UBKV

(Course No. AG 453)

TRICHO MANUAL

Mass Production of Trichocards

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Tricho Manual
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Published by

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FOREWORD

I take immense pleasure to write the Foreword for the book entitled, 'Tricho Manual' (Mass Production of trichocards), a practical workbook for students under Experiential Learning Programme on Bio Control Agent Production , authored by Dr. Moulita Chatterjee and Dr.Tapan Kumar Hath, Department of Agricultural Entomology, Uttar Banga Krishi ViswaVidyalaya, Pundibari,CoochBehar.

The content of the workbook has been arranged systematically with discussions considering the need of the students, researchers and other cognate disciplines. This learning module will provide practical guidelines to the students along with technical skill for implementation at the ground levels, as the increasing number of stakeholders require the products to satisfy their needs and expectations.

This practical workbook is written in a very simple and lucid language which will be very much useful for the students pursuing their degree programmes in Agricultural Universities and others. This manual will be a good source of practical material to understand the beneficial organisms- rearing programme in the laboratory and the compilation will obviously improve the practical knowledge of the students. The workbook will also be a source of information for those who are engaged in research and teaching with its novelty.

(Dibyendu Mukhopadhay)

ACKNOWLEDGMENT

We are extremely thankful to Prof. Dibyendu Mukhopadhyay, Dean, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya (UBKV) for constantly encouraging us to bring out the manual. Heartfelt thanks are due to Prof. J. Ghosh, Head, Department of Agricultural Entomology, Faculty of Agriculture of the Viswavidyalaya for his keen interest and valuable suggestions during the course of preparation of this manual. We would like to thank Mr. Atanu Maji, Mr. Samrat Saha and Ms. Soheli Sarkar, Ph.D. students of the Department of Agricultural Entomology (UBKV) for rendering their assistance. The cooperation received from Mr. Mijanur Rahaman, Jr. Assistant, Department of Agricultural Economics of UBKV in the preparation of this manual is sincerely acknowledged. Sincere thanks are due to Mr. Apurba Kumar Das, Stenographer (G-III), Coochbehar Krishi Vigyan Kendra (UBKV) for his *ad libitum* assistance in lay-out, designing and decoration of the document.

We believe that besides the students, those who are interested in learning about the mass production of the bio-agent will find this document to be of great use.

We would sincerely appreciate any and all suggestions to improve the manual.

Pundibari, Coochbehar, West Bengal — Moulita Chatterjee

Pundibari, Coochbehar, West Bengal — Tapan Kumar Hath

CONTENTS

1. Group:

2. Name and registration number of the students

Name	Registration No.

Host and Parasitoid: A short introduction

Mass production of natural enemy is the basic need for any biological control progr-amme. Natural enemies can be mass multiplied either on artificial diet or on any suitable host. However, the latter is preferred as artificial diet preparation is costly. Mass rearing of suitable host is necessary for maintaining a continuous production cycle of natural enemy to ensure its steady supply to the clients.

Host/rearing medium should be cheap and easily available locally so that cost of natural enemy is low priced in order to render its affordability to the farmers.

Host (Corcyra): Among different stored grain pests, *Corcyra cephalonica* Staint *i.e.* rice moth is the most common and it is commonly used to rear *Trichogramma* for commercial purposes. It is a widespread pest of stored foods, viz., cereals, cereal products, oilseeds, pulses, dried fruits, nuts and spices. Though it is a pest of economic importance, it has gained importance in Biological Control Programme because many of the natural enemies can be mass-bred in the laboratory on different life stages of Corcyra. Its rearing in the laboratory is very easy, simple and cheap.

Parasitoid (Trichogramma): The parasitoid belonging to the family *Trichogrammatidae* (Order-Hymenoptera) under the genus *Trichogramma* are primarily egg parasitoids and abundant in all terrestrial habitats but remain unnoticed due to their minute body size. They play a major role in natural control of many of the most economically important and destructive crop pestschie fly belonging to the order Lepidoptera; howbeit, they also parasitize eggs of beetles, flies, true bugs, wasps and lacewings (Neuroptera). Mass rearing of these parasitoids is quite amenable. They are the most utilized bio-agent in the world that resulted in the most successful biological control events. It has the distinction of being advantageous as it kills the pest in its egg stage and checks any further damage to the crop before the most destructive larval stage could emerge. *Trichogramma chilonis, T. japonicum, T. Pretiosum, T. brassicae* and *T. embryophagum* are some of the commonly used parasitoid species against different crop pests. The development of different *Trichogramma spp*. is almost similar with minor variations in fecundity and longevity which depend on the species and hosts.

