

# UTILIZATION OF *Trichogramma* PARASITOID AS BIOLOGICAL CONTROL AGENT AGAINST SUNFLOWER HEADWORM *Helicoverpa armigera* HUBNER

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## ABSTRACT

The study evaluated *Trichogramma* parasitoid as a potential biological control agent against sunflower headworm. The aims were to determine the most effective *Trichogramma* species in parasitizing sunflower headworm egg, to evaluate the effectiveness of different rates of *Trichogramma chilonis* application and to compare the use of *Trichogramma* and insecticide on the yield of sunflower .

Results show that of the four *Trichogramma* species tested *T. chilonis* proved to be the most efficient in parasitizing *Helicoverpa armigera* eggs. Percentage parasitism, percentage sunflower seed set and yield of sunflower were greatly affected by the rate of application of *T. chilonis*. When applied at the rate of 70,000 parasites per hectare per release, *T. Chilonis* was effective in parasitizing sunflower headworm eggs, thus increasing sunflower seed sets and consequently sunflower yield. Application of *T. chilonis* was superior to insecticide treatment in controlling sunflower headworm.

## 1. INTRODUCTION

### Importance of the study

Sunflower *Helianthus annus* L. is an important economic crop in Southeast Asia. The seeds are main product from which an edible, very high quality oil is extracted. Sunflower seed oil has been dubbed as the potential "oil king" from among other oil seeds, on the basis of the yield returns per given period of time as well as its health insuring qualities.

Sunflower headworm *Helicoverpa armigera* is one of the most damaging insect pests of sunflower. The larvae eat the leaves, buds, petals and the small green leaf-like bracts surrounding the head or feed on the top of the developing seeds on the face of the head. Damage to the developing seeds is usually slight unless infestations are particularly heavy.

However, larvae feeding on the back of the head can pre-dispose the crop to secondary fungal head rots.

At present, the control of sunflower headworm is primarily dependent on the use of synthetic insecticides. However, chemical insecticides pose several hazards to animals, human beings, beneficial insects and the environment; hence, there is a need to look for other methods of control. Even pollinators are not spared from the devastating effect of chemical insecticides. Use of pollinators is crucial since it spells the success or failure in sunflower production.

The use of parasites and predators in sunflower production may help reduce the application of insecticides which will minimize the various hazards of these toxic compounds (Torreno and Cadapan, 1984).

#### **Host range of *Helicoverpa armigera* Hubner**

*Helicoverpa armigera* Hubner is a major insect pest of a number of economic plants (Torreno and Cadapan, 1980) and is one of the most damaging pests of sunflower (Icayan, 1978). It also feeds on cotton, corn, tomato tobacco and sorghum. It is also found feeding on the heads of cabbage (Magallona, 1977). Depending on the host and plant part it attacks, this insect is commonly referred to as cotton bollworm, sorghum headworm, corn earworm, tomato fruit worm or sunflower headworm (Ramos and Morallo-Rejesus, 1981).

#### **Biology of *Helicoverpa armigera* Hubner**

The biology of *H. armigera* Hubner under laboratory conditions was studied by Icayan (1978). The life cycle of the insect ranged from 50 to 57 days. The eggs appear like an ordinary pin head and pearly white when first laid. The color changes as it develops. The larvae is hairy with black spots on the body. The pupa is reddish brown and bullet shaped. After 10 to 14 days, the pupa emerges into an adult moth with light brown color. The male moth can be distinguished by having dark circular spots on the middle of the forewing while the female does not show such spots.

#### ***Trichogramma* parasitoids**

The *Trichogramma* are hymenopterous parasitoids of lepidopterous insect pests (Cadapan and Gonzales, 1983). They are minute (0.1 – 0.5 minutes) endoparasitic insects which prey on other insects eggs. They complete their life cycle inside the eggs of other insects and kill the host before it is hatched. They belong to the chalcid group of hymenopterous insect in the family Trichogrammatidae (Cadapan, 1986).

The genus *Trichogramma* was described and discussed by Alba (1986) as follows: the venation and marginal fingers are reduced with a short stigmal vein. The stigmal veins are sometimes absent but the post marginal vein is always present, the venation never reaching beyond with pubescence of ten arranged in rows or line with oblique fine setae (R.S.) present below the stigma, foreleg with modified straight foretibial spurs. They are the only chalcididae with three segmented tarsi. The flagella are not segmented and the male genitalia has dorsal gonobase expansion. The aedeagus together with apodemes are longer than the entire genitalic capsule.

The term parasitoid refers to insect parasites which usually require only one host to complete their life cycle and ultimately would kill their host insect (de Bach, 1964).

