INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
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www.vmsindia.org

# Effect Of Ionizing (Gamma) And Non-Ionizing (UV) Radiation On Lepidopteran Host Eggs For The Efficacy Of Egg Parasitoid, *Trichogramma Chilonis* Ishii (Hymenoptera: Trichogrammatidae)

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**Abstract:**
The egg-parasitoids belonging to genus *Trichogramma* are of great importance in applied biological control in different parts of the world, because of their large scale feasibility in release programme against various pests. The parasitization efficacy of *Trichogramma chilonis* on different species of lepidopteran hosts, viz., *Spodoptera litura*, *Plutella xylostella*, *Corcyra cephalonica* followed by parasitoid's emergence was ascertained with a view to establish the biocontrol agent for environment friendly pest management. UV-sterilization of host eggs markedly enhanced parasitization capacity of *T. chilonis* with respect to unsterilized host-eggs. The emergence of *T.chilonis* from parasitized host had also significantly increased in lepidopteran hosts. UV-sterilization of host eggs helped in multifold manner in promoting parasitization efficacy e.g.,UV sterilization of host eggs arrested its further development including even those eggs that were not parasitized.This would completely reduce the probability of hatching of hosts that could have eaten the other parasitized eggs, by showing superparasitism. This would in turn ensure more chances for survival of egg parasitoids with no risk of being parasitized by their own hosts. Also due to no development probably more nutrients might be directed for the development of growing parasitoid, instead of nutrient utilization by the host embryo. Further, the parasitization behaviour and efficacy of *T.chilonis* on gamma sterilized eggs of different species of lepidopteran hosts were ascertained. For this study two gamma doses evaluated were 5Gy and 10Gy. Parasitization capacity of *T. chilonis* was similar on hosts like *S.litura* and *C.cephalonica* but it was slightly increased at 10Gy in case of *S.litura*. Results further indicated that gamma radiation did not adversely affect the parasitization capacity of *T.chilonis* when compared with the un-irradiated host. Further, the suitability of lepidopteran hosts for parasitization was similar in normal and sterilized eggs. It was also noticed that gamma irradiated host eggs were less parasitized as compared to the UV-irradiated eggs, indicating that UV sterilization was more effective than gamma irradiation induced host sterility in eliciting parasitization response by *Trichogramma*.These results indicate that *Trichogramma* spp. are particularly suitable for use as a "biotic insecticide" for lepidopteran pests, and their bio-efficacy can be improved by using nuclear techniques for pest management.
**Key words:** Biological control, biotic insecticide, Gamma sterilized, *Trichogramma*

## Introduction

Instant killing by immensely high doses of insecticides has not only created a hazardous impact in the environment, but also it has resulted into a great deal of expenditure on their procuring and application in the field by the farmers. As a consequence, it raised global awareness on the need for evolving non-chemical methods of pest control under the overall umbrella of Integrated Pest Management (IPM).

One such non-chemical approach is biological control that might be further improved and properly established by involving the use of nuclear techniques in improving the production, shipping and deployment of biological control agents or natural enemies, such as parasitoids, predators and pathogens to manage pests in order to facilitate trade and protect the environment. This method is ecofriendly, self-regulating, self sustained, long lasting and economically viable. Considerable technological advances have been made in mass rearing of parasitoids and predators for augmentative biological control. The large scale availability of natural enemies of key insect pests opens the way for environmentally compatible systems of pest control, where mass releases of natural enemies can suppress pest densities in the field within the context of area-wide integrated pest management programmes.

So far inundative biological control is concerned which aims at directly increasing mortality in the pest population, whereby the released natural enemies are used as a biological insecticide (Stinner, 1977). The species of the hymenopterous genus *Trichogramma* have been used more than any other natural enemy for inundative biological control. Among the parasitoids, the genus *Trichogramma* heads the list and has received the most attention because of its importance in the biological control (King et al. 1985).

*Trichogramma* has a wide range of hosts, especially among the Lepidoptera (Nagarkatti and Nagaraja, 1977). Experiments with *Trichogramma* were started in the beginning of this century in

the USA and the USSR. Development of a method for mass production of *Trichogramma* on eggs of lepidopterous storage pests of grains like the rice meal moth, *Corcyra cephalonica*, the Angoumois grain moth, *Sitotrega cerealella*, etc. gave rise to worldwide use of the pest parasitoids as a pragmatic biological control agent. In India about 26 Trichogrammatid species are recorded of which *Trichogramma chilonis* Ishii, *T. japonicum* Ashmead and *T. achaeae* are of significant importance.

The addition of sterile eggs of the target hosts or the eggs of an innocuous alternate host may refer as a possible way of increasing *Trichogramma* parasite populations, without creating risks of additional crop damage. Knipling (1966) proposed that sterile egg-laying female of the host species with the mass releases of natural enemies act synergistically within the context of area- wide integrated pest management. Parker et al. (1971) demonstrated that control of *Pieris rapae* (L.) by *Trichogramma evanescens* Westwood was better in field plots where both parasites and hosts were realeased. The delicate balance between an egg parasite and successful utilization of host eggs by using irradiation sterilization of the eggs.

In order to promote biological control and enhance bioefficacy of parasitoids using nuclear techniques, an attempt was made in the present investigation, to study the effect of irradiation of host eggs on parasitization efficacy of egg parasitoid, *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) towards three Lepidopteran hosts, *Corcyra cephalonica*, *Spodoptera litura* and *Plutella xylostella,* and various biological attributes of *T. chilonis* were ascertained. The differential response elicited by UV and gamma radiation of hosts was studied with respect to bioefficacy of *T. chilonis*.

**Materials and Methods**

**1. Maintenance of insect culture**

In the present study the effect of irradiation of Lepidopteran host on parasitization efficacy of egg parasitoid *Trichogramma chilonis* Ishii was explored. Laboratory culture of egg parasitoid, *T. chilonis*, which is a biological agent, and three Lepidopteran hosts, viz. *Corcyra cephalonica*, *Spodoptera litura* and *Plutella xylostella* (DBM) was maintained.

**2. Laboratory culture of egg parasitoid, *Trichogramma chilonis***

**2.1 Mass production of *Trichogramma chilonis***

The strain of *T. chilonis* used in this test was obtained from the Nuclear Research Laboratory, IARI, and New Delhi where it was already mass reared for many generations on eggs of the rice meal moth (*C. cephalonica*). Such culture was established at Radiation Biology and Applied Entomology Laboratory of Department of Zoology, University of Delhi, Delhi.

