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Trichogramma Achaeae and Pesticide Toxicity Test: A Review

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ABSTRACT

Trichogramma achaeae lay their eggs in lepidopteran eggs, killing the developing moth embryo before it hatches and therefore preventing the damaging larval stage. The parasitoid larva consumes the contents of the moth egg, pupates, and emerges as an adult wasp in 7–14 days. A single female wasp is capable of parasitizing up to 50 eggs in her adult life span of 3–14 days. Typically, the wasps are released as parasitized eggs affixed to a card (TRICHO CARDS), which can be stored at 8–12°C for up to seven days. *Corcyra cephalonica* commonly called as rice moth is a pest of stored foods, *viz.*, cereals, cereal products, oilseeds, pulses, dried fruits, nuts, and spices. Many of the natural predator's mass-bred in the laboratory for use in field against crop pests due to the simple reason that it is easier and cheaper to produce. Among various methods, two common methods are recommended to test the pesticides /insecticides toxicity on *Trichogramma achaeae* with the help of the host eggs of Corcyra cephalonica.

Key words: *Trichogramma achaeae, Rice moth, Mass production ,Insecticidal susceptibility , Test Methods*

INTRODUCTION

The family Trichogrammatidae is one of the most poorly known groups of super family Chalcidoidea. The family represents more than 80 genera and includes the smallest insects, ranging in size from 0.2 to 1.5 cm. however the genus Trichogramma is the best known in the family due to its use in the biological control of agricultural pest with over 200 described species.

Among them *Trichogramma achaeae*, is a Hymenopteron parasitoid implementing biological control practices have been reported to reduce the chemical use, environmental pollution and the pesticide exposure to animals and human beings. Trichogramma are small and very uniform in structure which causes difficulty in naming the separate species. A major advance in the systematics of male genitalia can be used to name the species. But these days body colour, wing venation, and features of the antennae serve as supporting characteristics. But females cannot be identified with such confidence.

1..1 Life cycle of Trichogramma achaeae

Trichogramma destroy eggs of over 200 pest moth species (cutworms, fruit worms, leaf worms, leafrollers, loopers, armyworms, borers etc.), preventing ravenous worms (caterpillars) from hatching out and devouring crops. Trichogramma larvae eat out the insides of pest eggs, pupate, and cut an exit hole in moth eggshells for winged adults to squeeze through. Males emerge first, wait for females, and immediately mate. The life cycle from egg to adult is completed in 7 to 10 days (longer in cool weather). Trichogramma attack freshly deposited moth eggs to locate host eggs, adult females use chemical and visual signals, such as egg shape and colour.

Trichogramma achaeae on Corcyra Cephalonica egg (alchetron.com)

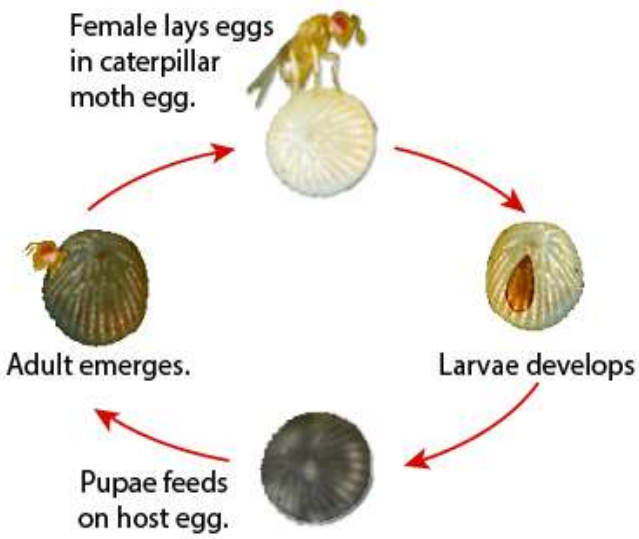
MODE OF PARASITIZATION

After she finds a suitable egg, an experienced female will attempt to determine if the egg has previously been parasitized using her ovipositor and antennal drumming (tapping the egg surface). Females also use antennal drumming to determine the Size and quality of the target egg, which determines the no. of eggs the female will insert. A single female can parasitize one to ten host eggs a day. Fresh moth eggs are usually white or a pale shade of yellow or orange. Moth eggs parasitized by Trichogramma darken to black within 48 hours. The ratio of black (contains pupating Trichogramma) to white (unparasitized) eggs is a measure of Trichogramma parasitism. Trichogramma wasps emerge from cards in two to five days, depending on temperature, which should ideally be 80º to 90º F. Emergence can be delayed by holding parasitized moth eggs at cooler temperatures (not less than 40º F). Emerging wasps are usually seen in the morning. Fecundity varies between 20 and 120 eggs per female according to the species, the host and the longevity of the adult. longevity is related to food supply (sugar, honey and water) availability of the host eggs temperature humidity and the longevity of the females. The adult female wasp uses chemical and visual clues to locate a host egg. The chemical clues called Kairomones. Shape and colour also are the clues to the wasp for parasitization.

