NRRI Research Bulletin No. 52

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ICAR-NATIONAL RICE RESEARCH INSTITUTE
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Correct Citation
Gowda B., Patil N., Pandi G.P., Annamalai M., Prasanthi G., Pradhan H., Rath P.C., Mohapatra S.D., Nayak A.K. (2024). Advanced Techniques in Mass Production of Bioagents. NRRI research Bulletin No. 52, ICAR-National Rice Research Institute, Cuttack-753006, Odisha, India. pp-44.

Published by
Director
ICAR-National Rice Research Institute,
Cuttack, Odisha, 753006, India

August, 2024

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Printed in India at Print-Tech Offset Pvt. Ltd.,
Bhubaneswar, Odisha 751 024.

PREFACE

The agriculture sector is very concern about the rise in incidences of insecticide resistance by insects and phytopathogens to the synthetic pesticides; and worry of public about the environmental and health hazards brought out by the synthetic pesticides exposure and residues. Sustainable agriculture practices are crucial to accomplish the zero-hunger goal makes the necessity to embrace environmentally friendly control agents against plant diseases and insect pests. Biocontrol, the use of living organisms to manage insect and disease pests, grips the potential possible way to minimize the hazards associated with the use of synthetic pesticides, is a way trajectory for sustainable agriculture at a lower ecological cost.

Over few decades, the flourish in education opportunities, training on the plant protection techniques and increased consumer demands through the awareness created by researchers, academicians, and non-governmental organizations influenced successful employ of biocontrol on a large scale, especially in developing nations where research and implementation of augmentative and classical biocontrol are gaining momentum. Momentum shift to adopt biocontrol and investment in biocontrol research, training, and adoption is accelerating. Biocontrol is a cost-effective, eco-friendly, and long-term solution for crop protection and biocontrol agents will remain indispensable and play a significant role in modern agriculture.

ICAR-NRRI, the premier rice research institute had made a promising achievements and way forward in terms of biocontrol. It accommodates well-established biocontrol production facilities and supplies different biocontrol agents to cater the need of the region and nation. Besides supplying biocontrol agents, the institute is in forefront to promote and encourage biocontrol agents' adoption through conduct of various training programmes for farmers, youth, women and other stakeholders. The institute spearheads in creating entrepreneurs on biocontrol by assisting them with the technology, necessary knowledge support and time-to-time technical assistance.

One part of it, the institute with the coordinated efforts brings out a research bulletin on production of various biocontrol agents. These research bulletin accommodates various contents, spreading out as the basics in biocontrol agents' production, safety requisites and procedures in host as well as biocontrol agents' production, quality standards and norms for various biocontrol agents, entrepreneurship development, and different initiatives at NRRI for accerlating the mass-production of these bioagents. The institute focuses on producing three biocontrol agents, *Trichogramma japonicum, T. chilonis* and *Habrobracon hebetor* which are of key relevance in rice crop. The authors believe that this bulletin will be of great interest to students, researchers, academics, entrepreneurs and other stakeholders for enriched knowledge on the practical production procedure of biocontrol agents, skill enrichment on biocontrol agents' production and step forward for entrepreneurship evolvement. The authors gratefully acknowledge the financial aid from RKVY-Odisha for establishment of the facility and ICAR-NRRI for round-the-clock support for the successful take out of this eco-friendly technology to the farmers field and entrepreneurship opportunities creation.

Authors

FOREWORD

Globally agricultural systems face greatest challenges in managing crop damaging pest populations with minimal impact and disturb on the ecosystem and environment. Biological Control is the eco-smart and highly host-specific tool in integrated pest management strategy of any crop. Biological Control Agents (BCAs) branch off as predatory insects, parasitoids, and microbial pathogens have grown up as a formidable weapon to curtail pest populations. Recent advances and breakthroughs in the mass production, formulation, and delivery mechanisms of the biocontrol agents favoured for large-scale promotion and adoption.

ICAR-National Rice Research Institute, formerly called as Central Rice Research Institute, is a pre-independence institute established at the back drop of Great Bengal Famine of 1943. The institute has attained its highest pride when Padma Vibhushan Professor MS Swaminathan stated “that the seed of Green Revolution in rice was shown in fields of CRRI”.

Since its inception, the institute and the researcher left no stone unturned in rice research to boost the rice production and productivity. The institute has carried out exemplary activities in crop improvement, production, protection, and technology dissemination etc. Protecting rice crop from the damage of diverse insect and disease-causing pathogens utilizing various components of integrated pest management strategy was investigated vehemently at this institute. Research on biological control has seen a perfect trajectory of growth at this institute. ICAR-NRRI has a well-established biocontrol laboratory and production facility catering needs of various stakeholders and fulfilling the supply of various biocontrol agents to the farmers of Odisha and other parts of India. This unit also extend their expertise in conducting various skill development training and entrepreneurship developmental activities on biocontrol agents’ production and assures the delivery of quality biocontrol agents to the end-user.

A piece of it, the scientists of the institute bring this research bulletin comprehending a variety of intelligence, orienting towards the necessary procedures in biocontrol production, entrepreneurship opportunities in biological control, and different quality standards for various biocontrol agents etc. I, from my core of the heart congratulates the attainment of the authors for bringing out this publication offered with more emphasis on the practical-oriented instructions and knowledge. I hope this bulletin will be very much beneficial for students, academicians, researchers, and entrepreneurs etc. to enrich their practical knowledge on biocontrol agents’ production procedures and excel in the field of biological control.

Director
ICAR-NRRI

CONTENTS

Safety and Precautionary Considerations in Handling of Biocontrol Agents

Handling and releasing predators and parasitoids, which are types of biocontrol agents, require specific safety and precautionary measures to ensure they are effective while minimizing risks. For any successful biocontrol program first and foremost thing is proper identification of insect pest and the selection of correct biocontrol agents. Because accurate species identification ensures that the predators or parasitoids are correctly identified and that they are appropriate for the target pest species. Further, suitability assessment confirms that the chosen biocontrol agents are suitable for the specific environment where they will be released. Likewise, source and quality control are also essential as farmers need to obtain biocontrol agents from reputable, certified suppliers to ensure they are healthy, free of contaminants, and appropriately reared. Additionally, appropriate temperature and humidity levels during transport and storage to be maintained to prevent stress or mortality of the agents. Similarly, proper containers and handling techniques to be used to avoid physical damage to the biocontrol agents.

Users need to evaluate the release site to ensure it is conducive to the survival and efficacy of the biocontrol agents, including factors like climate, vegetation, and the presence of the target pest. Release agents at the optimal time, usually when the target pest is most vulnerable, to maximize effectiveness and minimize unnecessary dispersal. Use controlled release methods, such as releasing agents in stages or in specific areas, to monitor their impact and prevent overpopulation or spread to unintended areas. Regularly monitor the area after release to assess the effectiveness of the biocontrol agents and to detect any unintended consequences, such as impacts on non-target species. Document all aspects of the release, including the species released, locations, dates, and observed outcomes, to provide data for future assessments and regulatory compliance. Ensure that personnel involved in handling and releasing biocontrol agents are properly trained in safe practices. By implementing these safety and precautionary measures, the risks associated with the handling and release of predators and parasitoids can be minimized, ensuring that these biocontrol agents effectively contribute to pest management while protecting the environment and public health.