*T. chilonis* adults were reared for more than 30 generations on *C. cephalonica* eggs (as the suitable Laboratory host) in a controlled environment, BOD was fixed at 27±1$^0$c, 65±5% relative humidity (RH), and a 12-h photophase (0600 to 1800 h and 1800 to 0600h). Large numbers of *C. cephalonica* eggs obtained by placing 1 to 2 day old adults in inverted 3.8-litres plastic funnel with screen bottoms, were collected after 24 hrs. The collection of host eggs was followed by sieving with mesh size 15, 30 and 40. Then these cleaned eggs were exposed to ultra violet rays (15 W UV-tube) for 10 minutes in a closed chamber maintaining a distance of 12.5 cm. between the eggs and the tube to make these eggs sterilized. The eggs could also be made inviable by exposing them to very low temperature, 0-4$^0$C in the freeze chamber of a refrigerator for 3-4 hours (Singh, 1969). The egg cards used for pasting the eggs comprised of 6 cm height × 2 cm diameter (Plate-1). These eggs were sprinkled on cards smeared with uniform layer of gum so as to enable uniform spreading of the eggs on the cards. The "egg card" after drying was kept in a container, a glass vial of size 10cm height × 3 cm diameter and exposed to freshly emerged Trichogrammatids under tube light (40W). Host parasitoids ratio of 6:1 was to be followed to avoid superparasitsm. *T. chilonis* which were about to emerge from Tricho - cards, (hereafter referred as 'mother cards') inside the glass vial and freshly prepared egg cards (hereafter referred as 'daughter cards') were kept just opposite to that of the mother card in the same vial. Fine streaks of 50% honey solution was provided regularly as food source on the inner wall of the Vial till the death of the *Trichogramma* adults. After 24h- 48h of exposition of daughter cards were transferred to the new vials. The parasitised eggs started turning black on the 3$^{rd}$ day of parasitization and the blackening was completed on the 4$^{th}$ day, normally 80-90% successful parasitization occurred.

**2.2 Life history and biology of *T. chilonis***

Detailed account of life history of *T. chilonis* (= minutum) including illustration of various developmental stages, development on

different age groups of *C. cephalonica* eggs viz. 24, 48, 72 & 92 hrs. old eggs were given by Krishnamurti (1938).The parasitoid completed its life cycle in 7-9 days, the egg, larval and pupal periods occupying 1, 2-3 and 4-5 days respectively.

Nagarkatti and Nagaraja (1978) studied life history of *T. chilonis* laboratory reared and wild type *T. confusum*. They reported net generation time are 11.55 and 11.32 days respectively and average longevity of 6.69 and 5.01 days *T. chilonis* completed nearly 34 generations per annum.

The parasitoid, *Trichogramma* species are free living tiny wasps usually found in most of the crops except tobacco, chick pea and pegion pea. The female *Trichogramma* is able to locate the insect pest egg on the crop and parasitizes it by inserting her egg in it. As a result of parasitism, the pest larva does not hatch. Instead *Trichogramma* wasp completes the development within the pest egg and emerges out after 7 days. Thus, the pest is killed in its egg stage itself. One female *Trichogramma* is capable of parasitizing 120 eggs of the pest in her life span of 4 to 5 days.

**3. Culturing of *Spodoptera litura* (Fabr.)**

**3.1 Adult pairing**

The male and female adults were paired singly or in-groups in the cages (20 x 20 x 20 cm.) made up of perspex and nylon. These cages were cleaned regularly with 30% alcohol and water. The base of the cage was covered with sheets of filter paper, which were made wet with water. In small plastic containers, cotton swabs soaked in 15-20% honey solution (w/v) were placed on which adults were fed. These swabs were replenished every day. Leaves of the castor plant were introduced in the cage to serve as ovipositional traps. The eggs were laid on the ventral surface of the leaves or on the nylon/ perspex walls of the cage. The eggs were collected with the help of a camel hairbrush (No. 0) and a thin strip of photographic film, in small plastic containers. The eggs were treated with 0.2% sodium hypochlorite (NaOCl) solution, followed by a rinse with distilled water for sterilization.

**2.1 Rearing of *S. litura* on castor**

All the glassware and plastic containers were washed with detergent, 5% formalin and water and then oven dried at about 70-80$^0$C.The eggs were kept for incubation (3-4 days) in plastic containers (10 cm dia x 13 cm ht). These containers were placed in trays with water so that optimum humidity conditions could be maintained. The newly hatched larvae were transferred to the glass jars having a layer of filter paper at the bottom. About 50-100 larvae were placed in each jar. These young larvae were fed with tender and soft leaves of castor. Castor leaves used for culturing were washed with water, $KMnO_4$ and again with water. When the larvae grew in size, gradual thinning of them was to be done to ensure healthy culture. As the larvae attained 4$^{th}$ and 5$^{th}$ instars, they acquired a voracious-feeding habit, thereby, the food consumption increased. Larvae were daily transferred to fresh jars. Regular removal of excreta, dead and infected individuals and the exuviae was done to maintain cleanliness of the culture. The sixth instar larvae fed voraciously for 2-3 days. The larvae, at this stage, showed random movements and entered in "wandering stage". Subsequently with a continuous purging out of material from their alimentary canal, the larvae entered into the pre-pupal stage. These pre-pupae were then transferred to the jars having a bed of soil, in which pupation was allowed to occur. The pupae thus formed were collected and surface sterilized (2 day old) by rinsing in 0.1% formalin solution and subsequently washed under running tap water. The pupae were sexed according to the location of gonopore, ( located on the 8$^{th}$ abdominal sternum of female pupa and on the 9$^{th}$ abdominal sternum in male pupa. The male and female pupae were kept in separate jars marked according to number and date of pupal formation. Emergence of adults was observed after a lapse of 7-8 days since pupation occurred. These adults were then kept for reproductive pairing for the continuation of the culture.

**3.3 Life cycle of *Spodoptera litura***

It is a polyphagus noxious pest belonging to family Noctuidae basically a leaf eater. The eggs were laid underneath the leaves in clusters of 200-300 which are covered with hair scales. The egg incubation period was about 3-5 days. The newly hatched caterpillars were tiny, blackish green and with a distinct band on the first abdominal segment. The insect had six in stars in the larval stage and the larval period extended for 15-17 days. The mature caterpillar was stout and smooth, dull grayish and blackish green in colour with pale white dorsal and lateral stripes. The head capsule was black with a typical inverted 'V' mark on it. Freshly formed pupa was pale green in colour and with gradual melanisation it turned into dark brown in colour. Pupal period lasted for 7-8 days after which adults were enclosed. Life span of adult moths was 9-11 days and the whole life cycle took about 38-40 days.