Rice moth (Corcyra cephalonica), female (Simon Hinkley & Ken Walker Museum Victoria)

The yolk and embryo of the parasitized egg are digested before the Trichogramma egg hatches. Eggs hatch in about 24 hours and parasites larvae develop quickly. Larva can consume the digested contents of a young host egg within 10 hours of hatching. Larvae develop through three instars. During the 3rd instar (3 to 4 days after the host egg was parasitized).dark melanin granules deposited on the surface of the egg chorion causing the egg to turn black, larvae then transform to the inactive pupal stage. After about 4.5 days, the adult wasps emerge from the pupae and escape the egg by chewing a circular

hole in the eggshell. the black layer inside the chorion and the exit hole are the evidence of parasitism by Trichogramma. The life cycle from egg to adult requires about 9 days but varies from 8 days when mid-summer temperature are high (90 degrees F) to as many as 17 days at 60 degrees. Adults are most active at 75 to 85 degrees.



Trichogramma Achaea. Life Cycle: (Clockwise) Female ovipositing a moth egg; → parasite egg inside host egg →developing larvae; → pupa; → adult wasp emerging from host egg. (Van den Bosch & Hagen, 1966).

Size and quality of the target egg, which determines the no. of eggs the female will insert. A single female can parasitize one to ten host eggs a day. Fresh moth eggs are usually white or a pale shade of yellow or orange. Moth eggs parasitized by Trichogramma darken to black within 48 hours. The ratio of black (contains pupating Trichogramma) to white (unparasitized) eggs is a measure of Trichogramma parasitism. Trichogramma wasps emerge from cards in two to five days, depending on temperature, which should ideally be 80º to 90º F. Emergence can be delayed by holding parasitized moth eggs at cooler temperatures (not less than 40º F). Emerging wasps are usually seen in the morning.

MASS PRODUCTION OF TRICHOGRAMMA ACHAEAE

1. Take 2.5 kg cleaned insecticide free, fresh bajra/sorghum/maize/paddy grains, sterilized at 100°C for 30 min and put into Corcyra rearing cages/plastic trays (one set used for 100 days)
2. Incorporate powdered groundnut kernel weighing 100g as a source of protein.
3. Adding yeast tablets (10 no or 5 g) as nutritional supplement +0.05g streptomycin sulfate as antibiotic. Supplementing 0.01sulfer as acaricide
4. Mixing one cc fresh viable eggs of Corcyra in each cage /tray maintenance of culture at 26 °C
5. After 40-45 days, emergence of adult moth commences.
6. Regular collection of adult moths manually and mechanically using vacuum cleaner.
7. Allow adult moths in fecundity cage used for three days supplementing with food swab impregnating honey 15% water and vitamin E (2 cap)
8. Daily collection Corcyra eggs
9. Pass the eggs through 15,30, and 40 mesh sieves and run over a slope of paper to eliminate dust particles or separation of scales admixture in eggs mechanically by motorized egg separator.
10. Harvest fresh eggs.
11. Storage of eggs at 10°C up to Seven Days
12. Freshly collected Corcyra eggs are subjected to UV treatment using 30-watt UV tube light for 45 minutes.
13. Pasting UV treating eggs on to the “TRICHO” cards measuring size 15x10 cm comprising six pieces (12x3cm each)
14. Introduction of single egg card inside polyethene balloon/pyritization chamber along with nucleus culture of Trichogramma stain maintaining in ratio of one female to 30 eggs for effective patriotization