#### **Species of *Trichogramma* in the Philippines**

Twenty-four species of *Trichogramma* are known worldwide. Of these, five have been reported in the Philippines, of which four are introduced and one indigenous (Cadapan, 1986). The different species are *Trichogramma chilonis* Ishii, *Trichogramma chilotheae* Nagaraja and Nagarkati, *Trichogramma evanescens* Westwood, *Trichogramma japonicum* Ashmead and *Trichogramma minutum* Riluz (Alba, 1986; Baltazar, 1981 and 1963).

The description of the different *Trichogramma* species and its close relative *Trichogramma toidae* was made by Alba (1986). According to her, *chilonis* has been erroneously identified as *australicum* Girault. She reported that *T. australicum* is not found in the Philippines and if this is true, all of the previous works on *T. australicum* (published or unpublished) in the Philippines before 1986 should apply to *T. chilonis*. This species was reportedly imported by Merino as *T. australicum* from China in 1934. Alba further stated that this species is being mass produced only by PHILSUCOM at La Granja, Negros Occidental for the control of sugarcane stem borers *Tetramesa schistaceana* and *Chilothea infuscatella*. She failed to mention that NCPC and the Department of Entomology Biological Control Laboratory (BCL) has been mass producing this species very cheaply (P1.00 per 10,000 parasites) since 1980 to control cotton, legume from PHILSUCOM, La Granja, Negros Occidental and from the BCL, and its population has been sent to the following: 1) Philippine – German Biological Control Project, and 2) Philippine Cotton Corporation to control bollworm, *H. armigera* Hubner, 3) Isabela State University, 4) Mariano Marcos State University, 5) Visayas State College of Agriculture, and 6) Central Mindanao University for the control of lepidopterous pests of legume and other crops. Moreover, it has been released in high population (80,000 parasites/ha) in Calauan, Bay and Los Baños, Laguna to control vegetable, rice and cotton pests, respectively. Parasitization ranges from 43% to 91%. It was

also released in La Union and Pangasinan in 1983 to control *H. armigera* at the rate of 50,000 to 80,000 parasites/ha where 80-98% of *H. armigera* eggs were parasitized (Cadapan, 1986).

In 1989, Aganon found that *T. chilonis* was the most effective in controlling lepidopterous insect pests of soybean with percent parasitism of 52.74.

Moreover, the different species of *Trichogramma* were described by Alba (1986) as follows:

*Trichogramma chilonis* Ishee. Male yellow with blackish abdomen and mesoscutum. Antennal hairs somewhat sharply tapering and moderately long; forewing with 4 to 6 oblique lines setae ( $R_{s1}$ ); fringe on tornus about one sixth width of wing. Genitalia with dorsal expansion of gonobase triangular, with lateral lobes very prominent, chelate structure (CS) markedly below level of gonoforceps, median chitinized ridge (CR) paired, extending anteriorly to about two-thirds length of genitalia; aedeagus as long as apodemes, both slightly shorter than hind tibiae.

Female yellow with first three abdominal terga black; antennae clubbed with short hairs on flagellum. Ovipositor as long as or slightly longer than hind tibiae.

*Trichogramma chitotraeae* Nagaraja and Nagarkatti. Male yellow with blackish pronotum, mesopleurum, mesoscutum, abdominal terga and hind coxae; antennal flagellum augmented with long hairs, longest hair more than three times the maximum width of flagellum; forewings with 3 to 4 obliquely lined setae ( $R_{s1}$ ). Genitalia with more or less triangular dorsal expansion of gonobase (DEG), apex tapering and not projecting beyond gonoforceps (GF), but extending only to level of chelate structures (CS); aedeagus prominent and long projecting beyond gonoforceps (almost as long as or slightly longer than the entire male genitalia); large chelate structures for below level of tips of gonoforceps (GF), protruberance at base of chelate structures very minute and inconspicuous; median ventral projection (MVP) very distinct and long, with two continuous ridges (CR) extending anteriorly only for a short distance from median ventral projection (MVP) aedeagus together with apodemes slightly shorter than hind tibia.

Female yellowish on anterior part and most of mesoscutum, one or more penultimate abdominal terga in median region blackish; antennae typically clubbed with few short hairs. Ovipositor slightly longer than hind tibia.

