Practical Manual

BIOLOGICAL CONTROL OF CROP PESTS AND WEEDS

APE 505 3(2+1)

For

M.Sc. (Ag.) Entomology

2023

Department of Entomology

College of Agriculture

Rani Lakshmi Bai Central Agricultural University

Jhansi- 284003

Practical Manual

BIOLOGICAL CONTROL OF CROP PESTS AND WEEDS

APE 505 3(2+1)

M.Sc. (Ag.) Entomology

M. Soniya Devi
Vijay Kumar Mishra
Usha
Sundarpal
Yogendra Kumar Mishra

Department of Entomology
College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi - 284003

Contents

Suggested Readings

1) Burges HD and Hussey NW. (Eds). 1971. Microbial Control of Insects and Mites. Academic Press, London.
2) De Bach P. 1964. Biological Control of Insect Pests and Weeds. Chapman and Hall, New York.
3) Dhaliwal GS and Arora R. 2001. Integrated Pest Management: Concepts and Approaches. Kalyani Publ., New Delhi.
4) Ignacimuthu SS and Jayaraj S. 2003. Biological Control of Insect Pests. Phoenix Publ., New Delhi.
5) Saxena AB. 2003. Biological Control of Insect Pests. Anmol Publ., New Delhi

PRACTICAL NO. 1

IDENTIFICATION OF COMMON PREDATORS OF CROP PESTS

Objective: To identify different type of predators present in crop ecosystem

Insect Predators

An insect predator is large in size, active in habits and has structural adaptations for catching the prey with well-developed sense organs and capacity for swift movements. They feed upon a large number of small insects every day. The important groups are as follows:

1. **Order : Odonata**

   Suborder : Anisoptera eg. Dragon fly

   Suborder : Zygoptera eg. Damsel fly

   * Relatively larger sized insects
   * Immature stages (Naiads) are aquatic feeding on aquatic insects
   * In naiads, labium is modified into a prehensile organ called mask for catching the prey
   * Adults feed on midges, mosquitoes, flies and small moths
   * Adults are capable of catching prey during flight with the help of basket shaped legs

2. **Order : Dictyoptera**

   Family : Mantidae

   * Praying mantids are large elongate insects
   * Nymphs and adults are cryptically coloured with long prehensile raptorial forelegs
   * Highly predaceous, feeding on variety of insects like flies, grasshopper and many caterpillars eg. *Mantis religiosa*

3. **Order : Hemiptera**

   Family : Reduviidae

   * Assassin bugs or cone nose bugs or kissing bugs
   * Usually blackish or brownish in colour
   * The beak or proboscis is short and three segmented
   * Most are predaceous and some are blood sucking
   * Both nymphs and adults are predaceous
   * Eg. *Harpactor costalis* on the red cotton bug *Dysdercus cingulatus*

   Family : Pentatomidae

   * Stink bugs
   * Bugs are shield shaped with 5 segemented antennae
   * Some of the species are predaceous on lepidopterous larvae
   * Both nymphs and adults are predaceous
   * Eg. *Eucanthecona furcellata* on the larvae of red hairy catepillar, *Amsacta albistriga* and gram caterpillar, *Helicoverpa armigera*

Family : Belostomatidae

* Giant water bug
* Elongate oval and somewhat flattened with raptorial forelegs
* Feed on variety of aquatic insects

Family : Miridae

* Elongated soft bodied insects
* A few species are predaceous
* Eg. Green mirid bug, *Cyrotorhinus lividipennis* feeds mainly on the eggs and early stage nymphs of green leaf hopper (GLH), brown plant hopper (BPH) and white backed plant hopper (WBPH) in rice

Family : Veliidae

* Ripple bugs
* Aquatic insects living on the surface of water
* Brown or black in colour
* Eg. *Microvelia atrolineata* feeding on the first instar caterpillar of lepidopteran pests and GLH, BPH and WBPH in rice ecosystem

3. Order : Neuroptera

Family : Myrmeleontidae

* Ant lions
* Larvae construct pit falls and remain buried in the soil
* Feed on the ants and other insects that fall into the pits
* Feed by inserting the mandibulo - suctorial mouth parts into the prey and sucking the internal contents

Family : Chrysopidae

* Aphid wolfs or green lace wings
* Adults are green in colour with golden or copper coloured eyes
* Feed on more than 18 families of insects
* The larvae are predaceous mainly on aphids and also on eggs of lepidopteran insects, psyllids, coccids, thrips and mites
* Larvae have sharp mandibles
* The eggs of aphid lions are stalked (pedicellate)

5. Order : Diptera

Family : Asilidae (Robber flies**)**

* Adults are mostly elongate with tapering abdomen
* Body is covered with dense hairs
* Legs are long, strong and well developed
* Adults are predaceous and attack a variety of insects like wasps, bees, grasshoppers,

flies etc.

* Mouth parts are piercing type. They feed by sucking the body fluid of the prey

Family : Syrphidae

* Hover fly adults are brightly coloured and resemble various bees and wasps
* Good pollinators
* Maggots are green in colour and feed on aphids by sucking their body fluids

6. Order : Coleoptera

Family : Coccinellidae

* Lady bird beetles
* Beetles are small, oval, convex and often brightly coloured
* Grubs are elongate, somewhat flattened and covered with minute barnacles or spines
* Adults and grubs feed on aphids, coccids, mealy bugs, whiteflies and other soft bodied insects
* Except one or two species in the family, all are predaceous
* Eg. ***Rodolia cardinalis*** on cottony cushion scale, ***Icerya purchasi***

Family : Carabidae

* Ground beetles
* Dark in colour and shiny and some what flattened
* Most of them feed on caterpillars
* Eg. ***Anthia sexguttata, Ophionea indica***

Family : Cicindelidae

* Tiger beetles
* Beetles are very active and brightly coloured
* They run and fly rapidly
* Both adults and grubs are predaceous
* Adults capture the prey with sickle shaped mandibles
* Eg. *Cicindela* spp.