1. Safety in mass multiplication of parasitoids

Safety in the mass multiplication of parasitoids is crucial for several reasons, each of which contributes to the overall success of biocontrol programs and the protection of human health, the environment, and agricultural systems. In order to prevent contamination and disease infection with pathogens, parasites, or unwanted organisms that can compromise the health and quality of the parasitoids. This can lead to the spread of diseases within the colony, reducing the effectiveness of the biocontrol agents. In case, different species of parasitoids or other biocontrol agents might be reared in the same facility; strict safety

protocols are required to prevent cross-contamination. Similarly, genetic integrity should be ensured and inbreeding depression should be reduced. Mass rearing can cause genetic drift, where certain traits become overrepresented. This might lead to a loss of desirable characteristics, so careful management is required to maintain the genetic integrity of the population. Inbreeding can lead to a decline in the fitness and effectiveness of parasitoids. Safety protocols ensure genetic diversity by managing breeding practices and avoiding genetic bottlenecks. Personnel involved in the mass multiplication of parasitoids must be protected from potential allergens, toxins, or other health risks associated with handling large numbers of insects. If chemicals are used in the rearing process (e.g., to sterilize equipment or control contamination), safety protocols ensure that these are handled and disposed of correctly to avoid harm to workers and the environment.

Safety measures are necessary to prevent the accidental release of parasitoids into the environment during the mass multiplication process. This is especially important if the rearing facility is located in an area where the parasitoids are not native. Even within controlled environments, safety protocols are required to ensure that parasitoids do not escape or interact with non-target organisms, which could disrupt local ecosystems. Safety protocols help maintain consistent production quality, ensuring that the parasitoids produced are healthy, effective, and capable of performing their intended role in biocontrol. Proper safety protocols include meticulous documentation, which is essential for tracing the origins of parasitoids and ensuring compliance with national and international standards. Poor safety practices can lead to the loss of entire colonies, which can be economically devastating, especially if the production facility relies on continuous output to meet demand. High-quality, safely reared parasitoids are more likely to be effective in the field, ensuring their continued demand and market viability. Safety in the mass multiplication of parasitoids is critical to maintaining the health and effectiveness of the biocontrol agents, protecting human health, and ensuring the sustainability and success of biocontrol programs. By adhering to safety protocols, potential risks are minimized, and the benefits of using parasitoids in integrated pest management are maximized.

2. Safety/ precautionary measures during mass production of the host insect

- Ensure wearing with a PPE jacket and covering the nose and mouth with a mask while working in the laboratory.
- Sterilize the rearing boxes (hot air oven at 100 degree Centigrade for 1-2 hours- wooden box; sterilize with formalin- plastic box)
- To prevent any bacterial infection in host, streptomycin sulphate is added to the feed and mixed thoroughly
- Disinfect the racks, cages, boxes etc. with formalin and place them in the sun for six hours before rearing.
- While separating the eggs from the scales, cover your nose with a mask.

- Collect the moths daily in the morning hours (preferably 9-11am).
- Also collect the eggs daily from the oviposition cage.
- Air dried muslin sheets dipped in dicofol (0.05% solution) and spread over the feed in rearing boxes, if noticed severe mites infestation.
- A table lamp with 60 or100 W bulb can be kept near racks containing host (*Corcyra*) rearing boxes along with a plastic tub filled with water below the lamp such that the light of the table lamp should face the surface of the water. Other insects/Parasitoids will be attracted to light and in turn will fall into the water and die.
- Remember not to open the sterilized rearing boxes before 40-45 days of rearing the host inside it.

3. Safety measures during using of instruments for mass rearing

- While using a UV lamp, never look directly at the beam
- Cover arms and neck and limit exposure to UV rays
- Wear protective eyeglasses and gloves while working under UV lamps.
- Do not tilt/ shake the rearing box after releasing host eggs to the feed
- Host rearing boxes should have provision of wire mesh for aeration
- The lid of the box should be tightly closed , so that there would not be chances of entering any insects from outside.
- The trays should be placed in a convenient position to collect the moths after adult emergence
- Plastic bucket for oviposition: one side of the bucket should facilitated in such a way that it would be useful to remove dead moths after oviposition.
- Egg-laying cage should have provision for honey feed to adult moths.
- Do not forget to check the host larval development, 10 days after the release of eggs to the rearing box, simply by tilting the box at a 45◦ angle.

4. Safety/ precautionary measures in the rearing laboratory

- Ensure that the laboratory has a controlled and monitored environment, including temperature, humidity, and light conditions, to support the optimal growth and development of parasitoids.
- Implement effective pest control measures to prevent contamination or competition from other insects or organisms that could harm the parasitoids.
- Design the facility with secure containment measures to prevent the accidental escape of parasitoids. This includes using screened windows, sealed doors, and secure storage areas.

- Required lab personnel to wear appropriate PPE, such as lab coats, gloves, and, if necessary, face masks, to protect against potential allergens, contaminants, or exposure to chemicals used in the rearing process.
- Encourage regular hand washing and the use of hand sanitizers to prevent contamination when handling parasitoids or their host organisms.
- Maintain strict sanitation protocols, including regular cleaning and sterilization of rearing containers, tools, and surfaces, to prevent contamination and the spread of diseases within parasitoid colonies.
- Handle parasitoids and their hosts gently to avoid injury or stress, which can affect their health and reproductive success.
- Conduct regular inspections of parasitoid colonies for signs of disease, deformities, or other issues that could affect their quality and effectiveness.
- Maintain detailed records of rearing conditions, production batches, and any issues that arise to ensure traceability and facilitate troubleshooting if problems occur.
- Ensure that all personnel involved in parasitoid rearing are properly trained in safe handling practices, and the protocols specific to the rearing of each parasitoid species.
- Train personnel on emergency procedures, such as how to respond to accidental spills, escapes, or exposure to injurious/hazardous materials.
- If chemicals are used in the rearing process (e.g., disinfectants, sterilization agents), ensure they are handled and stored safely, following all relevant safety data sheets (SDS) and guidelines.
- Ensure proper ventilation in areas where chemicals are used to prevent the buildup of fumes or exposure to harmful substances.
- Dispose of biological waste, including dead parasitoids, contaminated materials, and used rearing substrates, in accordance with biosafety protocols to prevent environmental contamination.
- Keep detailed records of all rearing activities, including the origin of parasitoids, rearing conditions, any treatments applied, and outcomes. This documentation is crucial for quality control, traceability, and regulatory compliance.

5. Precautionary measures during rearing

- Scales of laboratory host *C. cephalonica* may cause the respiratory problems; it is advised to wear a mask while rearing *C. cephalonica*.
- Trichocard should be properly sealed before keeping in the refrigerator to avoid any type of accident.

Implementing stringent safety measures during the handling of parasitoids in the laboratory is crucial for maintaining the health and effectiveness of the biocontrol agents, ensuring the safety of personnel, and preventing environmental contamination. These measures include proper use of personal protective equipment (PPE), maintaining clean and controlled rearing conditions, and adhering to strict protocols for handling and disposal. Regular monitoring and documentation, along with appropriate training for all personnel, are essential to mitigate risks such as contamination, disease, or accidental release. By prioritizing safety, laboratories can ensure the successful and sustainable production of parasitoids for pest management programs. Future safety strategies for handling parasitoids in the laboratory should focus on integrating advanced technologies and enhanced protocols to further mitigate risks and improve efficiency. Utilizing automated monitoring systems for environmental control, genetic screening to maintain healthy colonies, and AI-driven data analysis for early detection of potential issues can significantly enhance safety. Collaboration with international biocontrol experts and continuous updating of protocols based on the latest research will also be vital in addressing emerging challenges and ensuring sustainable biocontrol practices.

Infrastructure and Utilities of Bioagents Mass-production Laboratory

Biological control is an essential part of integrated pest management (IPM), which involves using natural enemies such as parasitoids to manage pest populations. In this context, *Trichogramma* and *Habrobracon* are two commonly used parasitoids. Effective rearing, handling, and application of these biological agents require specialized materials and equipment in a biocontrol laboratory. This document outlines the essential materials and equipment used for working with *Trichogramma* and *Habrobracon*, detailing their specific functions.

Rearing and Culture Rooms

Insect biocontrol laboratories require dedicated rearing and culture rooms designed to maintain optimal environmental conditions necessary for the successful propagation of *Trichogramma* and *Habrobracon* species. These rooms are equipped with environmental control systems to maintain temperature, humidity, and light conditions that mimic the natural habitats of these parasitoids.