*Trichogramma evanescens* Westwood. Male dull yellow with dark brown head, prothorax and abdomen; antennal hairs very long and finely tapering, the longest hairs being nearly four times maximum width of flagellum; forewing with 2 to 3 oblique lines setae ( $R_{s1}$ ), fringe on tornus about one sixth the width of the wing. Genitalia with dorsal expansion of gonobase (DEG) highly sclerotized, broadly triangular with broadly rounded sides and markedly constricted at base; chelate structures (CS) far below level of tips of gonoforceps;

median ventral projection (MVP) distinct; median chitinized ridge (CR) paired, extending anteriorly to three-fourths the length of genitalia; aedeagus slightly longer than apodemes together shorter than hind tibia.

Females have the same color as the males. The ovipositor is as long as the hind tibia.

*Trichogramma japonicum* Ashmead. Male dull brownish yellow with black thoracic sclerites and abdominal terga; antennal hairs long and sharply tapering; longest hairs about three and one half times maximum width of flagellum; forewings with 8 to 9 oblique lines setae ( $R_{s1}$ ) with fringe on tornus nearly one fifth width of wing, fringe on outer margin as well as on tornus nearly of equal length. Genitalia with dorsal gonobase expansion (DEG) highly sclerotized, horseshow-shaped with blunt posterior extremity; chelate structures (CS) far below level of tips of gonoforceps (GF); median ventral projection (MVP) inconspicuous, entire region highly chitinized; median chitinized ridge (CR) paired, extending about two-thirds the length of genitalia, aedeagus distinctly longer than apodemes, both as long as or very slightly longer than hind tibia.

Female dull brownish color with black thoracic sclerites; antennae clubbed with few short hairs on flagellum. Ovipositor nearly one and one half times longer than hind tibia.

### **The importance of *Trichogramma***

#### *Trichogramma* as biocontrol agent

The *Trichogramma* parasitoids and other egg parasites are desirable biological control agents due to the following reasons: (1) they attack and kill a variety of lepidopterous eggs before they can damage the crop; (2) they are relatively specific to the pest species and safe to use; (3) they can establish in a tropical ecosystem and can be integrated with other control strategies; (4) some species are locally available and (5) they can be mass produced cheaply and conveniently on unnatural host *Corcyra cephalonica* Stainton, by the farmers themselves (Cadapan *et al.*, 1986).

The efficiency of *Trichogramma* species against some rice insect pests was reported by Perez and Cadapan (1986). The insect pests affected were the eggs of yellow stem borer, *Tryporyza incertulas* (Walker); stripe rice stem borer, *Chilo suppressalis* (Walker); leaf folder, *Sesamia exiqua* (Butter); *Marasmia patnalis* (Bradley); green semi-looper, *Naranga aenescens* (Morre), rice caseworm, *Nymphula depunctalis* (Greenee).

Accordingly, the degree of parasitization on the different host eggs depends on morphological characteristics of the egg and location on the leaf where egg masses were deposited. Egg masses that were covered with bristle-like silky covering, secreted by the female moth during oviposition, prevented the female parasite from ovipositing her egg. They further observed that the host egg parasite ratio of three spp. of *Trichogramma* on the different

rice pest eggs were related to the size of the host eggs. Larger eggs like the egg of *C. suppressalis* accommodate more than one parasite per egg (Rissig *et al.*, 1986).

The potential of *T. australicum* and *T. chilostraeae* against the eggs of *H. armigera* was studied by Torreno and Cadapan (1984). Parasitization and subsequent emergence of adult *T. australicum* and *T. chilostraeae* were found to be highest in newly laid (less than 12 hours) eggs compared to the two and three-day-old eggs of *H. armigera*. As the host egg advances in its embryonic development, it becomes less attractive for parasitization (Torreno and Cadapan, 1984).

A study made by Morales (1991) showed that higher rates of *T. chilonis* application increased percent parasitization both for *H. armigera* and *L. orbonalis*. Recommended insecticide application outyielded all other treatments and as expected, the untreated control was the lowest yielder. Although the recommended insecticide significantly performed better in terms of yield, *Trichogramma* treated plants at either 50,000 and 75,000 parasitoids per hectare per release may prove economical considering the high cost of insecticides.

The dissection of papilio larvae parasitized by *Trichogramma papilionis* Nagarkatti and *T. dendrolomi* Matsumura revealed that the females inserted their ovipositor into host eggs but withdraw without laying on eggs of older hosts (Hiehata *et al.*, 1975). It was found out that *T. evanescens* preferred eggs of *Ostrinia* when they were at the vitellus stage (Benoit and Voegelé, 1979). Their rejections for old age egg was most probably due to physical and chemical conditions which cause them to withdraw their ovipositor without laying an egg.