**4.Culturing of *Plutella xylostella* (L.)**

**4.1 Rearing of *P. xylostella* on cabbage and cauliflower leaves.**

The larvae of *Plutella xylostella* were collected from the field at Research Farm, Indian Agricultural Research Institute, PUSA. Fifty larvae per glass jar (15cm ht X 10cm di) were released and provided with cauliflower leaves. The food was changed daily till pupation. The jars were covered with muslin cloth. The pupae, thus obtained were transferred to other jars having moist blotting paper at the bottom to avoid the desiccation of pupae. The male and female individuals were identified on the basis of brightness of Diamond stars on the forewing. The males have brighter diamond stars on forewing than those of females. The tip of abdomen of females is pointed whereas slanting in case of males. The adults of both the sexes emerging on the same day were released into jars (15cm ht × 10cm di). The jars were provided with moist blotting paper at the bottom so as to prevent the desiccation of eggs. The leaves of cauliflower were placed in each jar for oviposition and each jar was covered with muslin cloth. Cotton swabs dipped in 10% honey solution was given as food for adults. The leaves containing the eggs were removed daily and new leaves were provided for further oviposition. Fresh leaves were collected every day from research farm of I.A.R.I., PUSA. The eggs thus obtained were used for experimental purposes.

**4.1.1 Egg stage**

For each replicate, 50 freshly laid eggs were kept in petridishes (7.5cm di × 1.25cm ht) containing moist blotting paper for studying the incubation period and hatchability. There were 5 replicates for each treatment. The observations were recorded twice a day. Newly hatched larvae were counted and removed from petridishes at the time of observation and percent hatchability was worked out.

**4.1.2 Larval stage**

The period from emergence of larvae till they entered in pre-pupal stage was taken as larval period. Larvae were transferred into jars containing the tender leaves of cauliflower. The food was changed daily in morning. The number of larval instars and time taken for each instar was recorded. The time when a larva stopped its activity and feeding and spun a thread like structure around it to fix itself with host was considered to be near molting phase. The presence of exuviae confirmed the entry of larvae into next instar. The number of larvae which successfully entered into the next instar were recorded till they became pupae. Thus the percentage survival of different larval instars were calculated. To check the relative suitability of environmental conditions in different months laboratory rearing of *Plutella xylostella*, larval growth index was calculated.

**4.1.2 Pre-pupal stage**

The interval between end of larval stage and generation of pupa was taken as pre-pupal period. When the larvae entered the pre-pupal stage it stopped feeding became sluggish and its body got contracted. The larvae, which entered pre-pupal stage, were taken and released in glass jars having moist filter paper at the bottom and covered with leaves. Observations were made daily to record their duration.

**4.1.3 Pupal stage**

The period from formation of pupa to adult emergence was taken as pupal period. The pupae formed were transferred to separate glass jars (15cm × 10cm). The size of each pupa was measured by scale. Out of total pupae, the number from which moths emerged was counted and percent survival was worked out. The pupae were observed daily for recording the adult emergence and pupal period.

**4.2 Adult pairing**

5 pairs of DBM (*P. xylostella*) adults were released in glass jars (1 pair per jar) were having fresh leaves. The jars were covered with muslin and 10% honey solution on a cotton swab was provided as food. The observations were made daily. When female has oviposited for first day then pair was transferred to the new jar for recording further oviposition. The process was continued till end of oviposition.

**4.3 Life cycle of *P. xylostella***

Diamond back moth is an important pest of cruciferous crop and enjoys world –wide distribution. Minute yellowish-white eggs (0.5mm) were laid often singly on the leaves by the moths. The egg period varied from 3 to 6 days. The newly hatched caterpillars were pale-white with pale brown head. They could chew small cavities and holes on the leaves feeding mainly on the underside. The larval period varied from 14 to 21 days. A fully grown caterpillar measured about 10mm. long. It was light green in colour, moderately stout, attenuated at each extremity, smooth with short, scattered, bristly hair. It wriggled actively when disturbed and droped down suspending itself on a silken thread. Pupation took place inside a loose-silken cocoon

spun by the caterpillar. The pupa was about 6 mm long from which small grey-brown moths emerge in 7 to 11 days. The moth had narrow wings (wing expanse 14mm) with pale-white marks on them, which when the insect rests appear together as diamond-shaped median dorsal patches. Adult longevity lasted for 6 to 13 days in the laboratory.

**5. Culturing of *Corcyra cephalonica* (Stainton)**

In India, *Corcyra cephalonica* is being used as a laboratory host for multiplication of Trichogrammatids. The production procedure for multiplication of *Corcyra cephalonica* is detailed below:

**5.3 Production Procedure**

Sorghum with white bold grains meant for human consumption was procured and then was milled to make 3 to 4 pieces of each grain. Sorghum was heat sterilized in oven at 100$^0$C for 30 min. Sorghum was sprayed with 0.1% formalin. Sorghum was air-dried. Sorghum was poured @2 Kg / Box. Boxes containing sorghum were infested with Corcyra eggs.Boxes are kept in racks and lid was closed. On 40$^{th}$ day onwards moths started emerging. Moths were collected daily and transferred to specially designed ovipositional cages (a thick transparent polyvinyl funnel whose wide bottom portion was covered with mosquito net to prevent the escape of adult and the tip of inverted funnel was plugged with 20% honey cotton swab). Eggs collected and passed through 15, 30 and 40 mesh sieves. After the moth emergence was over, the boxes were reduced reused after cleaning. After sieving the cleaned eggs were collected in petridishes. Some of the eggs were used for maintaining the culture and some of eggs for rearing the parasitoids, Trichogramma chilonis in the laboratory.

**5.3 Life cycle of *Corcyra cephalonica***

The life cycle of *C.cephalonica* was near about 45 to 50 days. The eggs are hatched after 4 -5 days of egg laying near the larval food. The larva were dull white in colour with brown heads and had long fine hairs covering the body. They persisted for 15-20 days under favorable conditions . Rice moth's larvae produced large amounts of strong webbing and frass, before spinning a dense white cocoon in which they pupate. The pupal stage lasted for 7-10 days. The adult can survive up to 7-8 days.

**5.3.1 Features**

Two pairs of well-developed membranous wings, with few cross veins; Clothed with broad scales. Generally suctorial mouthparts; Metamorphosis complete with egg, larval, pupal and adult stages; Larvae frequently having eight pairs of limbs. Attack grain (especially rice), oil seeds, cocoa beans, dried fruits, etc.