Family : Staphylinidae

* Rove beetles
* Eg.: *Paederus fuscipes* feeds on rice leaf folder

6. Order: Hymenoptera

Family : Vespidae

* Wasps collect various insects and feed their larvae with them
* Mud wasps construct nests made of mud and provide caterpillars for the young ones in the nest

Family : Sphecidae

* Digger wasps construct nests made of mud and feed its young ones with insect caterpillars

Family : Formicidae

* About half the members of the family are predaceous upon insecta

Dragonfly

Lady bird beetle

Praying mantis

Syrphid fly

Green lace wing

Tiger beetle

Activities:

1. Collect the different predators present in the field.

2. Identify the collected predators and records characteristic features using morphological structure of insect.

PRACTICAL NO. 2

IDENTIFICATION OF COMMON PARASITOIDS OF CROP PESTS

Objective: To identify different type of parasitoids present in crop ecosystem

Insect parasitoids: Parasitoids require only one host, which it kills for its development into free living adult. Parasitoids are the same size as the host or sometimes even smaller. The adult food of parasitoids is different from that of larvae. Most of the parasitoids belong to Hymenoptera (90%) and Diptera (10%).

1. Order: Hymenoptera

The ovipositor originates and protrudes ventrally from the abdomen and is used to insert eggs into their hosts. There are three super families.

a) Super Family : Ichneumonoidea

* Possess long and filiform antennae
* Wings are veined

Family : Ichneumonidae

* Eg. ***Eriborus trochanteratus***, a larval parasitoid on coconut black headed caterpillar, ***Opisina arenosella***
* Trochanter two segmented
* Possesses two recurrent veins and rarely one
* Abdomen three times as long as the rest of the body
* Large slender black, yellow or reddish yellow insects
* Larvae are endo or ecto parasitic on many group of insects and spiders

Family : Braconidae

* Eg. *Bracon brevicornis*, a larval parasitoid on *Opisina arenosella*, *Chelonus blackburni,* egg larval parasitoid on cotton spotted bollworms, *Earias* spp.
* Adults are relatively small, more stout bodied than ichneumonids
* Abdomen is about as long as the head and thorax combined
* Not more than one recurrent vein
* Adults not as bright as ichneumonids
* Mostly endoparasitic on lepidopteran larvae

b) Super Family : Chalcidoidea

* Mostly smallest parasitoids and gregarious
* Antennae geniculate
* Abdomen very short or globular with very slender propodeum
* Wings without veins

Family : Chalcididae

* Eg. *Brachymeria nephantidis* a larval parasitoid on *O. arenosella*
* Abdomen humped

* Hind femur enlarged and toothed
* Ovipositor straight and short
* Parasitic on Lepidoptera, Diptera and Coleoptera

Family : Trichogrammatidae

* Eg. *Trichogramma chilonis*, an egg parasitoid on many lepidopterous pests
* Mostly egg parasitoids
* Minute insects (0.3 to 1.0 mm long) with three segmented tarsi and broad and elongated fore wings with rows of microscopic hairs on them
* Hind wings reduced with hairs

Family: Eulophidae

* Eg. *Trichospilus pupivora* and *Tetrastichus israeli,* pupal parasitoids on *O. arenosella*
* Adults have four segmented tarsi
* Many have brilliant metallic colouring
* Males of many species have pectinate antennae
* Mostly parasitic on aphids and scales and some are on pupae of Lepidoptera

c) Super family: Bethyloidea

* Smaller than Icheneumonoidea and larger than Chalcidoidea

Family: Bethylidae

* Eg. *Parasierola (= Goniozus) nephantidis*, a larval parasitoid on *O. arenosella*
* Small to medium sized, usually dark coloured wasps
* Females of many species are wingless and ant-like in appearance
* In a few species, both winged and wingless forms occur in each sex
* Parasitic on Lepidoptera and Coleoptera

2) Order: Diptera

Family: Tachinidae

* Eg. *Sturmiopsis inferens*, a larval parasitoid on sugarcane shoot borer, *Chilo infuscatellus*
* Large bristle flies
* Eggs may be macrotype or microtype
* Macrotype eggs are laid directly on the host's body usually attached to the neck region by a glutinous secretion
* Eg. *Spoggosia bezziana* on *O. arenosella*
* Microtype eggs are laid on the host plant and the host larvae feeding on the plant tissue ingest them

3) Order: Lepidoptera

Family: Epiricanidae

* Eg. *Epiricania melanoleuca*

* Parasitic on nymphs and adults of sugarcane leafhopper, *Pyrilla perpusilla*

Images of common parasitoids

Trichogramma chilonis

Epiricania melanoleuca

Bracon brevicornis

Goniozus nephantidis

Tachinid parasitoid

Aphelinus mali

Activities:

1. Collect the parasitoids present in the field.

2. Identify the collected parasitoids and records characteristic features using morphological structure of insect.

PRACTICAL NO. 3

IDENTIFICATION OF COMMON ENTOMO-PATHOGENS OF CROP PESTS

Objective: To identify different entomo-pathogens in crop ecosystem

Entomopathogens: Entomopathogens are widely used for the biocontrol of various insect pests of crops and are regular components in integrated pest management strategies. Entomopathogens include bacteria, fungi, viruses, nematodes, etc.