Requirements

Insectary

It is a place where insects are housed, multiplied or studied. It should have the fo following facilities.

1. Laboratory instruments and chemicals:

Hot air oven	UV chamber	Mixer grinder
Refrigerator	Weighing balance	Sieves
Formaldehyde 40%		

2. Rearing unit:

- Corcyra rearing basin: It may be boxes/trays/jars/any kind of vessel made up of plastic or wood with lid provided with wire mesh for aeration. Glass jar may also be used.
- Corcyra egg laying cages: It may also be plastic tub or any type of drum for oviposition.
- Working tables
- Racks for storing rearing cages/ egg laying cages
- Rubber tubes or aspirator for collecting adult Corcyra moths
- Nucleus Corcyra eggs
- Trichogramma nucleus culture

3. Feeding requirements

Broken sorghum/maize grains	Ground nut kernel
Yeast	Wettable sulphur
Streptomycin sulphate	Honey
Glycerine	Vitamin E capsule

4. Other accessories

Transparent polythene pouches	Mosquito nets	Measuring cylinder
Absorbent cotton	Scissor	Chart paper
Camel hair brush	Rubber band	Soap
Cotton	Gloves	Face musk
Shoe brush		

5. Personnel:

Persons to be engaged should haveinterest in dealing with insects and formal training in entomological works.

Corcyra is mass multiplied as a host for production of Trichogramma egg parasitoids.

The procedures are explained hereunder:

PART- I:Mass rearing of *Corcyra cephalonica*

1.Establishment of a nucleus culture of Corcyra eggs:

Corcyra eggs can be collected from reputed laboratories and commercial producers. However, to begin with the establishment of a nucleus colony, adult moths of Corcyra can be collected from warehouses or godowns where the food materials are stored.

For building up a healthy colony of Corcyra, the eggs should be free from any kind of contamination. Diseased or discoloured eggs, moth scales or any remaining broken limbs, if seen in the egg mass, should be removed.

The amount of eggs is required to be measured volumetrically in order to ascertain the number of boxes that can be inoculated. One cc (cubic centimetre) of eggs is equivalent to approximately 16000 - 18000 numbers of eggs.

2. Disinfection of rearing cages/rearing basins:

Plastic trays/tubs/basins/ glass jars to be used should be properly cleaned with any good detergent and then properly rinsed under running water followed by wiping with dry, clean cotton cloth or towel. Then shade drying is necessary. After each cycle of rearing, the emptied rearing vessels must be carefully cleaned, preferably with a disinfectant (2% formaldehyde) before these are returned to storage unit for further use. In case of wooden cages, sterilization can be done by exposing the boxes in hot air oven at 100^{0}C for 30 minutes.

3. Preparation of feed/medium for releasing Corcyra eggs:

Coarsely milled maize/sorghum/pearl millet collected from local market should be sterilized in hot air oven at 100^{0}C for at least 1 hour to eliminate any type of residual infestation by other stored grain pests (viz. *Tribolium castaneum*, *Rhizopertha dominica*) or fungi present in the purchased milled grain. After sterilization, the grains should be cooled and stored in a clean dry place. This grain stock should be used as feed while preparing rearing chambers for egg inoculation. Grains should be mixed and fortified with other essential food supplements to ensure supply of protein and fat for the developing and regulating larval growth.

Composition of ingredients required per box/vessel (18" X 9" X 5") to prepare food mixture

Milled maize/sorghum: 2.5 kg	Ground nut kernel powder: 100g
Dry yeast powder: 5 g	Wettable sulphur : 5 g
Streptomycin sulphate: 0.05 g (powder form)	

Groundnut powder may be prepared with the help of a mixture grinder. All the above ingredients should be thoroughly mixed by hand wearing gloves and 1 cc of Corcyra eggs per box/tray should be sprinkled on the surface of the culture medium.

4. Inoculation of the fortified medium with Corcyra eggs:

The media is inoculated with healthy, good quality and free flowing Corcyra eggs (collected from egg laying chambers) @ 1 cc per cage, which are sprinkled on the surface of the feeding medium. The inoculated rearing boxes covered with lids(or clean cloth tied properly with rubber band or any type of fasteners, in case of plastic basins) and labelled with serial number and date of inoculation are to be kept on the racks in a well aerated dry room. Favourable temperature for rearing is 28±2°C and RH (Relative humidity) 75±5%. However, optimum conditions for larval development are 30 32.5°C and 70% RH.