**Irradiation of host eggs**

**7.1Irradiation by UV-rays**

The *Corcyra* eggs were collected through mesh sieves 15, 30 and 40. The objective of using 3 mesh sieves was to remove any unwanted material like broken antenna or legs or any other part that must have come along with the eggs. After collection, these eggs were resorted to sterilization. The sterilization of *Corcyra* eggs was necessary so as to prevent the emergence of *Corcyra*. Since *Trichogramma* was an egg parasitoid, the *Trichogramma* females laid its eggs inside the host eggs (here *Corcyra* eggs). Therefore it became necessary to sterilize the host eggs. Host eggs were collected on filter paper and kept in a petridish. The sterilization of host eggs was done by UV light inside the chamber, exposing eggs at distance of 12.5cm.

**7.2 Irradiation by gamma rays**

The irradiation of cleaned eggs of *Corcyra cephalonica* was done at INMAS, New Delhi. There were two treatments of gamma-irradiation, such as, 5Gy and 10Gy by the source Cobalt-60. The dose rate was calculated and accordingly exposure time was set up for 5 Gy as 217.85sec. and for 10 Gy it was 435.73sec.

**8. Exposure of host eggs to *T. chilonis***

The freshly emerged (8-12hr old) *T.chilonis* from the nuclear culture were taken.

**8.1Collection of eggs and egg cards preparation**

Freshly (8-12 hr old) collected eggs were glued with the help of No.0 brush, gum and thick paper size, 2.5× 5 cm were taken. The card-paper had pasted egg at the rate of host eggs as 100 eggs, 50 eggs and 30 eggs on 1$^{st}$ day, 2$^{nd}$ day and 3$^{rd}$ day parasitization respectively by parasitoid on different insect hosts. And these egg-cards were allowed for drying and then kept inside the respective glass vials (homeopathic, 1drum size Vial).

**8.1.1 Exposure of host eggs to *T.chilonis***

In the glass vials containing *S.litura* egg cards were allowed a pair of *T.chilonis* with the help of brush, while they were mating, from the nuclear culture. Then 50% diluted honey strips at the inner wall of the glass vial were provided and then the vials were closed by cotton plugs. After

24 h of exposition for parasitization the old cards were transferred to new vials, and another fresh cards were replaced for further parasitization by *T.chilonis* on 2nd day, like-wise on 3rd day, it was repeated.

Similarly *P. xylostella* 'egg-cards' and *C. cephalonica* 'egg-cards' were prepared and parasitization efficacy of *T. chilonis* was determined by counting the number of eggs parasitized on 1st day, 2nd day and 3rd day on their respective specially made 'egg-cards'.

**9. Parasitization behaviour of *T. chilonis* on normal fertilized eggs**

**9.1 % parasitization capacity of parasitoids**

This was observed for three days of parasitization in the respective vial having egg cards. The data was recorded only for three consecutive days. The identifying characteristic for parasitization was the change of colouration from white to dark for *C. cephalonica*, light greenish to dark for *S.litura* and yellow to dark for *P. xylostella*. Then the darkened eggs were recorded.

**9.2 Emergence of parasitoids from host eggs**

The per cent emergence of parasitoids from host eggs was recorded after 7 days of parasitization. The emergence of parasitoids from the parasitized eggs were noted down daily.

**9.3 Developmental period of *T. chilonis***

The developmental period of *T.chilonis* within parasitized eggs of different lepidopteran hosts were recorded.

**9.4 Longevity of asitoids**

The adult pairs which were allowed for oviposition leading to parasitization in the different host eggs were daily observed and provided with 50% honey solution also. The male and female longevity was noticed while observing under compound microscope.

**9.5 Female emergence**

Out of the emerging parasitoids, percentage of female emergence was recorded.

**10. Parasitization behaviour of *T. chilonis* on UV sterilized eggs**

The eggs of different lepidopteran hosts were sterilized with UV-irradiation and exposed to parasitization by *T.chilonis*. Various parameters related to parasitization behaviour were assessed as per the methods described above.

**11. Parasitization behaviour of *T. chilonis* on gamma sterilized eggs**

The eggs of different lepidopteran hosts were gamma-irradiated with 5Gy/10Gy for sterilization and then exposed to parasitization by *Trichogramma*.Various parameters selected to parasitization behaviour were ascertained as per the procedure described above.

**13. Statistical analysis**

The data obtained in the above experiments were usually replicated ten times; and any variation in replicate number has been specified at an appropriate place in the text. The data were subjected to appropriate analysis of variance (ANOVA). Percentage data was transformed using arcsine √x value before ANOVA, but data shown in tables are back transformations. LSD post test was then performed to determine significant differences among the different treatments (Snedecor and Cochran 1989)

**Results**

**1. Parasitization efficacy of egg-Parasitoid, *Trichogramma chilonis* on different species of lepidopteran hosts**

Hosts have profound influence on the biological attributes of parasitoids through the quantity and quality of nutrients present in them. In the present study, the egg parasitoid *Trichogramma chilonis* was used as the biocontrol agent, with a view to ascertaining its efficacy in terms of the per cent parasitization capacity, per cent emergence, per cent female emergence, adult longevity & developmental period of parasitoid *T. chilonis* on normal fertilized eggs of three noxious lepidopteran insect pests *C. cephalonica*, *S. litura*&*P. xylostella*. (Table 1).

**1.1 Effect on Parasitism**

Parasitisation capacity of egg –parasitoid, *T. chilonis* was ascertained on normal fertilized eggs of different species of lepidopteran hosts. It was 27.2%, 33.5% and 22.7% in case of *Spodoptera litura*, *Plutella xylostella* and *Corcyra cephalonica*, respectively. (Fig. 1)

**1.2 Effect on emergence**

The per cent emergence of parasitoid, *Trichogramma chilonis* from host was determined to be 35.6%, 50.1% and 57.9% in case of *Spodoptera litura, Plutella xylostella* and *Corcyra cephalonica,* respectively.(Fig. 2)

**1.3 Effect on developmental period of parasitoid**

The developmental period of parasitoid, *Trichogramma chilonis* in host was observed to be 8.8-8.9 days in all the three lepidopteran hosts investigated.

**1.4 Effect on the longevity of adult parasitoid**

The longevity of adult parasitoid (*T. chilonis*) was 3.1-3.9 days for male, and 3.6-4.3 days for female.

**1.5 Effect on the female emergence**

The female emergence was observed to be 57.8% in host, *Spodoptera litura*, 48.3% in *Plutella xylostella*, and 55.6% in *Corcyra cephalonica*.