1.Entomopathogenic bacteria

a) *Bacillus thuringiensis:* This bacterium has been the most widely used insect pathogen to date. A complex of bacterial subspecies comprises this bacterium, all of which are typified by the production of a parasporal body during sporulation. This parasporal body contains one or more proteins, often in crystalline form, many of which are highly toxic to certain insects. In the insecticidal isolates, the toxins are known as **endotoxins** and often occur in the parasporal body as protoxins which after ingestion are activated by proteolysis in the gut. The activated toxins destroy midgut epithelial cells, causing death.

Following are some strains of Bt

Bt *kurstaki*, *entomocerus* (130-140 Kd) Lepidoptera

Bt *kurstaki* HD1 (71 Kd) Diptera, Lepidoptera

Bt *tenebrionides*, *sandiago* (77-73 Kd) Coleoptera

Bt *israeliensis* (125-145 Kd) Diptera

(b) *Bacillus popilliae*: Scarab milky disease was first discovered over 50 years ago (Dutky 1937, 1963). It is caused by *B. popilliae* and *B. lentimorbus*. The term "milky" derives from the opaque white color characterizing diseased larvae which results from the accumulation of sporulating bacteria in the hemolymph.

(c) *Bacillus sphaericus*: It is an aerobic rodshaped gram variable bacterium, first described by Neide in 1904. It produces terminal/subterminal spherical spores. It is highly persistent in the environment and has excellent control over *Culex, Psorophora and Mansonia* mosquito larvae.

2. Baculoviruses

I. **Insect viruses:** The important family among the viruses is Baculoviridae which include nuclear polyhedrosis viruses (NPV) and granulosis viruses (GV).

a) Nuclear polyhedrosis virus (NPV): The virus consists of proteinaceous polyhedral occlusion bodies inside which the virions or virus rods are embedded.

Symptoms: Insects become dull in colour, feeding rate is reduced and larvae become pinkish white especially in the ventral side due to accumulation of polyhedra. In advanced stage larvae become flaccid, the skin becomes very fragile and eventually ruptures. Larvae hang upside down from the plants. This is called tree top disease (or) Wipfelkrankeit Disease

Examples of baculoviruses isolated from different insect hosts:-

Nucleopolyhedrovirus

AcNPV	Lepidoptera: *Autographa californica*
LdNPV	Lepidoptera: *Lymantria dispar*
SlNPV	Lepidoptera: *Spodoptera litura*
HaNPV	Lepidoptera: *Helicoverpa armigera*
TnSNPV	Lepidoptera: *Trichoplusia ni*
BmSNPV	Lepidoptera: *Bombyx mori*
CnSNPV	Diptera: *Culex nigripalpus*
NsSNPV	Hymenoptera: *Neodiprion sertifer*

Granulovirus

TnGV	Lepidoptera: *Trichoplusia ni*
SiGV	Diptera*: Sturmiopsis inferens*

3. Entomopathogenic fungi: The first pathogens found to cause diseases in insects were fungi because of their conspicuous macroscopic growth on the surfaces of their hosts. The diseases caused by fungi are termed as mycoses. A large group of organisms belonging to the Phycomytes, Oomycetes, Zygomycetes and Imperfect fungi cause diseases of insects and can reduce their numbers.

i) White halo fungus, *Verticillium lecanii* infecting coffee green scale*, Coccus viridis.*

Symptoms: Body of scale insect is mummified and becomes hard. Body covered with filamentous white hyphae. Infected scale is found struck ot leaf veins with spores on the surface.

ii) Green muscardine fungus *Metarhizium anisopliae* infects coconut rhinoceros beetle / grub and **White muscardine fungus,** *Beauveria bassiana* attacks, silkworm, castor semilooper etc.

Symptom: Body is mummified and shrunk from original 'C' shape and becomes dried to hard structure. Body is covered with dark olive green powdery mass viz., spores.

Verticilium lecani

Green muscardine fungus

White muscardine fungus

<u>Activities:</u>

1. Collect the diseased larvae from the field.

2. Observe the symptoms under the microscope and identify the causal pathogens.

PRACTICAL NO. 4

IDENTIFICATION OF COMMON WEED KILLERS (PHYTOPHAGOUS NATURAL ENEMIES)

Objective: To collect and identify the weed killing insect present in the field.

Weed Killers: Insect which help in controlling weeds by feeding on them are called weed killers.

1. *Dactylopius tomentosus* cochnieal insect to control prickly pear *Opuntia dillenii*. This insect was introduced into India in 1925. Within 5-10 years it controlled the weed
2. Aristalochia butterfly, *Papilio aristolochiae* (Papilionidae:Lepidoptera). It feeds on Arista lochia which a weed.
3. Caotropis butterfly - *Danaus chrysippus* (Nymphalidae:Lepidoptera) - feeds on calotropis.
4. AK Grosshopper - *Poecilocerus* pictus (Actididae:Orthoptera) Feeds on Calotropis and controls ii.
5. Water hyacinth weevil *Neochetina eichhorniae* and *N. bruchi* **:** The larvae tunnel and feed inside the petioles. Ten pairs of adults and progeny controls plant growth in 0.58 m^2.
6. Parthenium weed killer, *Zygogramma bicolorata* (Chrysomelidae: Coleoptera**)** : Adults and grubs feed on leaves and flowers. 2 beetles controls and destroys one plant in 45 days.