### **Objectives of the study**

This study was conducted to:

1. determine the most efficient *Trichogramma* species in parasitizing *H. armigera* Hubner eggs;
2. evaluate the effectiveness of different rates of application of the most efficient *Trichogramma* species against sunflower headworm *H. armigera* Hubner; and
3. compare the use of *Trichogramma* and insecticides on the yield of sunflower.

### **Time and place of the study**

The study was conducted at the experimental area of the Central Luzon State University, Science City of Muñoz, Nueva Ecija.

## 2. MATERIALS AND METHODS

### Evaluation of different species of *Trichogramma* against *Helicoverpa armigera* Hubner

Four *Trichogramma parasitoid* species were evaluated as follows:

*Trichogramma chilonis* Ishii

*Trichogramma evanescens* Westwood

*Trichogramma chilostraea* Nagaraja and Nagarkatti

*Trichogramma japonicum* Ashwood

The parasitoids were acquired from the Biological Control Laboratory of the Department of Entomology, University of the Philippines at Los Baños. The application rate was 50,000 parasitoids per hectare per release.

A 4 x 5 m experimental plot per treatment repeated 3 times was utilized. A nylon net barrier was provided between treatments to prevent the parasitoids from transferring.

Each plot provided with five pairs of sunflower headworm adults at 55 days after emergence (DAE) and at 65 DAE and allowed to lay eggs. Paper strips containing about to emerge *Trichogramma* were introduced in the plots 10 days before full bloom (60 DAE) and at full bloom (70 DAE).

Parasitized and unparasitized eggs were counted and recorded daily after introduction of *Trichogramma* in sunflower plots.

The eggs were encircled using pentel pen to avoid double counting. Percent parasitization was computed and statistically analyzed by Duncan Multiple Range Test (DMRI).

### Rate of application of *Trichogramma chilonis*

The best *Trichogramma* species in terms of parasitization obtained in the first experiment was evaluated at different application rates as follows:

- 1) 40,000 parasites/ha
- 2) 50,000 parasites/ha
- 3) 60,000 parasites/ha
- 4) 70,000 parasites/ha

Field release of *T. chilonis* was done following the same procedure outlined in the first experiment.

### *Trichogramma chilonis* and insecticide application against *Helicoverpa armigera* Hubner

The experiment was conducted in a Randomized Complete Block Design (RCBD) with 3 replications. The treatments were:

- 1) No *Trichogramma* or insecticide application
- 2) *Trichogramma chilonis* application at the rate of 70,000 parasites per hectare applied at 60 and 70 DAE
- 3) Chemical application (Cypermethrin)

Cypermethrin application was done before the release of *Trichogramma*.

Percentage parasitization was computed using the formula:

$$\% \text{ Parasitization} = \frac{\text{Number of eggs parasitized}}{\text{Total number of eggs collected}} \times 100$$

Percentage seed set was obtained using the formula:

$$\% \text{ Seed Set} = \frac{\text{Number of filled} - \text{unfilled seed}}{\text{Total filled} + \text{unfilled seeds}} \times 100$$

### 3. RESULTS AND DISCUSSION

#### **Most effective *Trichogramma* species**

The percent parasitization of sunflower headworm eggs using different species of *Trichogramma* is presented in Table 1. Among the four species evaluated *Trichogramma chilonis* Ishii showed the highest percentage egg parasitization of 81.48%. Statistically, there was no difference in parasitization between *Trichogramma evanescens* and *Trichogramma japonicum* having means of 70.03% and 67.92%, respectively. *Trichogramma chilostraea* was least effective in parasitizing sunflower headworm egg.

**Table 1.** Percent parasitization of sunflower headworm eggs as affected by the different *Trichogramma* species. December 1998 – March 1999.

<i>Trichogramma</i> species	Percent Parasitism (%)
<i>Trichogramma chilonis</i>	81.48 <sup>a</sup>
<i>Trichogramma evenescens</i>	70.03 <sup>b</sup>
<i>Trichogramma chiloatraea</i>	63.06 <sup>c</sup>
<i>Trichogramma japonicum</i>	67.92 <sup>b</sup>

Means of the same letter are not significantly different at the 5% level.

According to Baltazar (1963, 1981), since its importation from Japan in 1934, *Trichogramma japonicum* was tested against *Helicoverpa armigera* in comparison with *Trichogramma chilonis* and the percentage parasitization obtained was 56.57% and 80%, respectively. This means that *Trichogramma chilonis* is more effective than *Trichogramma japonicum* against *Helicoverpa armigera* Hubner, (Cadapan, 1986).