15. Parasitation takes place within a week time.
16. About 2000 Trichogramma adults emerge out from single cards.

QUALITY CONTROL GUIDELINES FOR REARING

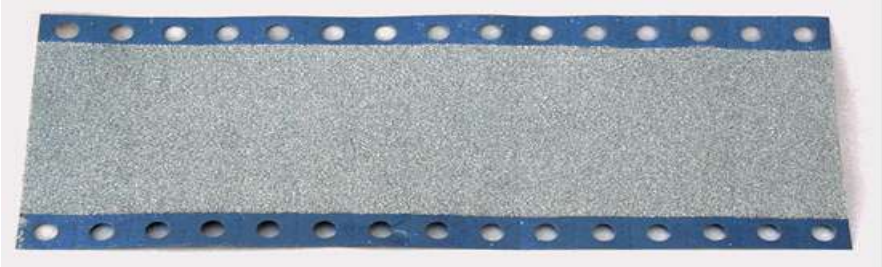
1. **ATMOSPHERIC CONDITION:** For rearing *Corcyra Cephalonica*, Atmospheric tempreture is recommended as 23 ± 2 ºC, Relative Humidity: 75 ± 10% and Light Regime be 16 hr. Light and 8 hr. Dark. The species are identified on the level and verified by the producer. Molecular techniques are available at laboratory of entomology. Tests are necessary once a year, sample size is considered as minimum 30 individuals. More than 50% females ;100 adults assess on ten release units each or 565x100 adult in bulk material at least weekly or batch wise test if batches were exposed to special treatment (e.g. storage). Fecundity and longevity are determined by the calculation as: Number of offspring per seven days per female and 80% of females should live at least seven days. Test is performed monthly or batch wise with 30 individuals.
2. **TESTING METHODS FECUNDITY AND LONGEVITY:** 30 female (age 24 h) are confined individually in the glass tubes : at least 200 factitious host eggs (> 24 hrs.) are glued with water on to a small cardboard strip: a small droplet of honey and water are added directly to the wall of the vial .eggs of C. cephalonica (<24 hr. old) are ultra violet irradiated and provided on day 1 and removed after day 7.:5.fresh eggs C. cephalonica are provided on day 1,3 and 5 .the number of living adults is recorded after day7.egg cards are incubated and number of black eggs is counted not earlier than day 10. Minimum fecundity after day 7 is 40 offspring per female; mortality after day 7 is <20% at least monthly test or batch wise if batches were exposed to special treatments (storage procedure or long- range shipments)
3. **NATURAL HOST PARASITISM :** 30 females ((age 24 h) are confined individually in tubes :two fresh egg s masses of at least 20 eggs per egg mass of *C. cephalonica* (< 24 h old) are added for 4 h: honey and water are provided as described above after separation of egg masses from females they are incubated for three days : the number of black eggs is counted : the mean number of black eggs is ≥ 10 per female .the host cluster acceptance rate (females parasitizing at least one host egg) should be ≥ 80% .this measure is important because parasitism drops drastically if a high proportion of female doesn't accept their hosts. This test is indirect measure of acceptance and suitability of natural host egg. The test should be performed two to four times per year depending on the rearing system (number of generations reared on the factitious hosts.)

PROCEDURE FOR THE STUDIES

Trichogramma spp. or species such as *Trichogramma Achaeae* can be used according to susceptibility towards a particular host egg are used as predator and rice moth eggs (*Corcyra cephalonica)* as host

TYPES OF TRICHO CARDS USED

1. Card containing Trichogramma larvae inside the host eggs (parasitized eggs), For Testing, test item is sprayed on it and kept in close container at optimum temperature and humidity. The larvae of Trichogramma Emerge and result of study is evaluated.
2. Card containing only host eggs, Trichoderma is released in a close container after the application of test item on the cards, at proper temperature and humidity, the wasps parasitize the eggs then result of the study is evaluated



Trichogramma card with 100,000 parasitized eggs, can be cut into 30 tabs, each with a hook for hanging.

Card is 4 X 11 inch, each tab is ¾ X 2 inch (www.rinconvitova.com)

TEST CONDITIONS

The test duration is 5 to 7 days. Temperature will be maintained in the range of 26 ± 2 ºC. Age of the host eggs should not be exceedingly more than 24 hr. Relative Humidity will be maintained in the range of 76 ± 10%. Light Regime will be maintained 16-hour Light and 8 hour Dark TRICHO" Cards in Test tubes will be used as the test vessels and each tube with card will be considered as a replicate. Administration test item is initiate as soon as possible

after the eggs get parasitized and terminated after hatching of Trichogramma spp. larvae. The host eggs should be immersed in the test solutions properly. At least three replicates of "TRICHO" cards of each test item (Pesticides) needed per treatment group are selected into the respective concentrations and controls. Parasitized eggs (black colour) should be separated and counted from unparasitized eggs before exposure of test items. Parasitized eggs must be observed with the help of binocular microscope. On one piece of cards 20-50 host eggs should be present for each test concentrations and control groups. Trichogramma should be euthanized by hypothermic shock in the refrigerator 2 - 0 °C for 10 min. approximately.