Dactylopius tomentosus

Zygogramma bicolorata

Neochetina eichhorniae

Danaus chrysippus

Poecilocerus pictus

Papilio aristolochiae

Activities:

1. Collect the weed killing insect from the field.

2. Identify and write their characters.

PRACTICAL NO. 5

MASS PRODUCTION OF RICE MOTH, *CORCYRA CEPHALONICA* (LABORATORY HOST)

Observation: To mass multiply the laboratory host (*C. cephalonica*)

Methodology for rearing of *C. cephalonica*:

1. Sterilize the rearing boxes (if wooden) in hot air oven for 30 minutes. If plastic trays are used, wash them before use
2. Heat sterilized broken sorghum grain @ 2.5 kg along
3. Add 100 grams of roasted ground nut powder, 5 grams of yeast, 5 grams of wettable sulphur, 0.05 gms of streptomycin sulphate in each box or tray
4. Sprinkle *Corcyra* eggs @ 1 cc per box or tray and cover with lid.
5. Label the date of inoculation in the box
6. The hatching larvae feed on the grain by webbing and larval period lasts for 30-35 days. The pupation takes place inside the web itself. Pupal period lasts for 5-7 days and adult moths emerge after 30-45 days from the date of egg inoculation.
7. The emerging *Corcyra* adults are collected every morning and transferred to a specially designed mating drum made of G.I. with wire mesh bottom
8. Provide cotton soaked 20% honey+ vitamin E solution as adult food in the egg laying chamber
9. The eggs are collected at the bottom on a blotting paper kept in a tray. Pour the eggs in a paper by tilting slightly downward so eggs come down side where as dust particles remain in upper side
10. Clean the eggs further by passing through different size sieves to 10, 15 and 40 meshes. Discard the moth after 4 days
11. Utilize the *Corcyra* eggs for *Trichogramma* production (or) host culture or store them in refrigerator at 10˚C for 7 days, if required.
12. Favourable temperature for rearing is 28±2 ˚C and Relative humidity, 75±5%

Activity: Perform and prepare a flowchart for mass multiplication of *C. cephalonica* and record its life stages

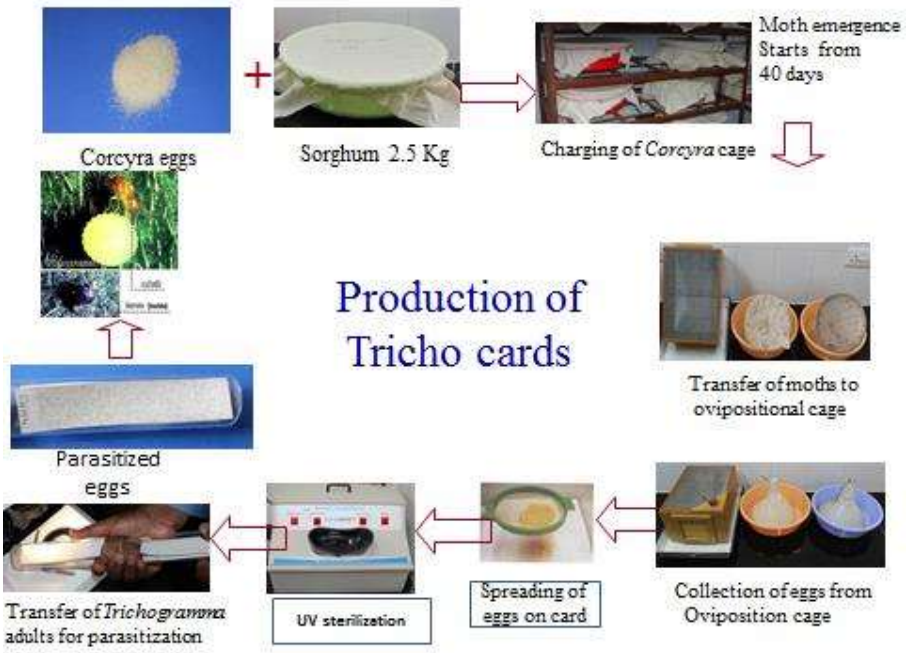
PRACTICAL NO. 6

MASS PRODUCTION OF *TRICHOGRAMMA SPECIES* (EGG PARASITOID)

Objective: To study mass multiplication technique of *Trichogramma* sp.

Methodology: Clean fresh *Corcyra* eggs by passing through 15, 30 and 45 mesh sieves. Prepare "Trichocard" by cutting card board sheet to the size of 10 x10 cm which can accommodate 1 cc of eggs. Apply gum on the card and sprinkle the cleaned eggs uniformly. Remove the excess eggs from the cards by using brush. Allow the card for shade drying for 30 minutes. Treat the eggs under UV lamp for 30 minutes. Take polythene bag, insert UV treated "Trichocard" and nucleus card at the ratio of 6:1 (6 *Corcyra* egg cards: 1 *Trichogramma* nucleus card) and provide 50% honey vitamin E in a soaked cotton swab. Remove the Tricho cards after 2 days *Corcyra* eggs changes black colour on 3rd day indicates the parasitization of eggs. Release the parasitized egg cards immediately in the fields (or) store them in refrigerator at 10°C up to 21 days. Place/tie/staple parasitized cards on leaf sheath of plant.