- **Temperature:** Typically maintained between 25-28°C for *Trichogramma* and *Habrobracon* rearing.
- **Humidity:** Generally kept at 60-70% relative humidity.
- **Light:** Controlled light cycles are crucial, often simulating a 16:8 hour light-dark cycle.

These conditions ensure the proper development of the insects, from egg to adult, ensuring a steady supply of parasitoids for biocontrol applications.

Processing and Laboratory Spaces

Separate laboratory spaces are crucial for different activities, such as insect collection, preparation of host eggs (for *Trichogramma*), and experimental procedures. Each space is designed to prevent cross-contamination between different stages of insect rearing and between different parasitoid species.

- **Diet Preparation Room:** For preparing nutrient-rich diets necessary for host insect rearing.
- **Culture Preparation Room:** Used for preparing and storing host eggs, crucial for *Trichogramma* and *Habrobracon* rearing.

Utilities (Power and Water)

- **Power:** Essential for operating incubators, laminar flow hoods, and other critical laboratory equipment. A backup power system is necessary to prevent disruptions in the rearing process.

- **Water:** High-quality water is required for cleaning, preparation of diets, and maintaining humidity levels in rearing rooms. Regular testing ensures that water does not introduce contaminants that could harm the insect cultures.

Essential Equipment in mass-production laboratory

Microscopes

Microscopes are vital for observing the morphology and behavior of *Trichogramma* and *Habrobracon* species at different developmental stages. They are essential for identifying and selecting healthy eggs and larvae, ensuring the quality of the parasitoid cultures.

- **Stereomicroscopes:** Used for examining and sorting larger specimens, such as host eggs parasitized by *Trichogramma*.
- **Compound Microscopes:** Employed for detailed observations of smaller structures, such as parasitoid eggs and larvae.

Insect Rearing Cages

Insect rearing cages are designed to provide a controlled environment for rearing *Trichogramma* and *Habrobracon* species. These cages allow for efficient feeding, observation, and collection of adult parasitoids.

Sizes: Typically range from small (30 cm x 30 cm x 30 cm) to large (60 cm x 60 cm x 60 cm) cages, depending on the scale of rearing.

These cages are designed to maintain the optimal environmental conditions and prevent the escape of the insects, ensuring a stable and controlled rearing environment.

Incubators

Incubators are critical for maintaining the precise temperature and humidity conditions necessary for the development of *Trichogramma* and *Habrobracon* species. They are particularly useful for hatching parasitoid eggs and ensuring the successful development of larvae and pupae.

- **Temperature Range:** 25-30°C, adjustable to meet the specific needs of the species being reared.
- **Humidity Control:** Integrated systems to maintain humidity levels around 60-70%.

Laminar Flow Hood

Laminar flow hoods provide a sterile environment for handling sensitive biological materials, such as host eggs and *Trichogramma* pupae. This equipment is essential for preventing contamination during the preparation of host materials and during the transfer of parasitoids.

- **Function:** Filters air through HEPA filters, ensuring a clean working environment.

- **Use:** Handling of *Trichogramma* eggs and larvae, preparation of sterile diets for host insects.

Rearing and Culture Materials

Insect Diets and Host Eggs

Specialized diets are essential for rearing the host insects, such as *Corcyra cephalonica* or *Sitotroga cerealella*, which are used as egg sources for *Trichogramma* and *Habrobracon* species. The quality of these diets directly impacts the health and productivity of the parasitoid colonies.

- **Diet Composition:** Typically includes grains (e.g., maize flour), vitamins, and sometimes antibiotics to prevent microbial contamination.
- **Host Eggs:** *Trichogramma* species are typically reared on the eggs of *Corcyra cephalonica*, while *Habrobracon* species may be reared on the larvae of specific host insects.

Autoclave

Autoclaves are used to sterilize equipment and other materials by subjecting them to high-pressure steam. This is crucial to prevent contamination, which could compromise the health of *Trichogramma* and *Habrobracon* cultures.

- **Function:** Sterilizes rearing trays, collection tools, and host eggs before they are introduced to the parasitoids.
- **Size:** Typically, bench-top autoclaves with a chamber size of around 30-50 liters are used in biocontrol labs.

Collection and Handling Equipment

Insect Collection Tools

- **Aspirator:** Used to gently collect small insects like adult *Trichogramma* or *Habrobracon* without causing harm. Aspirators are crucial for transferring parasitoids from rearing cages to release containers.
- **Collection Net:** Utilized for capturing flying insects, particularly when collecting host insects in the field.
- **Vacuum Pump:** Used for efficiently collecting large numbers of small insects in rearing setups.

Dissecting Tools

Dissecting tools, including scalpels, forceps, and scissors, are essential for preparing insect specimens for further study or for experimental procedures. These tools are also used for extracting *Habrobracon* larvae from their hosts or for dissecting host eggs parasitized by *Trichogramma*.

- **Sizes:** Forceps and scissors typically range from 10-15 cm in length, allowing precise manipulation of small specimens.

Storage and Maintenance Equipment

Refrigerators and Freezers

Refrigerators and freezers are used to store biological samples, host eggs, and reagents at low temperatures, preserving their viability and preventing degradation.

- **Temperature:** Refrigerators are typically set at 4°C to store host and parasitoid stages
- **Use:** Storing *Trichogramma* and *Habrobracon* pupae, preserving host eggs for future use.

Chemical Reagents and Preparation Tools

Chemicals

- **Industrial Alcohol:** Used for cleaning and surface sterilization, ensuring that equipment and work surfaces remain free from contaminants that could affect the rearing of *Trichogramma* and *Habrobracon* species.
- **Sodium Hypochlorite Solution:** A disinfectant used to sterilize tools and surfaces, critical for maintaining a clean environment in the rearing and culture rooms.

Insect biocontrol laboratories play a vital role in sustainable pest management by producing and studying these natural enemies of pests. The materials and equipment discussed are essential for the effective rearing, handling, and application of these parasitoids. Proper infrastructure, precise environmental control, and specialized tools ensure the success of biocontrol programs, contributing to more environmentally friendly agricultural practices.

Mass Production of *Corcyra cephalonica*: A Laboratory Host

The rice meal moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (Fig.1.), is the leading species of host insect in mass culturing of entomophagous insects. This is due to its suitability for large-scale production, adaptability to different rearing conditions, and its beneficial impact on the offspring of natural enemies.

Corcyra cephalonica (Stainton), a pest of stored grains, has been identified as one of the most effective surrogate hosts for breeding various biological control agents. Key agents reared on its larvae include egg parasitoids such as *Trichogramma* spp., egg-larval parasitoids like *Chelonus blackburni*, and larval parasitoids such as *Habrobracon hebetor, Goniozus nephantidis*, and *Apanteles angaleti*. Insect predators like *Chrysoperla zastrowii sillemi*, *Mallanda boniensis*, etc. are also reared on *C. cephalonica* larvae. Additionally, entomopathogenic nematodes like *Steinernema feltiae* are reared on these larvae. For effective production of these biological control agents, a well-managed and healthy rearing environment is essential. *Corcyra cephalonica* can be mass-reared throughout the year across all ecological zones of India at 28°C ± 2°C and 65 ± 5% relative humidity, ensuring both economic feasibility and high-quality egg production.