TEST AND CONTROL GROUPS

Tap water is considered as control group Test groups of different concentrations have 3 replicates each and for control one replicate is used A positive control at a fixed concentration of a pesticide (say dimethoate) is performed with each egg batch used for testing If solvent is us

VALIDITY OF THE TEST

The overall hatching rate of all eggs collected should be ≥ 70% in the batch tested. The room temperature should be maintained at 26 ± 2 °C. Overall survival of Trichogramma larvae in the control should be ≥ 90% until the end of the last day of exposure. Exposure to the control should result in a minimum mortality of 30% at the end of the last day of exposure. Hatching rate in the control (and solvent control if appropriate) should be ≥ 80% at the end of last day of exposure.

METHODS OF TREATMENT

There may be various methods to innoculate trichogrrmma eggs into the host to perform the studies. generally two methods are applied which are based on «TRICO» Card Methods.

1. **DRY FILM RESIDUE METHOD (Hassan, S. A, 1977)**

Dry film residue method of bioassay was followed to assess the contact toxicity of insecticides to adults of T. Achaea. Test tubes of 45 mL capacity with an internal surface area of 80 cm2 were used for preparing dry film residue. The test tubes were cleaned by soaking overnight in soapy water, reins with acetone and air dried for four hours before use. The test tubes were coated evenly with 0.5 mL of different concentrations of insecticides and dried thoroughly by rotating the test tubes between the palms. For the untreated control, 0.5 mL of water alone was used. Twenty freshly appeared adult wasps were transferred into each test tube and covered with a muslin cloth secured with a rubber band. The adults were anaesthetized by keeping in refrigerator for 15 minutes to enable counting and transfer of wasps to test tubes. After four hours of exposure to the insecticide residue in the treated test tube, the wasps were transferred to other clean test tubes and observed for the lethal effects. Cotton swabbed with 50 % honey was kept inside the tube as adult food. Mortality of adult wasps was recorded at 24 and 48 hours after treatment (HAT). The experiment was carried out in Completely Randomized Design (CRD) with five insecticide treatments along with an untreated control and four replications for each treatment.

2. **DIP METHOD (Singh, P. and Varma, G.C. 1986)**

Each egg card (10 X 2 cm2), with Corcyra eggs containing Trichogramma eggs, was cut into four bits of 5 X 1 cm2 size. The egg card pieces were treated by dipping in solution of five insecticides prepared at field recommended concentrations for three seconds . Four replications were maintained for each treatment and untreated control was maintained by dipping the egg card in water alone. The total number of eggs in each card bit was counted before insecticide treatment. After insecticide treatment, the egg card pieces were air dried at room temperature and kept in test tubes (45 ml capacity) closed with muslin cloth secured by a rubber band. The number of eggs parasitized, as indicated by black colouration of eggs, was observed at five days after treatment and the number of adults emerged from different treatments were also recorded at eight days after treatment. The longevity of adults and developmental period of parasitoids emerging from insecticide treated eggs of T. Achaeae were also determined. The rate of parasitism and adult emergence were computed based on the formula.

OBSERVATIONS

Observations are used for the determination of lethality and sub lethality at a particular Duration of time. Starting from 0 hr. to 48 hr.is observed EC_{50} is estimated for the examined insecticide. Rate of parasitization is determined. Additionally, hatching is recorded in treatment and control groups on a daily basis starting from 48 hrs. Rate of hatching of Trichogramma is evaluated Ratio of male and female is estimated. Longevity and fecundity in females are estimated. Statistical data for various parameters can be exhibited for research purpose.

GENRAL CALCULATION

No. of grubs hatched from each treatment:

% hatchability= [No. of hatched larvae/ total no. of eggs] x100

% Adult emergence= [(Ac-At)/AC] X100

Where:

Ac – adult emerged in control group.

At – adult emerged in treated group.

DATA ANALYSIS

In this test, the individual cards are considered independent replicates for statistical analysis. The percentages for mortality, hatchability, longevity and fecundity which at least one of the apical observations is positive at 48 and/or 96 hrs. are plotted against test concentration of different test items.

Mortality of Trichogramma spp. (%)	**Evaluation of toxicity**
Less than or equal to 50%	Harmless
More than 50%	Should pass on next test in the scheme
50-79%	Slightly harmful
80-99%:	Moderately harmful
More than 99%	Harmful

(According to Evaluation of toxicity by international organization for biological control 1994)

CONCLUSION AND FUTURE PROSPECTS

The success of biological control of plant pathogens using Trichoderma does not rely solely on effective antag-onists but also on the method of delivery or application on the seed, root and soil. In addition, all antagonists rely on their placement on the infection court to effect successful protection and control (Mathre et al., 1999) Future research on the biologi-cal control system of Trichoderma should look into for-mulation suitable to control foliar and aerial pathogens considering its endophytic nature (Evans et al., 2003). There is a need to strengthen research-industry partner-ship to scale up production systems including large scale promotion of Trichoderma formulation in farmers' fields particularly in the developing countries.

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