Field release: The parasitoids emerge 7 days after parasitization under room temperature. When cold stored, the cards are taken out and kept in room temperature for a day before field release. The egg card's cut into smaller cards along the lines and stapled on the plant.



Production of Trichocard under laboratory condition

Activities: Prepare a flowchart for mass production of *Trichogramma chilonis* and record the changes in the parasitized eggs.

PRACTICAL NO. 7

MASS PRODUCTION OF *CHELONUS BLACKBURNI* (EGG- LARVAL PARASITOID)

Objective: To study mass multiplication technique of *Chelonus blackburni*

Material required: Polythene bags, Rubber bands, Scissors, Gum, Brush, Tea strainer, 50% honey solution, Card, Refrigerator, UV lamp

Methodology for mass production of *Chelonis blackburni*

1. Paste a set of 100, 0-24 hr old eggs of *Corcyra* (not exposed to UV) to 5 x 5 cm card.
2. Expose the egg containing card to 30 *C. blackburni* adults in a 1.5 l plastic container.
3. Make sure the plastic container has windows with plastic mesh for aeration.
4. Place two cotton swabs, one soaked in 10% honey solution and the other in drinking =inside from the side opening which is closed tightly with a cloth covered cotton plug.
5. Remove the egg card after exposing to *C. blackburnii* for 24 hrs.
6. Then, place the egg card on 500 g sterilized cumbu medium.
7. In 30 days, 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.

Field release: The emerged parasitoids are collected daily and taken to fields for release @ 1/plant (or) 8000 parasitoids/ac larval parsitoid.

Activities: Prepare a flowchart for mass production of *Chelonus blackburni* and record the changes in the parasitized egg and larva.

PRACTICAL NO. 8

MASS PRODUCTION OF *BRACON BREVICORNIS* (LARVAL PARASITOID)

Objective: To study mass multiplication technique of *Bracon brevicornis*

Production of *B. brevicornis*

* *B. brevicornis* is amenable for mass rearing in the laboratory on the alternate host, *C. cephalonica*. For small scale culture, '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 paralyze 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 up to 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 50C and 50-60% RH.

Field release: *B. hebetor* is released @ 8000 adults/ac for cotton bollworm and *B.brevicornis* released @ 10 adults/tree for coconut black headed caterpillar.

Activities: Prepare a flowchart for mass production of *B. brevicornis* and record the changes in the parasitized larva.

PRACTICAL NO. 9

MASS PRODUCTION OF *GONIOZUS NEPHANTIDIS* (LARVAL-PREPUPAL PARASITOID)

Objective: To study mass multiplication technique of *Goniozus nephantidis*

Material required: Polythene bags, Rubber bands, Scissors, Gum, Brush, Tea strainer, 50% honey solution, Card, Refrigerator, UV lamp, BOD Chamber.

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 strips of paper in plastic boxes which are covered by muslin cloth.
* Considering the fecundity as 20-50, the female is capable of parasitizing 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).

Field release: The emerging adults are released in coconut garden @ 10 adults/tree.

Activities: Observe and record the changes in the parasitized larva.

PRACTICAL NO. 10

MASS PRODUCTION OF *BRACHYMERIA SPECIES* (PUPAL PARASITOID)

Observation: To study mass multiplication technique of *Brachymeria species*.

Production procedure

1. Release 50 adults of *B. nosatoi* comprising both sexes in a clean, dry cylindrical jar of 17.5x6.75 cm.
2. Insert a 12 cm long and 6.25 cm wide cardboard piece to facilitate the parasitoids to move and rest.
3. Secure the mouth of the jar with a piece of muslin cloth tightened with rubber bands. Keep the jar horizontally.
4. Transfer the parasitoids to fresh clean jar every 4 to 5 days.
5. For adult parasitoids, provide undiluted honey daily in minute droplets on wax coated paper.
6. Keep the jar containing parasitoid in diffused sunlight for 10-15 minutes daily for about 3-4 days after which only the host pupae are to be offered for parasitization. Exposure to sunlight stimulates mating.
7. Carefully remove the pupae of *O. arenosella* with cocoons and silken galleries intact or leaf-bits containing pupae within cocoons and silken galleries and place 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.
8. Insert the card board piece containing pupae into the horizontally placed glass jar containing the mated parasitoids for parasitization.
9. The parasitoids partially disorganize the pupal tissues standing on the galleries with their ovipositors by repeated thrusts and oviposit in the pupae.
10. Place the pupae without cocoons and silken galleries on the card board and cover with silken galleries as the parasitoid will not parasitize naked pupae.
11. Depending on the activity of female parasitoids, the host pupae can be exposed for a period of 4-6 hours for parasitization.

Field release: Release @ 20 adults/tree for coconut black headed caterpillar.

Activities: Observe and record the changes in the parasitized larva.

PRACTICAL NO. 11

MASS PRODUCTION OF *CHRYSOPERLA CARNEA* (PREDATOR)

Observation: To study mass multiplication technique of *Chrysoperla carnea*

Methodology: 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 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 10oC 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.

Field release: The first instar larvae of *Chrysoperla* are released in cotton fields at 20,000 to 40,000/acre for 3-5 times at 10 days interval to control aphids, whitefly, *Spodoptera, Heliothis*, pink bollworm, thrips and mites. The larvae are taken in plastic containers with 1-2 cc of corcyra eggs and loose paper strips. The paper strips along with larvae sticking on them are dropped in the field at random while walking across the field.

Exercise: Record the morphological and biological feature of different stages of *C. carnea*.