Fig. 1. *Corcyra cephalonica*, laboratory host

Table 1. Biological characteristics of *Corcyra cephalonica*

Stages	Range (days)
Egg	4-7
Larval instars	
I	4-5
II	5-6
II	3-4
IV	3-4
V	5-7
VI	8-10
Total larval period	28-36
Pupal period	9-16
Total development period (days)	41-59
Mating Period (minutes)	60-130
Pre-ovipositional period (days)	1-2
Ovipositional period (days)	6-8
Post oviposition period (days)	1-3
Adult Longevity (days)	7-9
Sex ratio (Female: Male)	1.5 ± 0.3

Common material required for rearing of laboratory host, *Corcyra cephalonica*

- Absorbent cotton
- Blotting paper
- Broken sorghum grain
- Camel hair brush
- Enamel Tray
- Honey
- Muslin cloth
- Formaldehyde 40%
- Filter paper
- Storing drums
- Coarse weighing balance
- Brush
- Soap
- Yeast powder
- Groundnut kernel
- Storage racks
- Streptomycin sulphate
- Measuring cylinder
- Oven
- Sieves
- Moth scale egg separator
- Face masks
- Sulphur (WP)
- Mosquito net
- Moth aspirator (collector)
- Oviposition cages
- Plastic basins
- Specimen tube
- Filter paper
- Animal supplement

The detailed procedure of mass rearing of *Corcyra cephalonica*

1 Square shaped transparent plastic boxes of dimension (38 cm L × 29 cm W × 36 cm H) having lid fitted with wire mesh (40 mesh size) has to be taken by creating empty space of 2.5-4.0 cm between lid and mesh. This empty space will avoid *Habrobracon* infestation during mass-rearing

2 Heat sterilized broken maize (2.5 kg), 100 g coarse groundnuts seed powder, 5 g of yeast and 2 g of tetracycline, 5gm animal supplement mixture (vitamins and mineral) are to be used as diet for the growing larvae.

3 The rearing tray consisting of with properly sterilized and chemical free diet is to be charged (inoculated) with clean and fresh 0.25 cc (~5000 eggs) *Corcyra* eggs.

4 After charging, the boxes have to be covered with muslin cloth before it is closed with its lid. The trays placed in the racks in the racks without disturbance of diet till the completion of larval development

5 A plastic container of approximately 15 litres capacity to be modified for preparing the oviposition cage. The mouth of the container (25 cm diameter) covered with wire mesh (15 mesh size) and a circular hole of 2.5 cm diameter to be cut open on the opposite face of the container to facilitate the entry of inlet pipe during moth collection using vaccum system.

Similarly, a small window of size 12 × 8 cm cut open on the side wall of the container and covered with wire mesh. This facilitates the free escape of air during vacuum suction.

6 After moth emergence, moths were collected through a vacuum based moth collection system (motor power: 120 W) consisting of an outer plastic circular container (50 L) and an inner oviposition chamber (15 L) covered with wire mesh on its base to drop the eggs.

For the collection of moths a larger plastic container of 50 litres capacity to be used which could accommodate the oviposition cage (15 L) inside it. A plastic pipe of 2 ft. length will pass through the hole on the lid of the larger container and ultimately connected to the oviposition cage placed inside.

To another hole at lateral side of the larger container, the hose of the vacuum cleaner to be fitted. By starting the vacuum cleaner, the moths will automatically be drawn into the oviposition cage along the air current.

7 Each oviposition cage has the capacity to harbour nearly 1000 moths. The oviposition cages with moths are to be kept in the lab and the moths are to be provided with 50% honey water solution mixed Vit. E tablet (one capsule/20ml of 50 % honey) soaked in cotton and attached onto the inner wall of the oviposition cage to boost the vigour of the adults and to get higher quantity of healthy eggs.

8 The eggs are loosely laid by moths and they are collected through the wire mesh bottom on a oviposition cage. Eggs are collected daily. The collected eggs are sieved 3-4 times to get cleaner eggs for further use. One CC of *Corcyra* eggs contain approximately 18,000-20,000 eggs. About l00 female moths produce 1-1.5 CC eggs in 4 days. From each basin an average of 2000-2500 moths emerge. In total, from each rearing tray 20-25 CC eggs are obtained during the period of 90 days.

Host moth collection: ICAR-NRRI initiative

ICAR-NRRI has developed a prototype 'Efficient Portable Insect Collector with Automated Counter' for which Indian patent has been granted (Patent No. 480911).

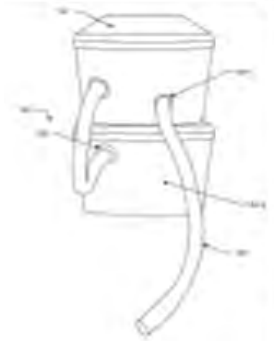
Summary of the product:

The *Corcyra cephalonica* rice meal moth is one of the crucial factitious hosts for the laboratory rearing of several parasitoids, including chrysopids, braconids, and trichogrammatids. Large-scale production of the host insects is necessary for the bulk growing of these parasitoids. Because *C. cephalonica*, the host insect, is essentially a moth and is raised in a hidden room, touching this insect—especially when it's an adult—emits a large number of scales, which handling staff may inadvertently inhale. Currently, moths are collected manually in test tubes and released into oviposition cages, a process that takes a lot of time. Additionally, the worker finds it challenging to count the moths.

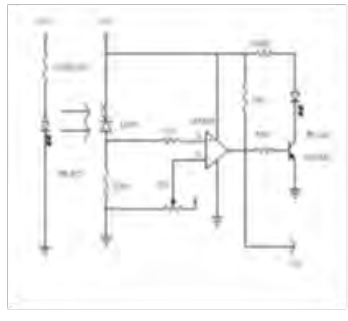
It's an insect collector, more precisely a battery-powered, rear-mountable insect collection system. An easy-to-use, lightweight device that requires to collect and count insects. It is a time- and energy-efficient portable device. Very useful for facilities that produce large quantities of insects and for collecting insects in the field at any time or place. This method provides an economical fix and does away with the labour-intensive procedure. It protects the worker's health throughout the moth collection process in a mass production facility, which could otherwise result in respiratory issues.

The product overcomes the drawbacks, shortcomings, and limitations associated with the conventional apparatus by providing a moth collection apparatus that includes a translucent Carboy tank of suitable capacity (38 cm L * 29 cm W * 36 cm H), its lid is fitted with wire mesh so as to make the entire tank an oviposition chamber. The entire apparatus is portable i.e., hand-held, and back mountable and battery-operated. The moths are being sucked through a vacuum device having a vacuum pump by adjusting the vacuum through an adjuster. The vacuum level is adjusted depending on the body size of the moth. The apparatus comes with a complete and flexible suction hose, which makes its entry into the Carboy tank and an electronic optical counter with a sensor to count the moths going into the oviposition chamber.

Further, the sensor configured in the bottom unit, the sensor adapted to detect the change in the light signal caused by the movement of the insect. The sensor is an optical sensor that includes a light emitting diode (LED). A counter coupled to the sensor, the counter adapted to count the number of insects detected and captured in the upper unit and the processor operatively coupled to the sensor and the counter. The processor is configured to receive, from the sensor, a change in the light signal caused by the movement of the insect. The processor can generate, by the counter, a count value of the insects detected by the sensor on the upper unit and display the count value of the insects present in the upper unit.



Outer view of the setup



Circuit diagram of the light sensor

Worker collecting moths

A microprocessor or other devices that can be programmed or configured to carry out calculations and instruction processing in line with the disclosure may be included in the processor. An Arduino processor might be the CPU in one example. In place of or in addition to a microprocessor, these other devices could be microcontrollers, digital signal processors (DSP), field programmable gate arrays (FPGA), complex programmable logic devices (CPLD), application-specific assimilated circuits (ASIC), discrete gate logic, and/or other assimilated circuits, hardware, or firmware.

