, while calculating the concentration of POB of stock suspension.

Problem :-
No. of POB/s /25 squares = 100
Therefore No. of POB's /ml = x/25 x 400 x 100 x 10 x 1000
= 4x / 25 x 10^{8}
Calculation

(Dx X) / Nx K
D – Dilution factor
X _ Total number of polyhedral counted
N_ No. of small squares counted
_ 16x 5 = 80 x 2 = 160
K- volume above one small square in cm^{3}

= Area x depth
= (1/25 x 1/16) x 0.1 mm = 1/400 x 0.1

= 0.0025 mm^{2} x 0.1

= 0.0025 mm^{3}
= 2.5 x 10 – 7 cm^{3}

Example :
D= 1000, x = 535. N= 160 , K= 2.5 x 10^{7}

$$= \frac{1000 \times 535}{160 \times 2.5 \times 10^{7}}$$

= 1.34 x 10 10 PIB /ml of undiluted sample

$$= \frac{4x\ 100}{25} \times 10^{8}$$

= 16 x 10^{8}
= 1.6 x 10^{9}
but 1 LE = 6 x 10^{9}
so number of LE in 1.6 x 10^{9} = $\frac{1.6 \times 10^{9}}{6 \times 10^{9}}$

= 0.266 LE

To make 0.266 LE to 1 LE we have to add 0.266 LE = 1 ml of suspension

Therefore 1 LE = 3.7 ml

For 100 LE = 3.7 x 100

= 370 ml of viral suspension needed.

G. Mass Culturing of Larval Parasitoid – *Bracon brevicornis*

Bracon brevicornis ***(*** Braconidae : Hymenoptera)

It is a larval parasitoid on ***Opisina arenosella*** and ***Helicoverpa armigera*** . For rearing Bracon 40 days old ***Corcyra*** larvae are used. ***Bracon kirkpatrichi*** is the parasitoid on spotted bollworm in cotton. ***Bracon greni*** is parasitised on 2 nd and 4 th instar of pink bollworm.

Rearing of *B.brevicornis*

Two mated female ***Bracon*** aduts are released to each ***Corcyra*** larva through the narrow end of the chimney which is closed with anther muslin cloth. After 3-4 hours the parasitised larvae are transferred to container having folder paper. The female ***Bracon*** lays about 8- 12 eggs on the ventral side of the larvae and egg hatches about 28-30 hours. The larval period lasts for 3-4 days, pupal period lasts for 2- 8 days. Life cycle is completed in 7-9 days. ***B. brevicornis*** is released @ 10 adults / tree.

H. Mass Culturing of *Chrysoperla*

Rearing of Chrysopa

Larval rearing : It is to be done in GI round basins (28 cm dia) at 250 larvae /basin covered with khada cloth . The eggs of ***Corcyra cephlonica*** are given as feeding material for the larvae in the laboratory. For rearing 500 Chrysoperla larvae the total quantity of ***Corcyra*** eggs required is 25 CC at the rate of 5.0 CC / feeding for 5 feedings in alternate days. The **Chrysoperla** larvae pupated into round white coloured silken cocoon in 10 days. The cocoons are collected with fine brush and transferred into a one litre plastic containers with wire mesh window for emergence of adults. From the cocoons, pale green colored adults with transparent lace like wings emerge in 9-10 days.

Adult rearing : The adults are collected daily and transferred to pneumatic trough (30 cm x 12 cm) or GI round troughs. Before allowing the adults , the rearing troughs are wrapped inside with brown sheet which act as egg receiving card. About 250 adults (60 % female) 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) dipped in water are kept. Besides an artificial protein rich diet is provided in semi solid paste form in three spots on the cloth outside. This diet consists of 1 part of yeast powder, one part of fructose, one part of honey and one part of protein. Water is mixed to make it as a paste . The adults feed the food and lay eggs in the brown sheet. The adults are collected daily and allowed into fresh rearing troughs with fresh food. From the old troughs, the brown sheets along with ***Chrysoperla*** eggs are removed.

Storage and Destalking of eggs : The brown paper sheets with eggs are stored at 10^{0} 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 sheet will be kept at room temperature for a day. The eggs will turn into brown colour and hatch on the third day. The first instar larvae are either taken for culture tray for recycling or for field release.

Field release of *Chrysoperla* : The first instar larvae of *Chrysoperla* are released in cotton, groundnut fields at 50,000 to 1,00,000 /ha for 3-5 times at 10 days intervals to control aphids, white flies, ***Spodoptera***, ***Helicoverpa***, pink boll worms, thrips and mites. The larvae are taken in plastic containers containing a small quantity (1-2 CC) of ***Corcyra*** eggs and loose paper strips. The paper strips along with the larvae sticking on them were dropped in the field at random while walking across the fields.