PRACTICAL NO. 12

MASS PRODUCTION OF *CRYPTOLAEMUS MONTROUZIERI* (PREDATOR)

Objective: To study mass multiplication technique of *Cryptolaemus montrouzieri*

Methodology for mass production of *C. montrouzieri*

1. **Colony establishment:** Collect the colonies of the mealy bugs from field initially. Guava plantations, vineyards, papaya, citrus and pomegranate gardens are good reservoirs of the mealy bug populations. Purify the mealy bug colonies in the lab to obtain the population free from parasitoids and scavenging ants.
2. **Culture maintenance:** Culture the mealy bugs on pumpkin (red) in the laboratory as it is very difficult to maintain the colony on the natural host plants. Select fleshy pumpkins with intact peduncle and deep ridges and furrows of weight 2.5 kg devoid of wounds and mouldy patches for multiplication of the mealy bugs. Soak the pumpkins in carbendazim 0.5% for 1 min. and shade dried. Plug the cut ends and wounds with molten wax. Provide pieces of paper.
3. Place the pumpkins in large sized cages over stainless steel stands. Set the cages in ant proof conditions as the mealy bugs secrete honey dews which attract ants invariably. Collect the ovisacs of healthy adults of mealy bugs and place on fresh pumpkin in the laboratory individually. From them, allow the eggs to hatch and multiply. In a month time, the mealy bugs begin to smother the entire surface of the pumpkin. From this stock, subsequent colonies are established.
4. Collect the ovisacs (when the colony is in active growth period with breeding females) with the help of camel hair brush and transfer to fresh pumpkins prepared as above. During the mass production care is taken to avoid fungal invasion. The cages, steel ware used are sterilized using common bleach. Used pumpkin fruits with symptoms of mould invasion are disposed of immediately. After 25 days of releasing the mealybugs, release 10 mated adult females of Cryptolaemus into the cage. For facilitating pupation of grubs, keep paper pieces on the bottom of the cage. After 1.5 to 2 months, collect the emerging beetles in the glass vials daily up to 5-10 days.

***Cryptolaemus* eggs** ***Cryptolaemus* nymphs**

***Cryptolaemus* pupae** ***Cryptolaemus* Adult**

Field release: For citrus mealy bug and grape vine mealy bug, release 10 beetles/tree (or) vine. Before releasing the predators, the ant movement should be arrested.

Activities: Record the morphological and biological feature of different stages of *C. montrouzieri*.

PRACTICAL NO. 13

MASS PRODUCTION OF *ZYGOGRAMMA BICOLORATA* (PHYTOPHAGOUS NATURAL ENEMIES OF WEED)

Objective: To study mass multiplication technique of *Zygogramma bicolorata*

Methodology:

1. Place 10 pair of adults (male and female) on bouquets of Parthenium leaves in 14 x 12 cm transparent plastic container.
2. Replaced leaves after observance of egg laying and proveide fresh bouquets.
3. Repeatation can be done for such replacement of bouquets in egg laying jars for one month.
4. Remove small Parthenium plants from soil and transplanted in 45 x 60 x 90 cm cages with zinc sheet trays at bottom. Fill these trays with soil and transplant the parthenium plants and water daily.
5. Once plants start growing, place leaves with eggs of *Zygogramma bicolorata* over them. Around 100-150 eggs can be transferred in one such cage.
6. On hatching larvae feed on leaves and pupate in soil. In such process they consume all plants inside cages. Generally ratio is 10-15 grubs to one plant. This way around 15-20 small plants are provided in each cage. Such cages yield around 100-125 adults. It can also be bred in open field by covering Parthenium plants with walk in field cages and by releasing 1 pair:2 plant

Eggs of *Z. bicolorata*

Grub of *Z. bicolorata*

Adult of *Z. bicolorata*

Activities:

1. Collect and rear *Z. bicolorata* in the laboratory
2. Illustrate the life cycle *of Z. bicolorata* and note its feeding behaviour.

PRACTICAL NO. 14

MASS PRODUCTION OF *NEOCHETINA SPECIES* (NATURAL ENEMIES OF WEEDS)

Objective: To study mass multiplication technique of *Neochetina species*

Methodology:

Neochetina eichhorniae and *Neochetina bruchi* are multiplied on water hyacinth plants in outdoor tanks ($4m^2$ x l m depth) cement cisterns or plastic fishing pools (120 cm dia x 60 cm depth). Addition of cowdung ($200g/m^3$) and superphosphate $40g/m^3$ and urea 10g/m3 helps in improving the growth of water hyacinth plants leading to faster and prolific multiplication of the weevils. To start with plants are exposed inside the laboratory by keeping about 25 plants in 61x 40 x 30 cm plastic throughs and releasing 50 adults. After a week the adults are collected and used for exposing fresh plants. The exposed plants are kept outside either in tank or plastic fishing pools. This process is repeated until the tanks/pools are filled up. Atleast 15 exposures can be done with the same set of adults. To start with plants are exposed inside the laboratory by keeping about 25 plants in 61x 40 x 30 cm plastic throughs and releasing 50 adults. After a week the adults are collected and used for exposing fresh plants. The exposed plants are kept outside either in tank or plastic fishing pools. This process is repeated until the tanks/pools are filled up. Atleast 15 exposures can be done with the same set of adults once fresh adults start emerging, after 3-4 months, they can be collected at fortnightly interval for field release. However, eggs laid by the adults before collection produces further generations. The tanks can thus be used for continuous multiplication just by the addition of fresh plants as and when older plants start drying. Between 100-250 adults can be collected per month by using a 120 cm diameter and 60 cm deep plastic fishing pool which can accommodate upto 100 plants.