5. Development of Corcyra larvae in fortified medium:

The larvae hatch out in 3-4 days after inoculation and start feeding on the fortified grain mixture by forming light webs on the grain surface. As the larvae grow up, they move downwards and are allowed to grow undisturbed.

Generally Corcyra moth starts emerging from 40thday onwards and continues upto 90 days after inoculation due to staggered development of the larvae. At optimum conditions, the period from hatching to adult emergence may take only 26-27 days when reared on sorghum. However, total developmental period was reported to be 41- 59 days when bred on foxtail millet at 24-28^{0}C and 70% RH.

When the boxes become ready for moth emergence, these are separated and shifted to the egg laying chamber.

6. Collection of adults for mating and oviposition:

The adult emergence generally starts one month after the release of the eggs in the medium. The adults are noticed sitting on the inner side of the covering cloth or on the netted portion of the lid of the rearing box. They are carefully and gently collected in the morning hours by using a glass tubes or a modified aspirator (TNAU model). During moth collection one should wear good quality face masks continuously to avoid inhalation of scales as this may cause different allergic reactions like coughing or breathing problem. Moth collection can be conveniently carried out within a mosquito net to prevent the escape of the moths. The moths are then transferred to the specially designed mating- cum-egg laying cages (14" X 10" X7") or oviposition drums made of plastic or GI sheets fitted with fine wire meshes at the bottom to help collect the eggs quickly and easily. To ensure proper ventilation, the side walls of the drum should have two vents covered with wire mesh opposite to each other. The lid of the drums is provided with handles and one or more slots on the lid are also necessary for easy handling and introducing moths and their food inside.

Fresh moths are collected regularly and released into mating/oviposition drums and kept as such on a separate rack for four to six successive days for mating and egg laying. Once the collection process is over, the contents of rearing basins and egg chambers/drums are emptied and thoroughly cleaned and disinfected with 2% formalin solution and dried thoroughly for next cycle of use.

The adults are provided with honey solution as their food to keep them energetic and to prolong their longevity. This feed prepared by mixing 50 ml of honey with 50 ml of glycerine water and 5 capsules of vitamin E (Evion) and may be stored in refrigerator and used as and when required. A small cottonswab soaked in the honey solution tied with a thread is hung inside the drum through the slot or on the lid. It is economical to collect moths upto 3 months from a rearing chamber after which the food quality becomes poor as well as the vitality of the

7. Collection and cleaning of Corcyra eggs:

The female moths lay eggs loosely in large numbers and these may be collected through the wire mesh provided at the bottom of the oviposition drum. It is often commonly found that shedded moth scales and broken limbs are mixed with the eggs. These may lead to potential health hazard to the laboratory workers after years of exposure to the Corcyra scales.

To minimize the risk of scales which float in the air freely, the oviposition cage/ drum is placed on a sheet of filter paper placed on a container/ enamel tray which will trap the scales. Moreover, several oviposition drums should be kept in well ventilated, clean and dry place near an exhaust fan to enable the workers to work comfortably with minimum risk of respiratory infection. These will separate the scales from the eggs to get clean eggs. However, egg cleaning may be done by a TNAU developed gadget at ease.

The bottom wire mesh of the oviposition drums fitted with wire-mesh are lifted up and cleaned gently on regular basis with a shoe brush so that the eggs and other remnants (dirt particles, shedded scales and broken limbs) settled on it can be dislodged and collected on the filter paper. After collection of entire material, clean eggs are separated by pouring the eggs gently on a paper by tilting slightly downward so that eggs freely descend whereas dust particles or other impurities are retained in the upper side.

After all these processes, the eggs are passed through 15, 30 and 45 mesh sieves serially and finally clean eggs are collected. The eggs are poured in measuring cylinders and quantified. These eggs can be stored and used for inoculating a fresh stock of rearing boxes or can also be used to prepare Corcyra egg cards depending upon the requirement.

Eggs should be collected daily for 4 days successively from each oviposition drum and moths should be discarded from 5^{th} day onwards, as egg quality starts deteriorating. About 100 pairs of adults (female-50%) produce 1.5 cc of eggs in a 4- days' laying period. From each rearing basin, an average of 2500 moths can be collected in 90 day and 18.00 - 20.00 cc of eggs can be obtained after 90 days, i.e. after completion of one rearing cycle.