**2. Parasitization behaviour of egg-Parasitoid, *Trichogramma chilonis* on UV sterilized eggs of different species of lepidopteran hosts**

UV irradiation was used to sterilize the host eggs in which embryonic development was stopped and these inviable eggs were evaluated as host for *T.chilonis* and parasitoids efficacy was determined (Table 2).

**2.1 Effect on parasitism**

Parasitization capacity of egg-Parasitoid, *Trichogramma chilonis* was determined on UV-sterilized eggs of different species of lepidopteran hosts, and it was noted as 31.1%, 29.4% and 43.9% in case of *Spodoptera litura*, *Plutella xylostella* and *Corcyra cephalonica* respectively. (Fig. 1)

**2.2 Effect on emergence**

Per cent emergence of parasitoid, *Trichogramma chilonis* from host was determined to be 56.4%, 48.8% and 65.0% in case of *Spodoptera litura*, *Plutella xylostella* and *Corcyra cephalonica*, respectively.(Fig. 3)

**2.3 Effect on the longevity of adult parasitoid**

The longevity of adult parasitoid (*T. chilonis*) was 3.6-4.6 days for male, and 4.1-5.6 days for female in the lepidopteran insects investigated.

**2.4 Effect on developmental period of parasitoids**

The developmental period of parasitoid, *T. chilonis* was observed to be 8.7 days in *S.litura*, 9.3 days in *P. xylostella*, and 9.1 days in *C. cephalonica*.

**2.5 Effect on the female emergence**

The female emergence was observed to be about 50% in all hosts tested.

**3. Parasitization behaviour and efficacy of *Trichogramma chilonis* on gamma sterilized eggs of different species of lepidopteran host**

With respect to Hosts, there might be some stimulating effects of low dose gamma radiation on host eggs on the biological attributes of parasitoids through the sterilization of host eggs and caused disinfection for the parasitoids. In the present study, The parasitisation behaviour and efficacy of *Trichogramma chilonis* was evaluated on radio-sterilized eggs of *Spodoptera litura* and *Corcyra cephalonica*. For this study two gamma doses evaluated were 5 Gy and 10 Gy. (Table 3,4)

**3.1 Effect on Parasitism**

The parasitisation capacity of egg-parasitoid, *T. chilonis* was similar on hosts, *Spodoptera litura* (29%) and *Corcyra cephalonica* (23%) at 5Gy, but it was slightly increased at 10 Gy in case of *Spodoptera litura* (31.3%), but remained almost similar in *Corcyra cephalonica* (21%). (Fig. 2 )

**3.2 Effect on emergence**

The per cent emergence of parasitoid, *Trichogramma chilonis* from irradiated host was determined as 30.5% and 51.4% at 5Gy; 31.2% and 66.6% at 10Gy in case of *Spodoptera litura* and *Corcyra cephalonica*, respectively. (Fig.4)

**3.3 Effect on the longevity of adult parasitoid**

The male and female longevity of the parasitoid was increased at 5Gy than at 10Gy.

**3.4 Effect on developmental period of parasitoid**

The developmental period of parasitoid, *T.chilonis* was not observed to be influenced by gamma-irradiation of host eggs of *S.litura*, or *C. cephalonica*.

**3.5 Effect on the female emergence**

The female emergence was reduced at 5Gy than at 10Gy where female emergence was almost equivalent to that in UV-sterilized host,i.e.,about 50% in the lepidopteran hosts experimented.

**Discussion**

The egg parasitoids belonging to genus *Trichgramma* are of great importance in applied biological control in different parts of the world, because of their large scale feasibility in release programme against various pests. Pest control programmes, which use *Trichogramma* as egg parasitoids depend on an efficient, reliable mass rearing system for augmentative and inundative releases. The maximum number of individuals of any genus cultured so far by men belongs to *Trichogramma* (De Bach and Hagan 1964). *Trichogramma* spp. are particularly suitable for use as a "biotic insecticide" through inundative releases, rather than as new natural enemies used in inoculative releases.

Manickavasgam et al. (1994) reported that based on pattern of oviposition, *Trichogramma chilonis* may be best suited parasitoid in ecological zones with extreme weather conditions occurring during the cropping season, since this parasitoid could deliver over 50% of its progeny within first day after

emergence. This characteristic was distinctly observed in the present findings as well although overall there was some influence of UV or gamma radiation on parasitisation capacity, but about 50% parasitization of total infectivity was evidenced on the first day of oviposition on three lepidopteran host pests tested.

**4.1 Various methods of sterilization**

Various methods for rendering eggs for *Trichogramma* rearing inviable have been employed, including cold treatment (Singh 1969) and gamma radiation (Marston and Ertle 1969) and ultra-violet (UV) radiation (Breniere 1965 and Voegele et al. 1974).

**4.2 Radiated host eggs as food source**

**4.2.1 UV-sterilization:** Breniere (1965 a) reported that exposing *Corcyra cephalonica* eggs for 15 min. to UV radiation killed the embryos and those eggs could be used for the multiplication of *Trichogramma australicum* Girault. Therefore, the UV radiation was also used as one of the sterilization means for treating eggs of three different lepidopteran hosts to ascertain the parasitization efficiency of *Trichogramma chilonis* and to evolve the strategy for augmenting this bio-control agent.

Eggs of *Ephestia kuehniella* (Zeller) killed with ultra-violet irradiation were also suitable for mass rearing of *Trichogramma* spp. (Voegele et al. 1974).

Goldstein et al. (1983) reported that UV-irradiated and non irradiated host eggs (*Ostrinia nubilalis*) were equally acceptable by the ovipositing females and were suitable for development of *Trichogramma nubiale* progeny. In the present studies UV-sterilization of host eggs of *Plutella xylostella* rendered treated eggs suitable to the same level as that of control; whereas the suitability of UV-treated host eggs of *Spodoptera litura* and *Corcyra cephalonica* was further enhanced in terms of parasitization capacity, parasitoid development and their emergence.

**4.2.2 Gamma Radiation:**

Elbadry (1965) was the first to suggest that moth eggs exposed to 3-9 krad of gamma radiation could serve as a food source for Trichogramma development. Marston and Ertle (1969) also tested the acceptability of irradiated (23.3 krad) moth eggs to *Trichogramma minutum* Riley, and reported that irradiated eggs were as suitable as control eggs for parasite development. This was true with present findings of parasitization efficiency of *T. chilonis* and their emergence from host eggs irradiated with 5Gy, 10Gy in case of *Spodoptera litura*, where as in case of *Corcyra cephalonica*, although parasitization efficiency was almost same as at 5Gy with respect to control but at 10Gy of gamma radiation the development and emergence of parasitoid were better than control.