Improving the Efficacy of Bio-Control Agents

The adults of any host or parasitoid is very important, since it is at the stage the eggs are laid and the generation is replenished. Adult should have a good reproductive capacity through out their life span whether it may be host insect or parasitoid or predator. Since their natural food source cannot be provided as such it is essential that an alternative source of adult food is provided to boost their vigour.

1**.** ***Corcyra cephalonica*** : This is one of the host suitable for ***Trichogramma*** and ***Chrysoperla* .**

Adult food : The adult moth needs to be provided with honey solution at a concentration of 50 % added with Vit E @ 1 capsule /20 ml of 50 % honey to boost the vigour of the adult and to get greater quantity of healthy eggs .

Method : The adult food is given by dipping cotton swab in it and allowing the swab to hang inside the oviposition drum with a twine thread.

Environment : The temperature and RH have influence on the egg laying by adults . The most optimum temperature and RH are 30 ^{o} C and 70 % respectively.

2. *Trichogramma sp.*

Adult food :- Normally ***Trichogramma*** adults are fed with honey in cotton swab which causes entangling of adults in the cotton lint. To avoid this a better adult feeding technique was developed.

Method : A thick mylar film sheet with holes of 1 cm apart in dotted lines are made with a sewing machine is cut into small bits (7 x 6 cm) . Honey solution is streaked on the smooth side with a camel brush . This sheet is folded and stapled in such a way that honey surface inside and the adults suck the honey through the holes from the eruptive surface.

Environment : A temperature range of 30 -32 ^{o} C and RH of 70 - 80 % is most suitable for breeding of ***Trichogramma*** .

3. Predator

Chrysoperla carnea :

The lab reared adult ***Chrysoperla*** is provided with a semi synthetic diet nutrient consisting of

Proteinex 1 part
Fructose 1 part
Honey 1 part
Yeast 1 part and a small quantity of water

Method : The nutrient mixture is applied as a streak on the georgette cloth of the adult oviposition cage . The adult feed from inside the cage sucking through the cloth. Water is also provided through soaked sponge placed over the same georgette cloth cover of the adult oviposition cage.

Environment : The optimum condition for the reproduction of ***Chrysoperla carnea*** are $20\ ^{0}$C temperature ad 80 % RH . Reproduction is maximum in a day length of 15- 17 hrs depending upon the ambient temperature.

Quality Control for Bio Control Agents

Quality control is a pre requisite for the ultimate success of bio-pesticides . The possible defective manufacture , contamination , poor storage conditions. Impurities , inefficient strains , improper formulation etc., will affect the efficiency of bio-pesticides. So the interest of the farmers is lost and the net result the technology development will be futile. Hence the adequate standards should be established to ensure the quality attributes of the bio –pesticides so that the interest of the framers are safegaurded. The following standards are adapted for the various bio-pesticides.

I Helicoverpa armigera - NPV - Aqueuous suspension

Tier I Test

Package Characteristics :

1. It should be packed primarily in Aluminium or low density polythene container . The cap should be sealed by blister packing .
2. The secondary packing should be either a paper board packing or simply the container itself.
3. The volume of suspension should be 1000 ml

Contents of pack and POB strength :

The POB should be suspended in sterile distilled water and it should be 3×10^{9} POB.Off odour of the content should be kept at the minimum level

Bio-efficacy :

The mean per cent mortality of second instar larvae of ***Helicoverpa armigera*** to a dose of 2×10^{6} POB /ml by a exposed leaf tip method , should produce a mortality ranging from 90 – 100 per cent for aqueous solution.

Tier II test :-

In addition to those envisaged in Tier I the following are contemplated ;

Physico – chemical characteristics

Sl.No.	**Characteristics**	**Requirement for ground spray grade**	**Cl.No. of IS : 6940 g 1973**
1	Ha NPV content as No. of POB /ml	Nominal value	-
2	Sieving requirement wet sieving (%)	95-99	11.1
3	Suspensibility after storage (%)	80	11.2
4	Wettability (Time in sec)	120	-
5	Acidity / Alkalinity	Near neutral	11.3

Methodology : As per Dulton (1967) WHO (1961) Shell (1957)
IS 6940 of 1973

Biological Properties : The results of bioefficacy list leaf disc method against 2 nd instar larvae to give a Lc of 1.5 – 2.0 POB/ mm ^{2}