Activity: Draw the different life stages of *Neochetina bruchi*.

PRACTICAL NO. 15

FIELD COLLECTION AND PRESERVATION OF PARASITOIDS AND PREDATORS

Objective: To collect and preserve various parasitoids and predators from the field

Field collection of parasitoids and predators on various aspects are given followed

1. Collection Areas

Carry out extensive field surveys for various insect pests of field crops and their insect parasitoids and predators in various district of bundelkhand region.

2. Collection Equipments

The minimum equipments required for the field collection of relevant insect materials comprised of collection kit containing a pair of scissors, forceps, soft camel brush, collection tubes, polythene bags, paper bags, specimen jars, and specimen tubes containing 75-90% alcohol as preservative, muslin cloth, rubber bands, hand lens, absorbent paper, field diary and camera, etc

3. Collection of Insect Predators of Crop Plant and weed Insect Pests

The insect predators of crop plant and weeds insect pests were collected either by rearing their immature stages along with host pest in the laboratory (some aphid, whitefly predators) or collected from the field while these were devouring the host pest.

4. Collection of Insect Parasitoids of Crop pest and weeds

The insect parasitoid of crop and weed insect pests will collected from the host-insect pests by rearing process in the laboratory.

5. Cataloguing of Field /Laboratory Data

It was useful to keep the breeding as well as field observation data in series in the form of catalogues. For this purpose, filling cards (6 cm X 4 cm) have been useful. Various information's about the sample like host-plant, host-pest, parasites and predators found, number of male and females of parasites and predators found, percentage of parasites and predators, location, altitude and remarks, were recorded in these catalogues.

Activities: List out the name of the parasitoids and predators collected from the field and preserve in the laboratory. Mention their hosts too.

PRACTICAL NO. 16

MASS PRODUCTION OF *BEAUVERIA BASSIANA* (WHITE MUSCARDINE FUNGUS)

Objective: To learn mass production of *Beauveria bassiana*

Material required: Chalk powder, Sorghum, water, autoclave

Methodology:

1. Soak 1 Kg of Sorghum in water for 48 hours.
2. Replace water after 24 hrs. After 48 hrs. rinse water completely.
3. Separate equally in 10-15 flasks and plug with hard cotton cushion and wrap with double aluminum foil. Sterilize for 40 minutes with 21 psi.
4. Inoculate each flask containing sorghum with 2-3 drop of nucleus culture after cooling.
5. *Beauveria* culture will grow fully after 20-25 days.
6. Mix 2 Kg of chalk powder in *Beauveria* culture and dry in shade

Insect infected with *Beauveria bassiana*

Dose : 1 gram/liter of water or 1 Kg/1000 liter of water/ha (Repeat application after 10-20 days interval)

Activities:

1. Write the symptoms of the insect infected with the fungus
2. What is the mode of action of the entomofungus?

PRACTICAL NO. 17

MASS PRODUCTION OF NUCLEAR POLYHEDROSIS VIRUS (ENTOMOPATHOGENIC VIRUS)

Objective: To learn the production technique of Nuclear Polyhedrosis Virus

Virus Preparation Method

Insect collection

HaNPV can be produced only in live host insects. Good quality HaNPV can best be produced by rearing large number of healthy *H. armigera* larvae in laboratories for NPV production but this is demanding and requires appropriate facilities. A low cost option is to use larvae collected from pigeonpea shaking operation or any other crop for virus production. For optimum HaNPV yield insects selected for production should be late fourth or early fifth instar larva (about 1.0 – 1.5 cm long).

Inoculation of virus

The larvae are supplied with well soaked (6-8 hours) chickpea seeds mixed with sufficient dose of virus inoculation (40 larval equivalent (LE)/kg of chickpea seed). Care should be taken to thoroughly mix the inoculum with chickpea seeds. Excessive inoculation is an unnecessary waste of NPV while under dosing is ineffective. Every insect kept in individual compartment is provided with 2-3 chickpea virus inoculated seeds, labeled and left for infection.

Insect rearing

Optimum rearing conditions such as 25-35^{o}C temperature are required for HaNPV production. Food should be available all the time to the infected larvae to minimize stress. To avoid cannibalism all the infected larvae are reared in multicellular trays with insect proof covering. The locally produced trays can fit one above the other and saves space. Monitoring sanitation is important while rearing and also through cleaning of apparatus after rearing. Cleaning the trays with disinfectant before and after rearing is necessary to avoid the buildup of contamination by bacteria and protozoa that can seriously reduce NPV yield. To avoid oviposition by flies on died larvae, fly proofing of insect rearing area is a great help.

Virus harvesting

Allow the infected larvae to die naturally so that the virus completes its life cycle to achieve maximum virus production. Dead larvae are soft, hence care should be taken to collect them in a separate container without causing any damage. Larvae with clear symptoms of NPV should be collected at or soon after death leaving those infected with other pathogens. The dead decomposed larvae produce often-bad smell due to the multiplication of bacteria and the decomposition of fat in the insect body. The collected larvae need to be kept in the refrigerated condition until further processing to slow down the bacterial activity in the dead larvae.