Fig. 2. Worker collecting moths using portable insect collector in Biocontrol laboratory at ICAR-NRRI, Cuttack

Advantages:

- Safeguards the health of the worker involved while collecting the moths which otherwise would lead to respiratory problems
- The product has potential to be adopted on a wider scale in all the mass production (Biocontrol) laboratory throughout the country

Proposed stakeholders

- Biocontrol laboratories:
- State agriculture universities/ICAR institutes
- Entrepreneurs who involved in biocontrol agent's business
- Commercial mass production labs abroad

पेटेंट कार्यालय,भारत सरकार
पेटेंट प्रमाण पत्र
(पेटेंट नियमावली का नियम 74)

The Patent Office, Government Of India
Patent Certificate
(Rule 74 of The Patents Rules)

पेटेंट सं. / Patent No. : 480911

आवेदन सं. / Application No. : 202211047342

फाइल करने की तारीख / Date of Filing : 19/08/2022

पेटेंटी / Patentee : Indian Council of Agricultural Research

आविष्कारकों का नाम /Name of Inventor(s) : 1.BASANA GOWDA G 2.TOTAN ADAK 3.PRAKASH CHANDRA RATH 4.NAVEENKUMAR B PATIL 5.GURU PIRASANNA PANDI G 6.ANNAMALAI M 7.HARMOHAN PRADHAN

प्रमाणित किया जाता है कि पेटेंटी को, उपरोक्त आवेदन में यथावर्णित *EFFICIENT PORTABLE INSECT COLLECTOR WITH AUTOMATED COUNTER* नामक आविष्कार के लिए, पेटेंट अधिनियम, 1970 के उपबंधों के अनुसार आज तारीख अगस्त 2022 के उन्नीसवें दिन से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled *EFFICIENT PORTABLE INSECT COLLECTOR WITH AUTOMATED COUNTER* as disclosed in the above mentioned application for the term of 20 years from the 19th day of August 2022 in accordance with the provisions of the Patents Act,1970.

अनुदान की तारीख
Date of Grant : 12/12/2023

Controller of Patents

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, अगस्त 2024 के उन्नीसवें दिन को और उसके पश्चात प्रत्येक वर्ष में उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained, will fall / has fallen due on 19th day of August 2024 and on the same day in every year thereafter.

Patent Certificate

Common problems encountered in mass rearing of *Corcyra cephalonica*

Common problems encountered in rearing *C. cephalonica* are;

1) Incidence of stored grain pest *Tribolium castaneum,*

2) The parasitoid *Habrobracon hebetor*

3) Mite *Pymotes ventricosus*

Therefore, some precautionary measures are essential to prevent them.

1. *Tribolium castaneum:* This stored pest competes with *Corcyra* sp. larvae for food and reduce the production considerably. Therefore;

- Get pest free grains
- Sterilise grains at 100°C for one hour using hot air oven.

- Spray grains with 0.1% formalin or fumigate.
- Place thick paper over the food to which the adult beetles congregate, then remove them and kill:
- Setup a 60 Watts lamp during nights over yellow pan with water. Lamp attracts beetles and the beetle fall in the water.
- Keep tray or basins at random containing 250 gm of wheat flour with 5% brewer's yeast to act as flour trap for beetle. Two such beetle traps are kept for every l00 rearing trays. Sieve the flour on alternate days and destroy the grubs and beetles attracted.
- Avoid using food grains which have more bran and husk.

2. *Habrobracon hebetor:* Larval endoparasitoid, parasitise larvae and multiply causing loss of host culture.

- Create an empty space of 2.5-4.00 cm between the lid and wire mesh of rearing tray
- Cover windows and all other openings with wire mesh
- Avoid using holed clothes for covering trays.
- Setup light trap with 60 Watts bulb over yellow pan with water. Switch on light in nights so that parasitoids are attracted and killed. In case of serious incidence destroy the culture.

3. Mites: *Pymotes ventricosus*

The mites cause serious problem in rearing *Corcyra*. These suck the sap from eggs laid and also spread in trays. To overcome the problem, mix 5 grn wettable sulphur regularly as prophalyactic measure along with diet. Dust sulphur over the trays, furniture's, rearing racks if incidence is severe. Frequent washing and changing of used cloth is essential to reduce mites. The laboratory should be free from dust and scales.

4. Scales: Prolonged inhaling of the scales during cleaning is highly detrimental to health leading to respiratory disorders viz., inhalation toxicity, swollen alveoli of lungs, irreversible damage and allergic reactions to eyes and skin. Therefore, utmost importance should be given for workers by covering face masks, fixing sufficient exhaust fans and devices to separate eggs and scales.

Other general practices:

- Recycle the culture trays once in 90 days
- Change the source of insect after 3-5 generations
- Regular inspection of trays
- Streptomycin sulphate should be added precisely since higher dose may lead to sterility of adults.

Mass Production of *Trichogramma japonicum* and *Trichogramma chilonis*

Introduction

Trichogramma (Hymenoptera) are egg parasitoids that are widely distributed in all terrestrial habitats. They belongs to Trichogrammatidae family, which includes 80 genera, with sizes ranging from 0.2 to 1.5 mm. *Trichogramma* are primarily used to control Lepidopteran pests, but they also parasitize the eggs of insects from the Neuroptera, Diptera, Hymenoptera, Coleoptera, and Hemiptera orders. Due to their widespread natural occurrence and effectiveness as biocontrol agents, they are critical for plant protection when released in large quantities. This parasitoid is the most commonly produced and used biological control agent worldwide because it targets pests in their egg stage before they can damage crops and can be easily mass-reared in biocontrol laboratories.

Biology of *Trichogramma sp.*

All *Trichogramma* species have a relatively similar life cycle. *Trichogramma japonicum* (Fig. 3.) and *T. chilonis* (Fig. 4.) are most promising biological control agents for managing rice stem borers, and rice leaf folders, respectively. Female *Trichogramma* parasitoids drill a hole in the chorion and lay their eggs inside the host egg, acting as egg parasitoids. A small drop of yolk is expelled from the oviposition hole due to the egg's internal pressure, which the female consumes to prolong her life. Under laboratory conditions, a female parasitizes between one and ten eggs per day, totalling between 10 and 190 eggs over her lifetime. Larger females parasitize more eggs than smaller ones. The number of eggs laid per host egg can range from 1 to 20 or more, depending on the host egg's size. However, due to the small size of stem borer eggs, only one or two parasitoids can develop inside a single egg.

Fig 2. *Trichogramma japonicum*

A female parasitoid can recognize already parasitized eggs in natural conditions, thus avoiding superparasitism or multiple parasitism. Parasites prefer eggs that are still in the developmental stage. Older eggs, especially those where the larva's head capsule is visible, are rarely parasitized, and if they are, the parasitoid survival rate is significantly reduced. The pre-digestion of the egg contents is mainly caused by the female's venom injected during egg-laying. Dark granular melanin forms on the egg's surface during the third instar, produced 3 to 4 days after the host egg is parasitized, causing the host egg to turn black. This color change is an important diagnostic feature for distinguishing between parasitized and non-parasitized eggs.

Fig. 3. *Trichogramma chilonis*

Adult parasitoids emerge from the pupae and exit the host egg by chewing through the shell, creating a circular hole. The parasitism of *Trichogramma* can be identified by the dark coating on the chorion and the escape hole. At 28°C, the egg, larval, and pupal stages of *Trichogramma* take about 1 day, 3-4 days, and 4-5 days respectively to complete, although it can be slowed down at very high temperatures or extended at lower temperatures. On an average adult parasitoids live for 10-12 days. After emerging, adults immediately begin mating, and oviposition occurs. In most cases, the sex ratio is 1:1.

Mass Production procedure

Trichogramma japonicum and *Trichogramma chilonis* are being mass-produced on *Corcyra* eggs. At ICAR-NRRI, they are being mass-produced and supplied in the form of NRRI Trichocard (T.j) [Fig.4.) and NRRI Tricho card (T.c) [Fig. 5.]