Microbial purity

a) The product should be totally free from ***Nosema***

b) It should be free from other contaminants . The permissible limit for contaminant bacteria is 10 ^{7} /g of product . The procedure for counting is as per Bergy's manual of determinative bacteriology .

iv) **Safety** : The product should be safe to parasitoid, predator , silkworm, honey and other invertebrates. The size to be tested is 10 times higher than the field dose .

v) **Stability** : The shelf life of the product under normal warehouse conditions should be 18 months. The product should not cake or emulsify . The samples should be drawn quarterly intervals for conductivity bioefficacy . LC 50 values should not fall below 1.5.-2.0 POB/mm ^{2} by soybean leaf disc method.

vi) **Compatibility** :
The product should be compatible with insecticide like endosulfan, chlorpyriphos , phosalone

vii) **Phytotoxicity** : The formulated product should not be phytotoxic . Scoring method should be adopted to list the phytotoxicity.

II. *Spodoptera litura* - NPV

Tier I Test :-

1. Package characteristic: As in the Ha NPV
2.Weight of formulation : ‘’
3.Content of POB : ‘’
4.Bio-efficacy test : Type methodology as in ***Helicoverpa*** NPV . Instead of chickpea , castor leaves are used/. The test dose is 4×10^6 POB/ ml
5.The results of the bio- assay against 2 nd instar should give a mortality of 90-100 per cent in 5- 7 days at the test dose of 4×10^6 POB/ml .

Tier II Tests : As in case of Ha NPV WP formulation. The bio-efficacy test in Tier II should be conducted by castor ; leaf disc method against 2 nd instar. The Lc 50 should fall within a range of 3.0 – 4.0 POB/mm^2 .

iii. Trichogramma sp.

Tier I Test :

1.The total No. of eggs / CC for ***Corcyra*** by volume should range from 16,000 – 18,000

2. Sampling method to be adopted – Random Size of the card including area for label and other details =

20 x 30 cmm = 600 cm^2
No. of Grids = 30
Area of each grid = 7 x 2 cm
= 14 cm^2
Total No. of eggs / cm^2 to be accommodated = 96,000 – 1,08,000
Mean number of egg / cm^2 of the card in the grid area excluding area for labeling
= 200 – 250
Number of counts/ card of size 20 x 30 cm to be taken
No. of parasitised eggs = 12

- 3-4 days old parasitised egg card has to be selected for examination
- count the number of eggs and eggs parasitised in an area by 1 cm^2
- Per card of size 20 x 30 cm count randomly in 12 positions
- Repeat the process for three different cards of same age
- Express the per cent parasitisation . The result should fall in range of 85-90 per cent.

Per cent adult emergence :

- Cut egg card with a surface area of 1 cm^2 in the above
- Put the cards individually in 25 ml screw cap vials and label.
- Watch out for adult emergence
- Work out the per cent adult emergence from the parasitised eggs

Express the result as mean per cent parasite emergence . The result should fall in the range of 85 –90 per cent .

Tier II tests :

In addition Tier I test contemplated work out the following :

sex ratio : The sex ratio should be 1 :1 to 1 : 5 (male : female) . Select at random card with an area of 1 cm 2 in triplicate after emergence of adult. Anesthetize with co_2 and characterize the sex as male or female . Express the results as ratio.

Parasitism potential :- Allow the parasitoids from the sampled colony at the ratio 1 : 6 to a fresh batch of eggs and watch out for the parasite potential. The results should yield a parasitic potential of 85-95 % .

Economics of Production of Bio Control Agents - *Trichogramma Spp.*

Introduction :

Trichogramma chilonis , ***T.japonicum*** has tremendous market in Bio-control . The raw material for preparation of these bio-control agents is ***Corcyra*** eggs. The simple economics for ***Trichogramma*** production is carried out.

Capital Investment : It is the initial amount of money to be spent for building , machinery's. The interest on capital investment is alone taken as fixed cost.

Fixed Investment : It includes depreciation values of machinery, building , taxes, insurance . It costs even when there is no production.

Total Cost :

Total cost = Fixed cost + Variable cost
Gross income :-
The total income is calculated based on the average cost of product /unit/year .

Net Income : This is calculated using the cost of production and gross income

Risk factor : Risk factor is taken in to account and it is analysed.