8. Maintaining record book:

The overall success of the quality production of Corcyra eggs depends largely on careful maintenance of daily records of accurate information about each and every rearing as well as egg laying events. It is the most important part of the entire rearing process. This systematic approach will ensure smooth running of the laboratory on a continuous and long term basis.

Important information to be recorded and mentioned (need based) are as follows:

1. Date of collection of Corcyra eggs/larvae
2. Source from where the eggs/ larvae have been collected
3. Date of feedpreparation for inoculation with eggs
4. Date of release of eggsinto rearing box
5. Expected date of Corcyra adult emergence
6. Numbers of collected moths on daily basis
7. Problems encountered, if any,with every single rearing basin or egg laying drum during the production process
8. Record about the workers handling the cages
9. Routine of assigned job for each worker on rotation basis

Rearing of Corcyra in the Biocontrol Laboratory under Experiential Learning Programme (Faculty of Agriculture) at Uttar Banga Krishi Viswavidyalaya

Corcyra rearing cage with netted cover

Preparation of rearing cage by pouring food mixture

Stacking of rearing boxes on metal racks

Developing larvae within the rearing box

Corcyra moths resting on inner side of the lid

Releasing adult moths inside egg laying cage

Egg laying cages

Adult moths

Collected eggs

9. Problems encountered with Corcyra rearing:

Insects, mites and pathogens may occur in the feed, rearing unit and cause damage to feed as well as attack the Corcyra larvae/eggs. Besides, the moth scales can cause health hazard to the laboratory workers. These can be managed suitability by the following procedures.

i. Red flour beetle: *Tribolium castaneum*

Management:

- Sterilize grains at 100 °C for 1 hour in a hot air oven
- Use a 4W UV lamp during night over a yellow water pan and destroy the attracted beetle
- Procured grain should be free from stored grain pest.

ii. Corcyra larval parasitoids: *Bracon hebetor*

Management:

- Keep windows and all other openings covered with wired mesh sieve
- If cloth cover is used, change it frequently if it is having small holes
- Use a 60W bulb during night hours over a yellow water pan and kill the trapped adults.
- In severe cases spray 0.1% Malathion over traps, furniture rack and inner side walls of the laboratory.

iii. Mites: *Pyemotes ventricosus*

Management:

- Apply sulphur dust over trays, furniture, racks in severe cases
- Mix wettable sulphur @ 5gm per basin as a prophylactic measure
- Keep the laboratory free form dust and scales

iv. Fungi, bacteria and viruses may infect Corcyra:

- The feed should be treated with 0.05% streptomycin sulphate to check bacterial contamination.
- If virus infection occurs the rearing basins should be immediately discarded.

v. Problems associated with moth scales:

Scales may cause breathing troubles to the persons engaged in rearing. It may also cause allergic reactions on skin and eye when exposed continuously.

Management:

- Use face mask during moth collection and cleaning of eggs.
- Provision of good number of exhaust fan in the rearing unit.
- Use TNAU moth scale and egg separator.

PART -II: Mass production of Trichocards

1. How to prepare Corcyra egg cards?

Clean Corcyra eggs are taken in glass petridish (15-20 cm diameter) and are sterilized in a closed chamber by exposing them to 15W UV light for 30 minutes to kill the embryo within the eggs so that the eggs do not hatch into larvae. Cards (size: 15 cm X 10 cm) are prepared by cutting good quality art paper/ board papers. After applying a thin layer of diluted gum acaciaon it, free flowing 1cc sterilized Corcyra eggs are sprinkled / smeared on each card. The extra loose egg on the cards should be separated by tilting and tapping the card with a finger and are collected in a petridish. The cards are then allowed to dry under shade for 30 minutes. These cards can be divided into 12 equal pieces and used in the field. These egg cards are called Corcyra egg cards and can be stored in refrigerator for some time. *Corcyra cephalonica* eggs stored up to 2 weeks at 4°C - 8°C was reported to have satisfactory level of parasitization.