**4.3 Radiation mediated influence on development:**

Elbadry (1965) stated that irradiation of host eggs had no effect on the development of Trichogrammatid egg –parasitoid, *Trichgramma semifumatum* (Perkins). Similar was the finding in our experiments where UV-irradiation or gamma irradiation of host eggs of *Spodoptera litura*, *Plutella xylostella* and *Corcyra cephalonica* did not influence the development of *Trichogramma chilonis*.

**4.4 Parasitization efficacy of *Trichgramma chilonis* on different species of lepidopteran hosts:** Parasitization of *Trichogramma chilonis* was almost similar in all the three hosts experimented, viz., *C. cephalonica*, *P. xylostella* and *S. litura*. Emergence of parasitoid from parasitised host was significantly more in *C. cephalonica*, followed by *P. xylostella* and *S. litura*. This reflects that host nutrients in *Corcyra* were presumably more suitable for parasitoid development than that of other hosts tested. That is also probably one of the reasons that *Corcyra cephalonica* is generally taken as a factitious host for *Trichogramma* sp. for its augmentative and inundative releases.

**4.5 Parasitization behaviour of *Trichogramma chilonis* on UV sterilized eggs of different species of lepidopteran hosts:** UV-sterilization of host's eggs markedly enhanced parasitization capacity of *Trichgramma chilonis* with respect to un-sterilized host-eggs. Emergence of parasitoid, *T.chilonis* from parasitized host was also significantly increased in lepidopteran hosts due to UV-sterilization of host. UV-sterilization of host eggs helped in multifold manner in promoting parasitization efficacy. Treatment with UV radiation sterilized the eggs of hosts, thereby checking the development of even those eggs that were not parasitized. This would completely reduce the probability of hatching of hosts which could have eaten the other parasitized eggs, by showing superparasitsm. This would, in turn, lead to more percentage and chances of survival of egg parasitoids with no risk of being parasitised by their own hosts.

Also due to no development occurring in sterilized eggs (inviable eggs), probably more nutrients might be directed to development of

growing parasitoid, instead of nutrient utilization by the host embryo. Therefore, *Corcyra cephalonica* could be more effectively exploited as a factitious host for *Trichogramma* sp. for its augmentative and inundative releases, by using radiation a tool.

**4.6 Parasitization behaviour and efficacy of *Trichogramma chilonis* on gamma sterilized eggs of different species of lepidopteran host. (Table-3 & 4):** Overall the gamma radiation did not adversely affect the parasitization capacity of *Trichgramma chilonis*, with respect to un-irradiated host, rather the suitability of lepidopteran hosts for parasitization was similar in normal and sterilized eggs.

The per cent parasitization by *Trichogramma* on gamma-irradiated host eggs at 5Gy or 10Gy was less than in UV-sterilized host eggs. This indicated that UV-sterilization was more effective than gamma irradiation induced host sterility in eliciting parasitization response by *Trichogramma*. Further among gamma-radiation doses, appeared to be 10Gy as a better dose than 5Gy for parasitoid development and its emergence. UV-sterilization of host resulted in enhanced parasitoids emergence in case of *Spodoptera litura*, whereas there was no significant difference between the UV-sterilization and gamma sterilization with respect to parasitoids emergence in eggs of host, *Corcyra cephalonica*.

The male and female longevity of the parasitoid where increased at 5Gy than 10Gy, where the response was almost similar to control. Further, it must be noted that UV-sterilization and 5Gy-gamma sterilization of host had a little stimulating effect on the longevity of the parasitoid adults. The female emergence was reduced at 5Gy than at 10Gy where emergence was almost equivalent to that in UV-sterilized host.

**4.7 Sterilization of host eggs and their introduction in parasite-host environment**

Knipling and Mc Guire (1968) suggested that it might be possible to maintain high population *Trichogramma* parasites by adding host eggs to the parasite-hosts environment. They further suggested that the addition of sterile eggs of the target host or the eggs of an innocuous alternate host as a possible way of increasing *Trichogramma* parasite populations without creating risks of additional crop damage. Knipling (1966) proposed that sterile host eggs might be provided by releasing sterile males, and sterile egg laying females of the hosts species.

**4.8 Advantage of host sterilization**

Radiation sterilization is not harmful (Goldstein et al. 1983) and that incorporating the method into a mass rearing system for various *Trichogramma* species would increase the production efficiency for eliminating the loss of developing parasitoids to predation by host larvae emerging out of non-parasitised eggs. This refers the phenomenon of super-parasitism that could be avoided which would lead to better conservation of bio-control agent.

**CONCLUSION**

An approach of combination of parasite releases with release of radio-sterilized insects, has been proposed and advocated (Knipling 1966, 1979; Knipling and McGuire 1968, Brower 1982). Here is a great need to test the hypothesis of Knipling that the combination of *Trichogramma* release with release of sterile or partially sterile moths might be more effective than either technique used alone.

The use of *Trichogramma* has made significant strides during the past 20 years, and this bodes well for the next decades. As with *Bt* and chemical insecticides, significant commercial achievements have been made with *Trichogramma*. This achievement suggests that we have every chance of succeeding in those order host/parasitoid systems that remain unexplored. One of the most important areas is the insect control – nuclear technique and bio-technology by which we focused on the use of radiation as a method to eradicate or suppress insect population in a total ecosystem was first initiated in 1949 that how low dose gamma radiation has been proved to be more effective method for insect disinfestation purposes of food and agricultural products than some suspected mutagenic and carcinogenic chemicals. The most important factor is the development of extension support to deliver the product to the use of and allow them to get into the field in a form that can have an effect. Information regarding where, when and how to release in different growth situation should be included with the product. This package, which will provide a service rather than a product alone, could come from the producers, government extensions or private consulting.

Although release of *Trichogramma* is currently one of the most benign approaches to pest control, more attention must be paid to the population dynamics of the pest, the other natural mortality factors at work, and the native complex of natural enemies, in particular native *Trichogramma* species. Finally, perhaps the greatest need is that of setting guidelines and

standardizing terminology and measurements; this includes issues of taxonomy, quality control, and assessments of efficacy.

**Acknowledgements**

The authors gratefully acknowledge financial support from the International Atomic Energy Agency, Vienna for conducting this work under Research Contract No. IAEA-RB-10847.