Fig. 4. NRRI Tricho card (T.j)

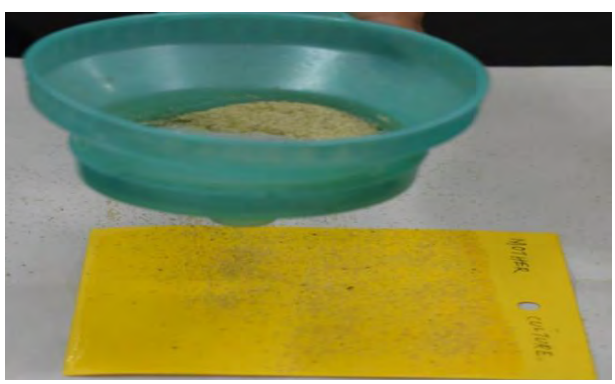
Fig. 5. NRRI Tricho card (T.c)

Material required for rearing *Trichogramma japonicum* and *Trichogramma chilonis*

1) *Corcyra* eggs	9) Muslin cloth
2) Mother culture of *T. japonicum* and *T. chilonis*	10) Honey
3) Tricho cards	11) Mesh sieve/ Tea strainer
4) UV Sterilization Chamber	12) Scissors
5) Refrigerator	13) Rubber bands
6) Chart Papers	14) Glass tubes
7) Gum Arabic	15) Plastic trays
8) Camel hairbrush	16) Zipper Bags

The detailed procedure of mass rearing of *Trichogramma japonicum* and *T. chilonis*

1 To arrest the embryonic development of freshly collected (0-4 hour old) eggs of *C. cephalonica* these are to be irradiated with ultraviolet light of power 15 W till a period of 45 minutes. 30 cm of distance should be maintained between the eggs and UV tube in sterilization chamber.



2 The amount of sterilised eggs is measured volumetrically with the help of a measuring cylinder. Smear 50 % Arabic gum on card grids, and eggs of one cc are sprinkled uniformly using a tea strainer or through automatic egg dispenser. These cards are considered as a commercial tricho card.

The card is divided into four 9 x 8 cm sections (LxB). It is separated into 8 2.5cm grids throughout its length. Each grid measures 4 x 2.5 cm in size. 0.125 cc of eggs can be placed in each square.

3 These eggs are exposed to female wasps in a 100:1 ratio till mortality of adults occur. The insect population is maintained in the glass tubes which are to be used for mass production of tricho cards. The eggs turning black indicate parasitism and their number is to be recorded to know the rate of parasitism. To achieve successful parasitism, the parasitoid-host card ratio is adjusted to 1:6. The parasitized eggs in the Tricho Card turn back in three or four to five days, and the adult parasitoids emerge eight to ten days after parasitization.

4 The parasitized eggs at the pupal stage i.e. post turning black can be stored at 5°C for about three weeks without losing their quality.

How to Use Tricho cards?

When the parasitoids reach the pharate stage or a few number of adults emerge from the host egg in the evening time. For managing yellow stem borer in rice, three NRRI Tricho-card (T.j) may be applied from 30th day after transplantation. Whereas for managing rice leaf folder, NRRI Tricho-card (T.c) may be applied from 45th day after transplantation. Tricho-cards having 1 CC eggs in each card (consisting of ~ 60000 parasitized eggs) per hectare are applied usually. Four such releases are made at every 7-10 days interval till egg masses or moth activity is not seen, whichever is earlier. Without damaging the eggs, the cards are split into pieces along the dotted lines and stuck in an inverted plastic cup (Fig. 6) in different sites of the field.

Fig. 6. Field release of Tricho-cards in field

Advantages of using Tricho cards

- Less expensive, more effective.
- Suitable as organic farming input
- Field application (releases) is quite straightforward.
- No environmental contamination

Availability and sale

- Bioagents are available in the Division of Crop Protection, ICAR-NRRI, Cuttack. Prior indent (before 45 days) is expected if the required number of cards exceeds 50. Cards will be supplied after the quality check at the production laboratory.
- Trichocard [NRRI Tricho-card (T.j); NRRI Tricho-card (T.c)] - Rs. 90 per card (contains 18000-20000 parasitized eggs).

Precautions while producing and using Trichocards

- Control failures can be caused by poor quality of mass-reared *Trichogramma*.
- Growers and pest specialists are unable to detect low quality trichogramma prior

to release, with the exception of addressing challenges like as the absence of adult emergence or wing abnormalities.

- To ensure that the percent host egg parasitization, the sex ratio of emerged adults are all within permissible limits, suppliers should examine them.
- The parasitized surface of Tricho cards should be on the inner side of the pack while shipping.
- The batch wise quality standard data needs to mentioned on the backside of the cards for the users' convenience.
- Tricho cards should be placed in such a way to avoid direct sunlight.
- To avoid predation, the card should be placed early in the morning and immediately before emergence.
- Pesticides should not be used in fields where Tricho card have been released. Selective and safer pesticides can be employed if necessary, and pesticides must be applied 15 days before or after the discharge of Tricho card.

Fig 7. – Graphical representation of mass rearing of *Trichogramma sp.*

Mass Production of *Habrobracon hebetor*

Habrobracon hebetor is a ectoparasitoid wasp that parasitize on the larval stages of numerous lepidopteran pests. The parasitoid, *H. hebetor* augmentation biological control is the most promising technique for controlling the primary insect pest of rice and stored grains. Because *H. hebetor* survival is difficult during the nine-month off-season when the hosts are in diapause, a sufficient supply of parasitoids must be available for fresh release each year. As a result, to develop a small-scale parasitoid rearing technique that is adapted to the environmental circumstances and can be scaled up as needed. Research are being conducted to smooth and standardise the *H. hebetor* rearing technique for commercialization.

Biology of *Habrobracon hebetor*

It feeds on the insect host's larvae stage and lays eggs on the host's surface. It is a small parasitic wasp that grows to be two mm long. Female have shorter antennae and are larger in size than males, which have 22-27 cylindrical segments. *H. hebetor* eggs are hymenopteriform in shape, size is 0.52mm length and 0.12 mm breadth and are often connected to paralysed host larvae. Larvae start eating on host body fluids as soon as they hatch by putting their mouth parts into the host. The last instar are with a length of 2.64 mm, a head capsule of 0.29 mm, and a width of 0.95mm lasts roughly four days before spinning little white cocoons for pupation. Two or more parasitoid larvae grow and pupate from each host larval. The egg stage lasts 1-2 days, the larval stage 2-4 days, the pupal stage 7-8 days, and the adult stage 20-70 days. Eggs can be arranged individually or in groups of two to eight. During her lifetime, a female can lay 250-300 eggs on 30-40 host larvae. On average, a female lays 2-27 eggs every day. During the first ten days of oviposition, the female lays the most eggs. The different stages of *H. hebetor* are described in Fig.8.

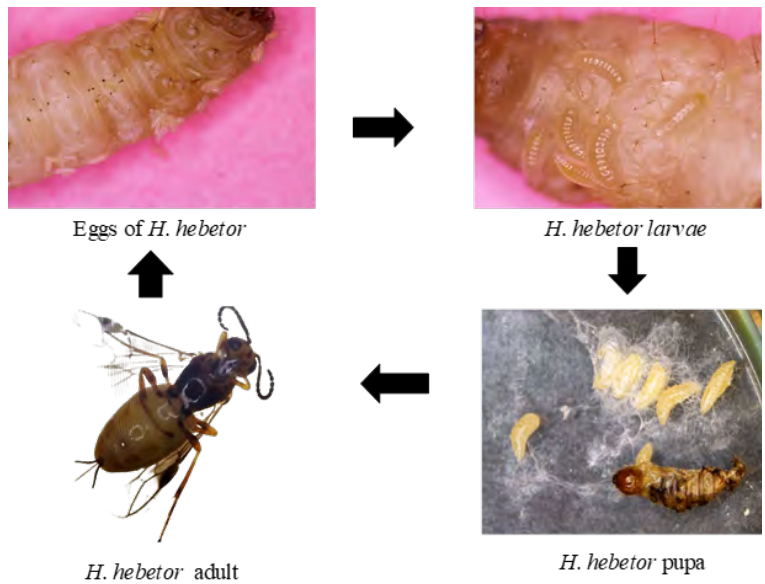


Fig. 8. Different stages of *H. hebetor*

Material required for rearing of *Habrobracon hebetor*

1) *Corcyra* larvae	8)Plastic containers
2) Nucleus culture of *Habrobracon*	9) Honey
3) Printed Bracon cards	10) Camel hairbrush
4) Plastic tubs	11) Scissors
5) Refrigerator	12) Rubber bands
6) Chart Papers	13) Zipper Bags
7) Muslin cloth	14) Forceps

Mass Production procedure

Generally, *Habrobracon hebetor* are mass-reared on grown up larvae (fourth and fifth instars) of *C. cephalonica.* At ICAR-NRRI, they are being mass-produced and supplied in the form of NRRI Braconcard (B.h) [Fig. 9.]