Inference : It is written based on the profitability of the product

Capital Investment :

Cost of terraced building @ Rs 500/sq .foot	= 40 ' x 20' x 20 '= 800 ' = Rs. 4,00,000
Refrigerator 165 lit	= Rs 8000/ unit
Exhaust fan 1 HP UV lamp box	= Rs 4500/= + Rs .1200/=
Wooden table 1 No. with 3 chairs @ Rs 750/table Rs 150 / chair	= Rs 1200/=
Iron stands with 5 racks , dimension } 9'x2 'x 7 ' Rs 200/ stand } 40 kg/stand @ Rs 50 /kg 900 stands }	Rs. 60,000/=
Iron netted doors Rs 15/sq. m 4 x Windows = Rs 80 /= } 3 x doors = Rs 72/= } with fitting charges	Rs 3275 /=
Telecom deposit	Rs 2500
Glass wares Measuring cylinder	Rs 40/=
Cost of plastic basin 30 cm diameter Rs 15 x 900	Rs. 13,500

Test tube 8 Nos. Rs 80 /=

Mating drum plastic @ Rs 75/= bucket Rs. 1600/=
Plastic tray Rs 20/= 8
Hand sprayer = Rs 50x 2 Rs 100/=

Fixed investments

Fixed investments includes depreciation , values of machinery , building , taxes, insurance . It costs even when there is no production .

Depreciation of house 1 year period at 1 % Rs 4000/=

Refrigerator @ 10 % depreciation Rs 720/=
UV lamp box @ 20 % Rs 240/=
Exhaust fan @ 5 % depreciation Rs 405
Table and chair @ 3 % Rs 36/=
Iron stand @ 5 % (20 yr life period) Rs 2160 /=
Iron netted doors @ 2 % Rs 65.50
Depreciation for trays (15 yrs) Rs 2700/=
Glass wares 50 % Rs. 60/=

Variable costs

Eggs cost for inoculation @ 1 CC /tray
For 4 seasons 1 x 900 x 4 = 3600 CC Rs 54, 000
@ Rs. 15 /CC

Broken cumbu grains @ 2.5 kg /basin x 4 = 92250 kg
For 1 year Rs 45,000

Khada cloth 2.25 sq.ft /basin @ Re1/basin Rs 900/=
Specimen tube 20 tubes @ Rs 4/= /tube Rs 80/=
east tablets 1 kg Rs 80/=
4.5 kg / yr Rs 1440/=
Rubber band /twine Rs 30/=
Groundnut kernel powder @ 100 g .bed Rs 3600/=

Sieves (plastic) @ Rs 5/ unit 5 units Rs 25/=
Wettable sulphur 5 g basin x 90 = Rs 1836 /=
4.5 x 4 @ Rs 120 /kg

Streptomycin sulpahte bottle @ Rs 7 / bottle Rs 28/=
1 Formalin 40 Y @ 300 ml / month Rs 60/=
For I yr Rs 720/=
Blotting paper sheet 1 yr 50 sheets x 2 Rs 100/=

Honey solution 1 kg Rs 60/=
10 kg Rs 600/=

Sugar solution 5 kg sugar @ Rs 15/ kg		Rs 75/=
Camel hair brush Rs 5 / piece x 5 nos.		Rs 25/=
Vitamin E capsule		Rs 3600
Rs 1/= capsule		
Hair brush Rs 3 x 5		Rs 15/=
Absorbent cotton	Rs 30 x 4	Rs 120 /=
Chart paper 5400 charts Rs 1.50 . chart		Rs 8900/=
Mylar film strips @ Rs 8/ sheet		Rs. 8/=
Gem clips / box		Rs 5/=
Plastic cover (100 gauge) 2400 sheets		Rs 648/=
Stapler pin box @ Rs 5/box		Rs 40/=
Other miscellaneous cost		Rs 3000/=
Electricity charges		
For exhaust fan 402 units		Rs 704
Other electric charges	Rs 1004 x 12	Rs 12,048
Telephone charges Rs 300 / 2 months		Rs 1800/=
Transport charges @ Rs 50 / day	1500 x 12	Rs 18000

Labour charges :-

1 Graduate accountant cum supervisor @ Rs 2500 / month	Rs 30,000
10 female labourers (B Type) Rs 25 day Rs 750 x 12 = 9000 x 10 =	Rs 90,000
Miscellaneous cost Rs 500 / head/yr	Rs 5000/=

Income :-

Corcyra egg production is about 15 CC / basin 900 x 15 x 4 = Rs 10/=		
Total income from parasitised egg	Rs 5400 x 10	= 5,40,000
Disposal of cumbu @ 1000 /tonne	=	Rs 8000
Capital investment		Rs 5,41, 925
Fixed cost		Rs 23,946.50
Interest on fixed capital @ 14 % annum = 56, 479/=		
Total variable cost		Rs 1,56,848
Labour		Rs 1,25,000
Electricity/telephone		Rs 13,848
Transport		Rs 18,000
Total cost = TVC +FC+CI		
156848 + 80425 =		Rs 2,37, 273/=
Total income =		Rs 5,48,000/=
Net profit	= Rs 5,48,000 – 2,37,273	Rs.3,10,727 /=

BC Ratio = Rs.5,48,000 / 237, 273 = 2.3

Inference: Hence it is clear that setting up Bio - control agents production centre is profitable . The BCR is 2.3 and net profit is Rs 3,10, 727 / per annum.