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**Parasitization behaviour and efficacy of *Trichogramma chiloni* on lepidopteran host**

- ***Spodoptera litura***
- ***Diamond back moth***
- ***Corcyra cephalonica***

**Table:1** Parasitization behaviour and efficacy of *Trichogramma chiloni*s on normal fertilized eggs of different
species of lepidopteran host

| Nature of host for *Trichogramma* | **Parasitoid's parasitization capacity (%)** | | | | **% Emergence of parasitoids from host eggs parasitized on** | | | | Develop-mental period of *T.chilonis* (Days) | Longevity of parasitoid (days) | | % female emergence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st day** | **2nd day** | **3rd day** | **Total** | **1st day** | **2nd day** | **3rd day** | **Total** | | Male | Female | |
| Eggs of *Spodoptera litura* | 25.4a ±2.3 | 32.7ab ±2.6 | 24.3a ±4.5 | 27.2ab ±2.6 | 29.7a ±3.1 | 45.4a ±2.7 | 30.3a ±1.7 | 35.6a ±4.8 | 8.9a ±0.1 | 3.9a ±0.5 | 3.6a ±0.3 | 57.8a ±1.9 |
| Eggs of *Plutella xylostella* | 34.4b ±2.2 | 34.3b ±2.9 | 29.5a ±2.2 | 33.5b ±2.0 | 46.1b ±4.9 | 54.8b ±4.1 | 56.7b ±5.1 | 50.1b ±4.5 | 8.9a ±0.1 | 3.7a ±0.4 | 4.3a ±0.6 | 48.3b ±3.1 |
| Eggs of *Corcyra cephalonica* | 18.8a ±2.5 | 25.8a ±2.4 | 24.6a ±2.6 | 22.7a ±2.2 | 63.2c ±5.9 | 62.4b ±4.8 | 44.7b ±4.4 | 57.9b ±4.8 | 8.8a ±0.1 | 3.1a ±0.3 | 4.0a ±0.4 | 55.6ab ±5.6 |

NB: Some eggs hatched because of their non-parasitization by *Trichogramma chilonis.*

On Day-1, 100 eggs pasted on paper card (known as *Tricho*-card) were provided for parasitization by *Trichogramma*

*chilonis*;On Day-2, 50 eggs on *Tricho*-card were provided for parasitization by *Trichogramma* ; On Day-3, 30 eggs

on *Tricho*-card were provided for parasitization by *Trichogramma chilonis*;

Means ± SE followed by the same letter in a column are not significantly different at P =0.05 level (ANOVA followed

by LSD post-test); n = 8

**Parasitization behaviour and efficacy of *Trichogramma cheloni* on UV-sterilized eggs of host**

- ***Spodoptera litura***
- ***Diamond back moth***
- ***Corcyra cephalonica***

**Table:2** Parasitization behaviour and efficacy of *Trichogramma chiloni* on UV sterilized eggs of different species of lepidopteran host

| Nature of host for *Trichogramma* | **Parasitoid's parasitization capacity (%)** | | | | **% Emergence of parasitoids from host eggs parasitized on** | | | | Develop-mental period of *T.chilonis* (Days) | Longevity of parasitoid (days) | | % female emergence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st day** | **2nd day** | **3rd day** | **Total** | **1st day** | **2nd day** | **3rd day** | **Total** | | Male | Female | |
| UV-sterilized eggs of *Spodoptera litura* | 30.5a ±2.3 | 35.2a ±3.0 | 25.3a ±2.5 | 31.1a ±1.8 | 57.1a ±5.5 | 60.4a ±5.4 | 42.9a ±3.6 | 56.4a ±2.7 | 8.7a ±0.1 | 4.6a ±0.8 | 5.6a ±0.9 | 51.8a ±3.7 |
| UV-sterilized eggs of *Plutella xylostella* | 32.2a ±1.7 | 28.3a ±3.1 | 25.0a ±3.3 | 29.4a ±2.2 | 48.3a ±3.8 | 55.0a ±3.6 | 47.5a ±3.5 | 48.8a ±3.9 | 9.3b ±0.1 | 3.6a ±0.5 | 4.1a ±0.5 | 51.0a ±4.7 |
| UV-sterilized eggs of *Corcyra cephalonica* | 42.8b ±2.5 | 56.0b ±2.5 | 47.3b ±6.6 | 43.9b ±1.4 | 72.4b ±4.9 | 60.1a ±1.5 | 50.4a ±6.6 | 65.0b ±2.1 | 9.1ab ±0.1 | 4.2a ±0.3 | 5.2a ±0.4 | 49.9a ±1.9 |

NB: Some eggs hatched because of their non-parasitization by *Trichogramma chilonis,* but relatively it was very less as

compared to normal fertilized eggs when subjected to parasitization.

On Day-1, 100 eggs pasted on paper card (known as *Tricho*-card) were provided for parasitization by *Trichogramma*

*chilonis*;On Day-2, 50 eggs on *Tricho*-card were provided for parasitization by *Trichogramma* ; On Day-3, 30 eggs

on *Tricho*-card were provided for parasitization by *Trichogramma chilonis*;s

Means ± SE followed by the same letter in a column are not significantly different at P =0.05 level (ANOVA followed

by LSD post-test); n = 8

**Parasitization behaviour and efficacy of *Trichogramma cheloni* on gamma- sterilized eggs of host**

- ***Spodoptera litura***
- ***Diamond back moth***
- ***Corcyra cephalonica***

**Table:3** Parasitization behaviour and efficacy of *Trichogramma chilonis* on gamma sterilized eggs of different species of lepidopteran host [Gamma radiation dose administered was 5Gy].

| Nature of host for parasitoid, *Trichogramma* | **Parasitoid's parasitization capacity (%)** | | | | **% Emergence of parasitoids from host eggs parasitized on** | | | | Develop-mental period of *T.chilonis* (Days) | Longevity of parasitoid | | % female emergence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st day** | **2nd day** | **3rd day** | **Total** | **1st day** | **2nd day** | **3rd day** | **Total** | | Male | Female | |
| Gamma-sterilized eggs of *Spodoptera litura* | 25.0a ±1.8 | 43.6a ±4.2 | 16.3a ±2.3 | 29.1a ±1.6 | 37.0a ±2.6 | 21.6a ±2.5 | 42.9a ±4.7 | 30.5a ±2.7 | 9.3a ±0.1 | 4.8a ±0.7 | 6.6a ±0.5 | 43.8a ±2.8 |
| Gamma -sterilized eggs of *Corcyra cephalonica* | 22.6a ±2.5 | 32.4a ±4.4 | 15.3a ±3.4 | 23.6a ±1.6 | 45.0b ±2.2 | 57.2b ±2.7 | 63.0b ±5.9 | 51.4b ±7.9 | 8.9a ±0.2 | 5.6a ±0.6 | 5.3a ±0.8 | 39.9a ±2.5 |