Precautionary measures to alleviate UV risk:

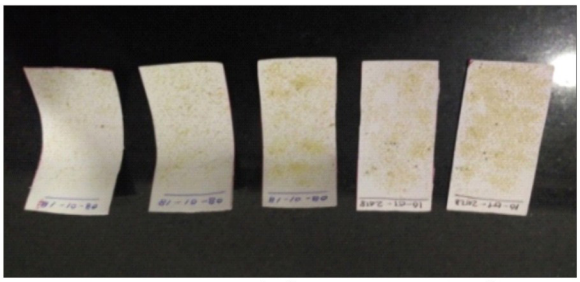
- Always enclosed beam path should be used as far as possible
- Protective eye wear and gloves should be used
- Arms and neck should be covered and neck and exposure time should be limited
- Looking directly at the beam should be avoided
- An electronic or a manual shutter should be used to close the beam

2. How to make Trichocards?

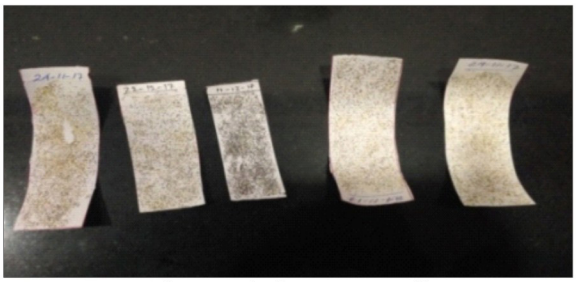
In transparent polythene pouch or bags UV treated Corcyra egg cards and Trichogramma nucleus cards are kept at the ratio of 6:1 as such for 24-48 hours (6 Corcyra egg cards: 1 Trichogramma nucleus card). The parasitoids will emerge from the nucleus card and the mated females will parasitize Corcyra eggs glued on the egg card. Parasitized Corcyra egg cards *i.e*. Trichocards are to be removed after 2 days. The colour of the Corcyra eggs turns black on 3rd day indicating successful parasitization. This darkening of colour happens due to deposition of dark melanin granules on the inner surface of the host egg chorion which indicates that the growing parasitoids larvae (inside the egg) has entered third instar of their developmental cycle. Adult emergence takes place nearly 3-6 days after blackening of the eggs. Entire life cycle is generally completed in 8 to 9 days, but it may be prolonged at lower temperatures. The tiny adults live for 6-8 days only. After successful parasitization, these Trichocards can be released immediately in the fields orcan be stored in refrigerator.

3. How to store the Trichocards?

Parasitized card can be stored at 10° C up to 15 days. Adult longevity and fecundity of the successive progeny can be impaired due to prolonged storage exceeding 15 days. Pupal stage of the parasitoids is the best stage for storage. Parasitized egg cards at blackening stage can be stored in a refrigerator at 12°C -15°C for 10-15 days.



Corcyra egg cards (Non-parasitized)



Trichocards (Parasitized)

Special Notes:

A. How Trichogramma kills the pests?

From a Trichocard, about 12,000 Trichogramma adults may emerge out within a week after parasitization. The tiny adults are very efficient in searching the pest's eggs (i.e. host eggs) in the field and parasitize them by laying their eggs within the eggs of the pest. Parasitoid larvae then develop by feeding on the embryonic contents of the pest egg and complete their development inside the egg within 7-8 days.

B. Precautions to be taken during preparation of Trichocard:

- ✓ Healthy eggs of hosts should be used to obtain healthy as well as energetic parasitoids
- ✓ Super parasitization can be avoided either by allowing the host eggs for parasitization up to 8 hours or by maintaining the ratio of 6:1 (fresh cards: nucleus card)
- ✓ Frozen Corcyra eggs should not be provided directly to the parasitoids as it affects their development
- ✓ Storage duration of parasitized eggs should not exceed 15 days as prolonged storage impairs biological attributes of the parasitoids and reduces their field performance
- ✓ Excess gum should not be used on the egg card while sprinkling the host eggs as the excess gum layer may interfere with the normal movement or activity of parasitoids
- ✓ To reduce the chances of damage during transport/ handling, Trichocards should be packed carefully in such a way that the parasitized surface remain on the inner side.
- ✓ Emergence date of the parasitoids must be mentioned on the cards to guide the user

C. Release of trichocards (frequency and dose):

It is suggested to release the parasitoids at periodic interval for borer pest management. Early in the season, for low level of infestations, 10,000-20,000 Trichogramma per acre per week can give satisfactory control over the pests when the release is continued throughout the season or for about eight to ten weeks. For medium to heavy infestations a minimum of 50,000 to 1,00,000 Trichogramma per acre should be released biweekly, or more frequently if necessary, throughout the season. The doses and frequency should be standardised depending upon the pest density, stage of the crop and environmental conditions.