Fig. 9. NRRI Bracon card (B.h)

The detailed procedure of mass production

1 Take a clean, dry round base and wide mouth plastic tub of diameter 30 cm. The cards containing pupae of *H. hebetor* are to be placed in the plastic containers and cover with a muslin cloth with the help of rubber bands. The adult parasitoids are to be provided with 50% honey solution by dipping cotton balls and stick it to the wall of the container

2 After the adult emergence and mating (full grown pupae will general emerge in 24-48 hrs), late instar larvae of *C. cephalonica* i.e. 4^{th} and 5^{th} instars are to be sandwiched between fine muslin clothes on the mouth of the container, with the help of another muslin cloth over the larvae and again secure with a rubber band (larvae needs to be sandwiched between two layers of muslin cloths)

3 The female parasitoids are then allowed to attack or parasitize the host larvae for 48 hrs. Females generally paralyze the host first and then lay eggs on them. The parasitized larvae containing eggs of *H. hebetor* are collected on to a card (8 × 10 cm) using forceps and maintain separately for further development of their life stages.

4 After the *H. hebetor* turned to pupal stage (creamy white colour pupae), the cadaver of *C. cephalonica* needs to be removed using forceps to obtain clean and healthy pupae

5 When the pupae turn to black colour after development, these can be can be stored at 5°C for about three to four weeks without losing their viability.

How to Use Bracon cards?

H. hebetor is an important larval parasitoid of lepidopteran pests of rice specially rice leaf folder. Eight bracon cards (consisting of ~4000-4500 pupae) per hectare (2 cards/week, four time release) are generally recommended. Additional releases may be made if pest activity persists, depending upon the need weekly releases need to be undertaken. Card should be placed in the field before expected date of adult emergence mentioned on the card. Farmers should refrain from using pesticides in the field where *H. hebetor* are released.…….

Availability and sale

- Bio agents are available in the Division of Crop Protection, ICAR-NRRI, Cuttack. Prior indent (before 45 days) is required if the order exceeds 50 cards. Cards will be supplied after thorough quality check. Cards need to be purchased by visiting the institute only.
- Bracon card- Rs. 100 per card (contains ~500 pupae).

Fig 10. Graphical representation of mass rearing of *Habrobracon hebetor*

Quality Control in Mass-production of Biocontrol Agents

Biocontrol agents are the eco-smart tool for integrated insect and disease pest management. Biocontrol agents are the cardinal approach in suppressing phytopathogens and plant damaging insect pests to achieve the goal of sustainable agriculture. A successful biocontrol formulation should possess features such as economically viable and cheap to produce, safer to environment, stable and act effectively and consistently under varied environmental conditions, easily deliverable, and good shelf-life etc (Rajendran et al., 2011).

The quality of biocontrol agents is the major factor which influences the performance of biocontrol agents and ultimately the success of the biocontrol programme. Since, quality control occupies a crucial role in bioagents production, it is a matter of concern where standardized protocols for the quality control of many of the biocontrol products are lacking. Thus, quality control systems for biological agents always need to be much comprehensive and specialized. Though biocontrol strategy holds exceptional potentiality in eco-friendly pest management strategies, quality is the major concern limiting its wide scale adoption by the farmers. Many researchers have attributed the variations in the degree of success of biocontrol programmes mainly to the poor or inconsistent quality of the biocontrol agents used (Ballal *et al*., 2013). The successful performance of these biocontrol agents in the field greatly depends on their consistent quality maintenance in the laboratory during its various phases of mass-production, thereby affecting the whole success of field suppression of the insect pests and disease-causing pathogens. There many factors which defines the production efficiency and quality of the reared biocontrol agents.

Establishment and maintenance of the good quality biocontrol agents is the major criteria to attain apical level of commercialization of the biocontrol agents. Quality of an organism can be defined as the ability to function as intended after release in to the field. The main aim of the quality control programme is,

1. to check whether the overall quality of a species is maintained
2. to determine the factors that affect the overall quality
3. to determine whether a natural enemy is still in condition to properly control the pest to an acceptable level rather than maximum or optimum control

The quality assessment procedure of the final product needs, from all levels of the production process. The quality criteria are the critical process and can be defined as product control, process control and production control. The following flowchart explains the different steps in any quality control programme (Fig 11).