Processing virus

Blend the dead larvae collected in a suitable blender to crush the insect tissue and release the NPV. This process also helps to break down the cells to extract PIBs. Blend the NPV insects in cool room condition and collect the material by washing with distilled water. The blended material is filtered through a muslin cloth to remove the remnants of insect body parts. The muslin cloth is placed in a funnel to get the filtrate. Once the filtrate is collected, gently squeeze the cloth to extract the remaining liquid. This solution is centrifuged at 3000 – 5000 rpm for 15 minutes to separate virus from the homogenized liquid. This helps in the removal of bacteria, insect body fats that together are the major cause for bad smell.

After centrifugation, the liquid which is turbid and loose at the top of the tube is poured off and the paste like substance at the bottom full of NPV is saved. The virus thus obtained should be stored in UV opaque bottle and stored in refrigerated condition with proper labeling.

Measurement of quality of virus

Assessing the quality of virus produced is highly essential. A haemocytometer is an essential tool to measure the quality of virus in a sample. Either dark field or phase contrast microscope is needed to count the PIBs per ml. However, it is a common practice to quantify NPV in India in terms of larval equivalents (LE). This represents the average NPV content of a single optimally infected host larva.

While the use of LE should not replace actual counting of NPV the use of the term LE is widespread and may be used as an additional description for an NPV product, provided the LE figure is supported by direct counts. The currently proposed Indian standard for an LE is $6x10^9$ PIBs. It is important also that batches of NPV be bioassayed to confirm the potency of virus to the target insects.

Application of NPV

NPV is sensitive to ultra violet rays of sun. Options for improving the effectiveness of the NPV include spraying late in the day after peak sunshine. Additionally adding UV absorbents such as 1ml of robin blue to a liter of spray solution has been reported as improving the effectiveness of the NPV. For pigeonpea HaNPV should be used at 3×10^{12} PIB (500 LE ha^{-1}) and chickpea 1.5×10^{12} PIB (250 LE ha^{-1}).

Activities:

1. Draw a flowchart for the production of NPV
2. Enlist the commercial formulations of NPV available at the market

PRACTICAL NO. 18

QUALITY CONTROL AND REGISTRATION STANDARD FOR BIOLOGICAL CONTROL AGENT

Objective: To understand the quality control and registration standard for bioagents

The use of biological control agents has been increasing worldwide and there are now many companies mass-producing such organisms, particularly for the control of insect pests. However, there is a great need for quality control in the production and use of these natural enemies, which include insect parasitoids and predators, fungi and viruses. Quality of an organism can be defined as the ability to function as intended after release into the field. Mass-rearing of natural enemies often takes place in small companies with little know-how and understanding of conditions influencing performance, which may result in natural enemies of bad quality and failures with biological control. This makes robust quality control programmes a necessity. Background information is presented on the activity of mass-producing natural enemies, the emergence of the development of quality control worldwide is sketched, basic considerations for quality control are outlined and difficulties encountered when developing quality control are discussed. Several examples of poor functioning of organisms when quality control guidelines are not applied and that resulted in failure of biological control were observed. Eg: Failure of Trichogramma brassicae in swiss in controlling the European corn borer (Ostrinia nubilalis) in maize. The aim of quality control programmes is • To check whether the overall quality of a species is maintained (general). • To determine the characteristics that affect overall quality (straightforward). • To determine whether a natural enemy is still in a condition to properly control the pest to an acceptable level rather than maximum or optimum level.

Development of quality control: The problem of quality control can be approached from two sides

: • Measure how well the insect functions in its intended role, if it does not function well enough, trace the cause and improve the rearing method.

• List the changes we can expect whn the mass rearing is started, measures these and if the changes are undsired, improve the rearing method.

Registration standards:

Bio-pesticides Registration: At present, microbial pesticides like fungi, NPV etc., are included in the schedule of Insecticides Act, 1968 and Insecticides rule, 1971. This ensures the quality of bio- pesticides at farmers level. Directorate of Plant Protection Quarantine and Storage, Department of Agriculture and Cooperation, Ministry of Agriculture, GOI have issued guidelines/data requirements for registration of bio-pesticides in the country. As per this, all the units have to meet the Indian standards and technical specifications to be eligible for registration under the Insecticides Act, 1968.

Registration of Insecticides: Under Insecticides Rules, 1971 1.a. An application for registration of an insecticide under the Act shall be made in Form I and the said Form including the verification portion, shall be signed in case of an individual by the individual himself or a person duly authorized by him in case of partnership firm by the managing partner ; in case of a company, by any person duly authorized in that behalf by the Board of Directors ; and in any other case by the person in-charge or responsible for the conduct of the business. b. The Registration Committee may, if necessary, direct inspection of the 'testing facility' for establishing the authenticity of the data. 2. An application form duly filled together with a bank draft of Rs. 100 only, drawn in favour of the Accounts Officer, DPPQ&S, payable at Faridabad towards registration fee shall be sent to the Secretary, Registration Committee, DPPQ&S,, NH-IV, Faridabad-121001, Haryana. One Self addressed stamped envelope and one stamped envelope must be enclosed along with the application. 3. The registration fee payable shall be paid by a demand draft drawn on the State Bank of India, Faridabad, in favour of the Accounts Officer, Directorate of Plant Protection, Quarantine and Storage, Faridabad, Haryana. 4. The certificate of registration shall be in Form II or Form II-A, as the case may be and shall be subject to such conditions as specified therein. Issue of duplicate certificate of registration A fee of rupees five only shall be paid for a duplicate copy of the Certificate of Registration if the original is defaced, damaged or lost.

Activities:

1. Rear any biocontrol agent in the laboratory.
2. What are the points to be considered while rearing a natural enemy for maintaining its quality.