Release of trichocards can give good control over notorious pests like sugarcane early shoot borer, bollworms of cotton, sorghum stem borer, rice yellow stem borer and many other borer pests of crops.

Examples: For controlling sugarcane early shoot borer in the beginning of crop season (4th week of planting), releasing 6,000 parasitoids per week per acre for a period of 5 weeks can give satisfactory reduction in pest population. A total of 30,000 parasitoids are to be released per acre. Whereas in cotton, a total of 3releases of Trichocards @ 5 cards / ha (*i.e.* one lakh eggs) starting from 45 days after sowing is recommended.

Recommended release rate of Trichogramma:

Crop/Insects	Trichogramma species	Recommended dose/ ha
Rice yellow stem borer	*T. japonicum*	1 lakh adults
Rice leaf folder	*T. chilonis*	20-30 DAT (Days After Transplanting) at 10 days interval
Sugarcane *Chilo species*	*T. chilonis*	0.5 lakh adults
Tomato fruit borer (*Helicoverpaarmigera*)	*T. pretiosum*	1.5 lakh adults at 10 days interval
Cabbage diamond back moth	*Trichogrammatoidea bactrae*	1 lakh adults at 10 days interval
Cotton boll worm	*Tr. Bactrae*	

D. Precautions to be taken at the field level:

- ✓ Parasitoids should be preferably released in the evening or morning hours in windward direction
- ✓ Insecticide spraying should be avoided in the crop at least 15 days before and/ or after release of Trichogramma
- ✓ To avoid direct sunlight, the cards should be stapled or pinned on the ventral side of the leaves in the middle region of the plant
- ✓ The card should be tied in different spots of the field avoiding the border rows/ plants.
- ✓ Trichocards may be stapled inside a plastic tea cup which can be tied to the leaf/shoot of the crop in an inverted manner to protect the eggs from the adverse climatic conditions, hyper- parasitism and attacked by the spiders or other natural enemies.

E. General life cycle of rice moth *(Corcyra cephalnonica)*

Larval period : 25-35 days
Pupal period : 10-14 days
Adult longevity :
Male : 5-7 days
Female: 7-10 days
Fecundity (eggs/female) : 150-200
Incubation Periods : 4-5 days
Viability of eggs (%) : 76.0%

F. Life cycle of parasitoid (*Trichogramma sp.)* on rice moth eggs

Fecundity (eggs/female) : 20-200
Incubation period : 24-36 hours
Larval period : 2-3 days
Pupal period : 3-4 days
Adult longevity :
Male : 5-7 days
Female : 5-20 days

Job: Draw pictures of Corcyra moth and Trichogramma adult here in the given space.

PART- III: Skill development of students on mass rearing of Corcyra and production of trichocards

Phase -1 : Laboratory mass rearing of host insect *Corcyra cephalonica*
Phase -2 : Preparation of Corcyra egg cards and Trichocards
Phase -3 : Repetition of the processes

Phase wise job schedule to be followed by the students

Phase - 1

Step 1: Prepare rearing box. Label the box mentioning its serial number and date of preparation. Inoculate the box with 1 cc Corcyra eggs and mention the date of inoculation in the label. Leave the box undisturbed for about 45 days for adult emergence.

I. Write date of preparation of the box:

II. Write box number:

III. Write about composition of feed material (mentions all ingredients and mention their amount):

IV. Mention the amount of Corcyra egg inoculated:

V. Write the date of inoculation with Corcyra eggs:

VI. Label the box mentioning the necessary information and take picture and paste here.

VII. Take photographs while doing the job in group and paste here.

Step 2: Start collecting Corcyra adults from any one of the previously maintained rearing boxes available in the laboratory.

I. Write the following information about the box from where you are collecting adults:

A. Mention box number:

B. Date of preparation of that box:

II. Write about the condition of that box. Is it free from any other secondary pest? If not, mention the other pests found in that box. Give picture of the pest.

III. Count the total number of adults collected for releasing into the egg laying cage. Try to differentiate male and female moths. If you can differentiate, give separate pictures of male and female moths.

IV. Have you faced any difficulty while collecting moths? If yes, mention it. Suggest about the possible solution to overcome this difficulty.