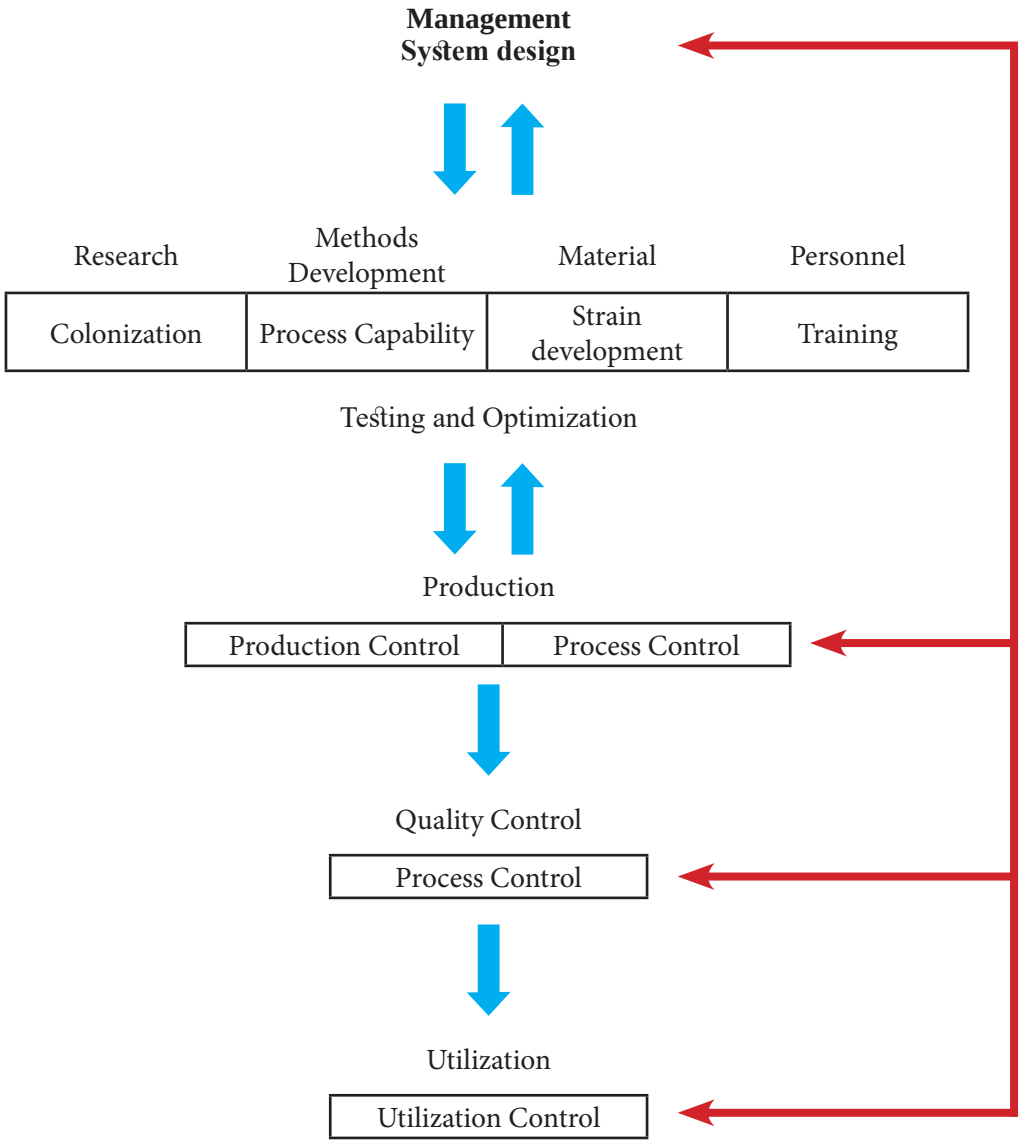


Fig. 11. Flowchart on total quality management

Quality check of the insect biocontrol agents

Maintaining the quality of natural enemies that reared for many generations is one of the most important problems which should be considered, because continuous mass rearing in insectaries with stable conditions may decrease their performance under field conditions (Bertin *et al*., 2017). In quality control process some traits including parasitism rate, sex ratio, fecundity, longevity, body size and weight and flight activity or host-searching ability have to be evaluated (Lu *et al*., 2017).

The following are the criteria should be taken in to account while assessing the quality of the biocontrol agents (natural enemies).

- Quantity
- Sex ratio
- Emergence rate
- Fecundity
- Longevity
- Parasitism rate
- Predation rate
- Adult size
- Flight capacity
- Field performance

Quality control is normally done under standardised test conditions of temperature (22 ± 2 °C or 25 ± 2 °C), relative humidity (usually 75 ± 10%) and light regime (usually 16L: 8D). All numbers/ratios/sizes should be mentioned on the container or packaging materials. Expiration date for each shipment should be given on packaging material.

As a part of quality protocols, the following aspects need special attention:

- Have a large founding population to minimize the immediate effect of inbreeding;
- Maintain different genetic strains adopted to different agro-climatic conditions and hosts;
- Routine rejuvenation of parental stocks of the parasitoids and predators as well as their factitious hosts after determining the periodicity of such requirement for each of them;
- Need to segregate the rearing rooms for individual species to avoid the risk of contamination between species and also prevent competitive displacement (eg. more aggressive species like *T. chilonis* displacing others)

- ❖ Determine a safe temperature regime and duration for optimum cold storage of host eggs before and after parasitisation; and
- ❖ Quality assessment through regular monitoring of attributes like per cent parasitisation, per cent adult emergence, sex ratio, etc.

Some simple recommendations formulated for ascertaining the quality of some of the potential biocontrol agents are as follows: for Trichogrammatids - parasitism > 90%, adult emergence > 90%, sex ratio 1:1 and no. of eggs per parasitised egg-card 16000 to 18000; for coccinellid predators - sex ratio: 1:1, fecundity 75-100 eggs/beetle; for *Chrysopids* - 85% hatching and fecundity > 400 eggs / female; for *Goniozus* - 80% emergence and a minimum of 60% female progeny. Ensuring high quality of biocontrol agents is essential as it plays an important role in the success of biological control and the reception of biocontrol by producers (Ballal *et al.*, 2013).

Quality control: NRRI initiative

Though no quality control guidelines available currently for quality assessment of the microbial biocontrol agents in India. However, the quality needs to be thoroughly checked before the biocontrol agents are dispersed to stakeholders. NRRI biocontrol agents come with quality parameters on the reverse of the card. A quality control standard was established for *Trichogramma* sp. reared on *Corcyra cephalonica* are minimum of 95% parasitization, 90% adult emergence, a male-to-female ratio of 0.50:1, and longevity of female 8-10 days. The cards will be mentioned with quality control information for every batch of cards before they are distributed to stakeholders.

Reaching Last Mile: NRRI Initiative on Developing Biocontrol Entrepreneurs

The availability of biocontrol agents in India faces several challenges, including limited production infrastructure, poor distribution networks, and low awareness among farmers about the benefits of these agents. To improve the situation, it is crucial to invest in production capabilities, develop robust distribution networks, conduct awareness and training programs. These steps can enhance the availability and adoption of biocontrol agents, promoting sustainable agricultural practices in India.

Entrepreneurs can create robust and scalable production and distribution networks, ensuring widespread availability and accessibility of these agents. By fostering competition, entrepreneurship encourages continuous improvement and cost reduction, making biocontrol agents more affordable for farmers. Additionally, entrepreneurial ventures can bridge the gap between research institutions and the market, facilitating the commercialization of new biocontrol technologies. This not only supports sustainable agricultural practices but also generates employment and economic growth in the sector.

Memorandum of Agreement (MoA) with the Biocontrol Entrepreneurs by Honourable Director, ICAR-NRRI

Ultimately, entrepreneurship plays a vital role in overcoming the challenges associated with the mass production and adoption of biocontrol agents, contributing to a more sustainable and environmentally friendly agricultural ecosystem.

ICAR-NRRI has created five entrepreneurs on mass-production of Biocontrol agents under the RKVY project "BioBank: Production and promotion of biocontrol agents and entrepreneurship development in aspirational districts of Odisha". These entrepreneurs are from different aspirational districts of Odisha viz., Kalahandi, Koraput, Balangir, Kandhamal and Malkangiri. In this regard Memorandum of Agreement (MoA) has been established between these entrepreneurs and ICAR-NRRI, Cuttack, and were supported with basic equipment to setup biocontrol units. We will be hand-holding them for successful establishment of mass-production units and subsequent biocontrol agent's production.

Future Thrust

The mass production of host insect, predators and parasitoids is essential for effective biological pest control in agriculture. Future research thrusts could focus on various aspects to enhance the efficiency, scalability, and sustainability of their production. Some of the suggested areas are

Genetic Improvement and Breeding: Developing a breeding programs to enhance desirable traits such as predation efficiency, reproductive rates, and adaptability to different environments. Exploring genetic modification to improve traits like resistance to diseases, tolerance to environmental stresses, and increased lifespan.

Nutrition and Diet Optimization: Formulate cost-effective and nutritionally balanced artificial diets to replace or supplement natural prey, reducing reliance on natural food sources. Investigation on the role of vitamins, minerals, and other supplements in improving the health and productivity of insect predators and parasitoids.

Rearing Techniques and Environmental Control: Designing and optimize rearing systems that maximize space utilization, minimize labor, and ensure high survival rates. Study on the effects of temperature, humidity, light, and other environmental factors on the development and behavior of insect predators and parasitoids.

Behavioral and Ecological Studies: Research should focus on the behavioral ecology of predators and parasitoids to understand their hunting, mating, and oviposition behaviors for improved rearing practices. Study on the interactions between biocontrol agents and their prey/hosts, as well as their impact on non-target species and ecosystems is essential

Automation and Mechanization: Developing an automated system for mass rearing of host and parasitoids to reduce labor costs and improve efficiency.

Field Performance and Release Strategies: Optimization of methods for the release of biocontrol agents in the field to ensure their survival, dispersal, and effectiveness.

Economic and Market Analysis: Conduct economic analyses to evaluate the cost-effectiveness of mass-producing and using insect predators and parasitoids compared to chemical pesticides.

Regulatory and Policy Frameworks: Research the regulatory requirements for the production, release, and use of biocontrol agents to ensure compliance and facilitate market access. Also, to develop quality standards for different biocontrol agents for Indian context

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