Field Application of Bio-Control Agents

Introduction :

Today we practice the modern tecnologies , but these technologies have serious repercussions . The chemicals based agriculture diverted the environments mostly unhealthy. Serious environment hazards like pollution , bio-magnification , increased rsidues of pesticides , pests developing tolerance to a spectrum of insecticides etc., cropped up. Conventional methods as a result had to give way to integrated approach . Our search for other means has yielded valuable resources. Viz., the insect pathogenic viruses. To-day , they are used widely on a variety of crops in IPM approach.

Rice : Release of 1 lakh of parasitsed eggs of ***Trichogramma japonicum*** against stemborer and ***T.chilonis*** against laffolder 1 lakh/ha at weekly intervals starting from the appearance of 2-5 moths in light trap or around 30 days after planting whichever is early six to eight releases may be made at weekly intervals depending upon the availability of host eggs in the field. Cut the parasitised "Tricho cards " into equal bits and release the parasitoids by stapling individual cardbits in to the leaves in the middle region of each sub-plot of 125 sq. m area every week. Collect one egg mass/ subplot at weekly intervals , starting from 30 th to 50 th DAT and again from 80 th to 110 th DAT . Collect the egg masses on the observation dates , bring them to laboratory, record all the emerged parasitoids and work out per cent parasitism due to different parasitoids .

Cotton : Release of ***T. chilonis*** @ 1 lakh / ha / release starting form 70 th day after sowing at weekly intervals or based on the moths caught in the light traps or pheromone traps . So that ***Trichogramma*** release will help to synchornise the egg stage of the pest and the parasitoid to lay eggs. The number of release 8-10 may be decided based on the moths activity / intensity of damage by the bollworms. Collect the 125 eggs / ha at 10 days interval and observe the parasitism .

Pulses : Place 1 lakh egg parasitoids of ***T. chilonis*** / ha by stapling egg card bits on the lower side of the leaf at 50 % of the crop flowers or the first moth is trapped in the pheromone trap. Impose the treatment in the evening or morning hours and repeat the treatment 5 times at 10 days interval. Record the number of eggs and parasitised eggs by examiming the eggs collected at each count and work out the per cent parasitism.

Sugarcane : Six releases of 2.5 C C of parasitised eggs of ***T. chilonis*** at 15 days interval starting from 4 th month after planting or early , depending upon the availability of eggs of internode borer or after the appearance of the pest is to be made. After each release , the egg cards of the host insect at 10 spots containing 100 eggs / card and retrieve after 2 days Bring the parasitised card with host eggs to the laboratory and record the per cent parasitism by ***T. chilonis***.

Helicoverpa armigera : The application of NPV depends on the crop and the stage of the crop. With low crop canopy the dose administered is less. When the pest occurs in as a foliage feeder the dose required is also less on crops like cotton the pest preferentially feeds on the fruiting parts. Under such situations higher dosages are needed. To protect the virus from unfavourable conditions numerous adjuvants are advocated . The steps involved are :

Crop	Recommendation
Chickpea	NPV 250 LE /ha + crude sugar 2.5 kg /ha
Pigeon pea	NPV 250 LE /ha + crude sugar 2.5 kg /ha
Groundnut	NPV 250 LE /ha + crude sugar 2.5 kg /ha
Sunflower	NPV 250 LE /ha + crude sugar 2.5 kg /ha
Cotton	NPV 500 LE /ha + NSKE 10 % + Glycerol + egg white + ranipal + teepol 1 %

Spodoptera litura NPV* :-** Unlike ***Helicoverpa here 250 LE /ha is applied along with 2.5 kg of crude sugar . The application technique is similar to that of the ***H.armigera*** .

Chilo infuscatellus* GV : -** The ***chilo GV is applied at the rate of 250 LE + Teepol 0.1 % . The spray should be directed towards the basal portion of the stem. Two sprays on the 35 and 50 th DAP gives effective control.