#### Effective application rate of *Trichogramma chilonis*

**Table 2** presents the percentage parasitization of sunflower headworm eggs at different rates of application of *T. chilonis*. Percentage egg parasitization increased corresponding to the increase in the number of parasites released per hectare. The means were 43.12%, 54.37%, 71.87% and 86.56% for 40,000, 50,000, 60,000 and 70,000 parasites, respectively. The results imply that with increasing rate of application of *T. chilonis* percentage parasitization corresponding increases.

**Table 2.** Percent parasitization of sunflower headworm eggs as affected by the different rates of *Trichogramma chilonis*. March 1999 – June 1999.

Rate	Percent Parasitism (%)
40,000	43.12 <sup>d</sup>
50,000	54.37 <sup>c</sup>
60,000	71.87 <sup>b</sup>
70,000	86.56 <sup>a</sup>

Means of the same letter are not significantly different at the 5% level.

These findings proved that *Trichogramma chilonis* have the ability to parasitize *Helicoverpa armigera* Hubner especially at high rate of application. Similar findings were

likewise established in the studies of Perez and Cadapan (1986) on the efficiency of *Trichogramma* species against lepidopterous insect pests in rice.

#### Percentage seed set and yield

The percentage seed set and yield as a result of the different rates of application of *T. chilonis* application is shown in Table 3. The highest percentage seed set was 83.93% attained from plots which received 70,000 parasites per hectare. The lowest percentage seed set was from 40,000 parasites per hectare.

**Table 3.** Percentage sunflower seed set and yield as affected by the different rates of *Trichogramma chilonis* application. March 1999 – June 1999.

Rate	Percentage Seed Set (%)	Yield (ton/ha)
40,000	56.05 <sup>c</sup>	0.97 <sup>c</sup>
50,000	70.68 <sup>b</sup>	1.06 <sup>bc</sup>
60,000	70.02 <sup>b</sup>	1.29 <sup>b</sup>
70,000	83.93 <sup>a</sup>	1.55 <sup>a</sup>

Means of the same letter are not significantly different at 5% level.

The high seed set of sunflower as affected by the highest rate of *T. chilonis* application implies the effectiveness of the parasite in controlling sunflower headworm. The parasite successfully parasitized sunflower headworm eggs and thus prevented damage to the sunflower head itself.

The highest yield attained was 1.55 tons per hectare which was obtained from plots with 70,000 parasites per hectare. This was followed by 60,000 parasites with yield of 1.29 tons per hectare. The significant results obtained among treatments indicate the positive effect of increasing rate of *T. chilonis* application.

#### Comparison between *Trichogramma* and chemical application

The comparative effect of *T. chilonis* and insecticide application is presented in Table 4. Application of *T. chilonis* significantly produced higher percentage seed set and yield.

**Table 4.** Percentage sunflower seed set and yield as affected by *Trichogramma chilonis* and chemical application. March 1999 – June 1999.

Treatments	Percentage Seed Set	Yield (tons/ha)
No Application	70.59 <sup>b</sup>	0.82 <sup>c</sup>
Insecticide application	67.07 <sup>b</sup>	1.20 <sup>b</sup>
<i>Trichogramma chilonis</i> application	82.80 <sup>a</sup>	1.52 <sup>a</sup>

Means of the same letter are not significantly different at 5% level.

Results confirmed the efficiency and superiority of *T. chilonis* as a good non-chemical warfare against sunflower headworm without the negative effects of chemicals to environment. The higher percentage seed set obtained from *T. chilonis* application could be attributed to the presence of pollinator bees which are not decimated as a result of the parasite application. This indicates that application of *T. chilonis* at the rate of 70,000 parasites per hectare per release is very effective in controlling sunflower headworm. This further supports the findings of Terreno and Cadapan (1984) that the use of parasites and predators indeed could reduce the application of insecticides which will minimize the various hazards of these toxic compounds.

#### 4. CONCLUSION

*Trichogramma chilonis* is recommended as a potential biological control agent for sunflower headworm *H. amigera* Hubner. Specifically the use of 70,000 parasites/hectare per release is highly recommended for maximum results. The use of higher rate of application is recommended beyond 70,000 parasites/hectare per release is however recommended for further study to test whether this could yield optimum results. More biological and ecological studies are needed in *Trichogramma* species. Incorporate *Trichogramma* to the Integrated Pest Management (IPM) package for crop production whenever applicable. Season of planting must be considered as one of the possible factors that could have influenced some of the variable in the study.

With the result and conclusion drawn from this study which serves as baseline information in formulating researches of this kind, further studies in a wider scale are therefore recommended to determine the effects of the treatments.

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