On Day-1, 100 eggs pasted on paper card (known as *Tricho*-card) were provided for parasitization by *Trichogramma chilonis*;On Day-2, 50 eggs on *Tricho*-card were provided for parasitization by *Trichogramma* ; On Day-3, 30 eggs on *Tricho*-card were provided for parasitization by *Trichogramma chilonis*;

Means ± SE followed by the same letter in a column are not significantly different at P =0.05 level ((Student's t-test);

n = 8

**Table:4** Parasitization behaviour and efficacy of *Trichogramma chilonis* on gamma sterilized eggs of different species of lepidopteran host [Gamma radiation administered was 10Gy].

| Nature of host for parasitoid, *Trichogramma* | **Parasitoid's parasitization capacity (%)** | | | | **% Emergence of parasitoids from host eggs parasitized on** | | | | Develop-mental period of *T.chilonis* (Days) | Longevity of parasitoid | | % female emergence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st day** | **2nd day** | **3rd day** | **Total** | **1st day** | **2nd day** | **3rd day** | **Total** | | Male | Female | |
| Gamma-sterilized eggs of *Spodoptera litura* | 34.5a ±2.8 | 30.4a ±3.0 | 22.3a ±2.3 | 31.3a ±2.5 | 33.9a ±5.5. | 33.6a ±3.6 | 11.9a ±5.2 | 31.2a ±4.8 | 9.1a ±0.1 | 3.3a ±0.5 | 3.5a ±0.4 | 45.5a ±4.3 |
| Gamma -sterilized eggs of *Corcyra cephalonica* | 22.4b ±2.2 | 23.2a ±2.4 | 11.3b ±2.6 | 20.7b ±2.4 | 72.3b ±5.9 | 62.1b ±4.4 | 44.2b ±6.2 | 66.6b ±3.4 | 9.2a ±0.1 | 4.9a ±0.7 | 4.6a ±0.7 | 49.8a ±4.3 |

On Day-1, 100 eggs pasted on paper card (known as *Tricho*-card) were provided for parasitization by *Trichogramma chilonis*;On Day-2, 50 eggs on *Tricho*-card were provided for parasitization by *Trichogramma* ; On Day-3, 30 eggs on *Tricho*-card were provided for parasitization by *Trichogramma chilonis*;

Means ± SE followed by the same letter in a column are not significantly different at P =0.05 level (Student's t-test);

n = 8

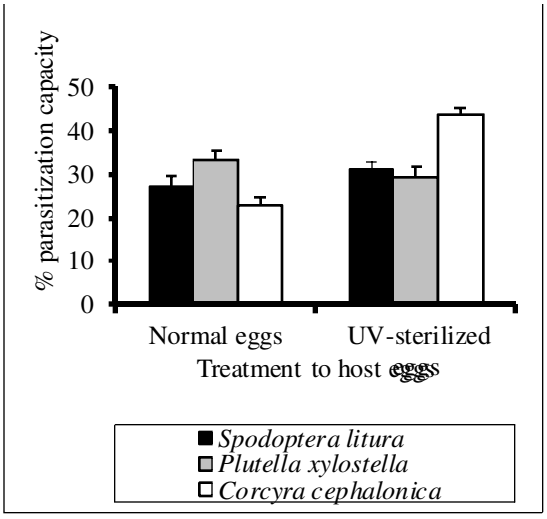


**Figure-1**

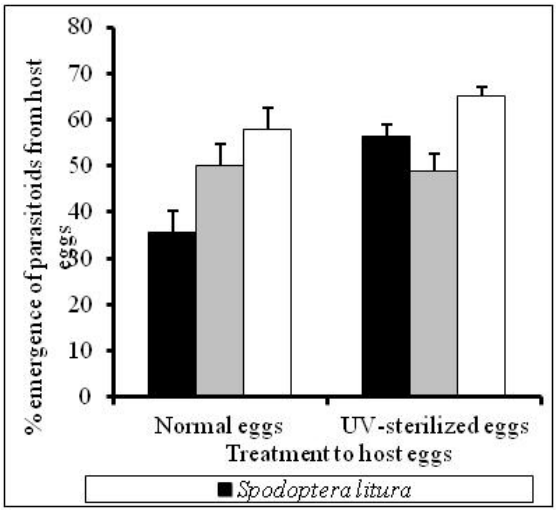


**Figure-2**

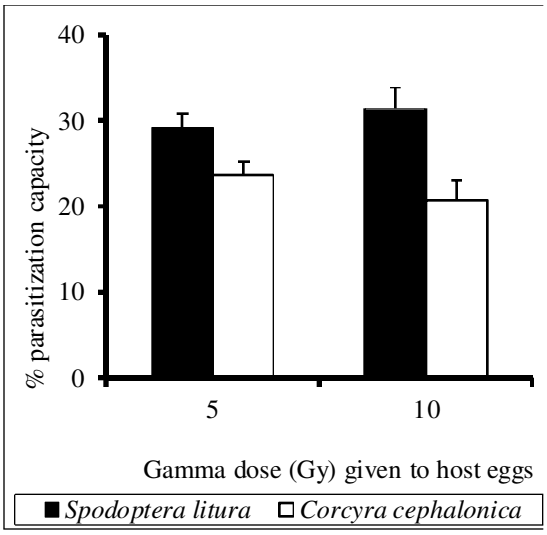


**Figure-3**

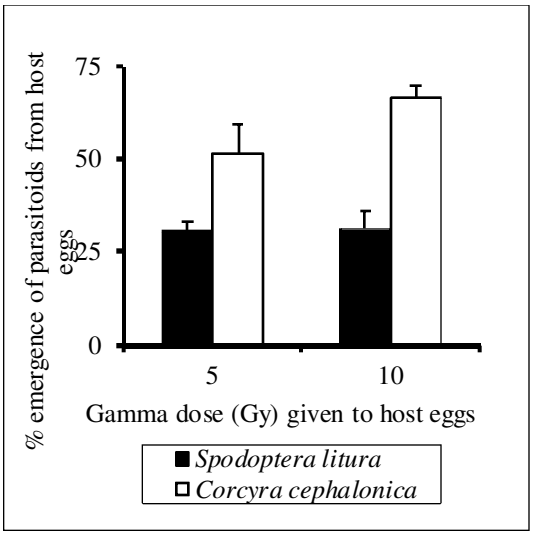


**Figure-4**

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