V. Take pictures of the group activity and paste here:

Step 3: Clean an egg laying chamber. Release the adults into that egg laying chamber. Provide cotton balls soaked in honey solution as an energy source for those adults.

I. How did you clean the egg laying chamber? Discuss and provide photograph:

II. Write the date of releasing Corcyra moths into the egg laying cage?

III. How many moths are being released?

IV. Mention box number:

V. Have you placed the cage on a white paper?

VI. Give photograph of the collected eggs. Properly label the cage and take photograph of the cage along with the label and paste it here.

Step 4: Start egg collection process. Clean the eggs by passing through 15, 30 and 45 mesh sieves serially and store in refrigerator in a glass vial/ good quality plastic tube until and unless collected eggs reach 1cc in volume.

I. After how many days of moth release first egg laying was noticed? Write the date.

II. Collect eggs every day and measure it volumetrically. Write day wise collected amount of eggs.

III. Finally, how much egg was collected from the box? Give volumetric measurement.

IV. Discuss the egg cleaning process.

V. Mention the time taken to collect 1 cc eggs.

VI. Take good photograph of the collected eggs and paste it here.Provide some photographs of group activity.

Phase - 2

Step 1: The eggs should be treated inside a UV chamber for 30-40 minutes to kill the embryo. For preparing Corcyra egg cards, white art paper should be cut into pieces of the size of 15 cm × 10 cm which can accommodate 1 cc eggs. Gum (preferably 10% gum arabic) should be applied on the cards leaving 1.25 cm area at the top and bottom side of the card. Clean eggs should be sprinkled uniformly in a single layer and the cards should then be air dried for 30 minutes. The excess eggs should be removed by gently passing a shoe brush over the card after sufficient air drying under fan.

I. Take photographs of your freshly prepared Corcyra egg cards and paste here.

II. Compare your egg cards with other groups. Observe whether the card is very neat and clean with evenly sprinkled eggs or not. Now Grade your egg card accordingly (A/B/C) by putting a tick mark:

Grade A: Very good
Grade B: Good
Grade C: Not so good or poor

III. Now clarify why you have given such grade.

Step 2: Take transparent polythene bags/plastic container and insert UV treated fresh cards/ Corcyra egg cards and also a nucleus Trichocard at the ratio of 6:1(6 Corcyra egg cards: 1 Trichogramma nucleus card). These should be left for another two days undisturbed.

I. Observe the eggs daily and mention day wise changes you found in your card. Is there any change in colourof the eggs? Share your views.

Step 3: Remove the Trichocards from the polythene bags/plastic container/ glass tubes and keep those separately. The eggs of these cards will turn black in colour on 3rd day indicating the successful parasitization.

I. After completion of 3 days (after parasitisation), take a clear photograph of the card and pasteit here.

II. Try to calculate the percent parasitization by counting the number of parasitized and unparasitized eggs.

$$\textbf{Rate of successful parasitisation (\%)} = \left(\frac{\text{No. of eggs turned black}}{\text{Total number of eggs}}\right) \text{X } 100$$

Step 4: Complete the labelling process.

I. Mention information in the given space of the card about the production unit, species of the parasitoids, date of parasitization and expected date of emergence. Take photograph of the labelled card and paste here.

Step 5: Subdivide the cards lengthwise into six grids of size 2.5 cm. Thus, the dimension of each grid becomes 10 cm×2.5 cm which can easily be handled.

I. Take photograph after subdividing the card and paste it here.

Step 6. Release the parasitized egg cards in the field or store these in refrigerator at 10^{0}C upto 21 days. Tie or staple cards on the leaf of the plant where you are going to release the parasitoids.

I. Mention whether you have directly used those cards in the field or you have stored.

Phase - 3

Step 1. Continue the egg collection process and follow the above described steps repeatedly, as applicable and necessary, depending upon the rearing condition or situation.

I. Write about the step(s) you repeated. Also mention the reason(s).

II. Write about the benefits as well as problems regarding the use of Trichocards from your own perception.

Extra space for providing pictures taken during the group activity

Step wise evaluation sheet

WORK PHASES	STEPS	OBTAINED MARKS	SIGNATURE OF THE EVALUATORS
Phase 1	Step I		
	Step II		
	Step III		
	Step IV		
	Special Comments:		
Phase 2	Step I		
	Step II		
	Step III		
	Step IV		
	Special Comments:		
Phase 3	Step I		
	Special Comments:		

TNAU Corcyra moth collector with minimum health hazard

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