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# Effectiveness of *Trichogramma chilonis* Ishii against spiny bollworm in Okra and susceptibility to insecticides

**Muhammad Qasim, Dilbar Husain, Saif Ul Islam, Habib Ali, Waqar Islam, Mubasher Hussain, Fangfei Wang and Liande Wang**

**Abstract**
The present study had evaluated the effectiveness of *Trichogramma chilonis* against *Earias insulana* in Okra field during 2014-16. The whole study was conducted by distributing ninety egg cards acre$^{-1}$ with 300, 400, 500 and 600 eggs.card$^{-1}$. The effectiveness of the egg parasitoid was boosted after the fourth-week release of *T. chilonis* at the rate of 600 eggs.card$^{-1}$, showing 43.09±0.51%, 25.65±1.59% and 39.13±1.48% control during 2014, 2015 and 2016, respectively. Whereas, infestation in the controlled field was recorded up to 62.89±1.39% during the first year of the trial, and the next year infestation was lesser (39.83±1.19%) as compared to the previous year. Susceptibility study revealed that six out of ten insecticides were extremely toxic to *T. chilonis* in the laboratory, and other four insecticides (Triflumuron, Emamectin, Spinosad and Imidacloprid) were least toxic, causing only 10-24% and 12-44% mortality than controlled conditions after 24 and 48 hrs of application. It is concluded that parasitism is high in the fields of more eggs per card throughout the trial as compared to less number of egg inoculation. It is observed that Emamectin benzoate is a safer insecticide for *Trichogramma*.

**Keywords:** *Earias insulana*, Egg parasitoid, Organic agriculture, Pesticide free, *Sitotroga cerealella*, *Trichogramma* emergence

## 1. Introduction

Okra (*Abelmoschus esculentus* L.) is one of the most important vegetables grown across 14000 ha of Pakistan, along with 7.72 tonne/ha. Pakistan is a world's fifth largest country in the production of Okra, with production 108080 Million Tonnes yield per year [1]. Vegetable production is hindered due to inefficient management of pests and diseases, which cause severe losses in the yield [2, 3]. These losses can surpass the 25 percent of the whole yield, which leads towards awful income loss to farmers. Chaudhary and Dadheech [4] reported that certain losses in okra are reached up to 54 percent due pests, whereas 18.45 percent damage is caused by spiny bollworm (SPB) alone on Okra [5].

*Earias insulana* (Boisduval) (Noctuidae: Lepidoptera) is obliterating and multivoltine lepidopteron crawly insect pest of Okra [6-8], which feeds on terminal shoots, flower buds and fruits. It has been accounted for several countries of Asia and Africa, and is also known as a cotton pest [9]. Therefore, chemical insecticides were being used to decrease the infestation brought about by SPB [10-12].

Much of the pyrethroids [13], organophosphorus [14] and other new insecticides (acetamiprid and indoxacarb) [15] were observed to be effective against this pest. However, repetitive utilization of insecticides has led to develop resistance in insect pests [16-18]. Because of such caveats, it is pivotal to include distinctive management approaches, for example, the use of transgenic crops, plant extracts and biological agents in Integrated Pest Management programs [19, 20].

*Trichogramma* is being utilized as a natural enemy as a part of natural control programs [21], which prevents adult emergence from host eggs [22], and leads toward slightest insect infestations on host plants [23]. Because of this specific property, *Trichogramma* is known as natural control specialist of lepidopteran insect pests [24]. Its effectiveness can be affected by natural variables such as temperature [25], habitat and the host [26-28]. Although, it has been successful especially against lepidopteran insect pests in the research center and nursery showing up to 50% parasitism [29, 30]. Few studies, however, has been conducted so far to observe the efficiency of *Trichogramma chilonis* in the field condition against SPB.

The present study was designed to assess the performance of *T. chilonis* against SPB in the field conditions, and to test the mortality of *T. chilonis* after exposure to various insecticides to scrutinize more secure insecticides.

## 2. Materials and Methods

Commercially accessible ten insecticides were collected from two distinct organizations and diluted with distilled water to prepare a solution of field prescribed dosage for each insecticide (Table 1), separately. Each insecticide was measured with the pipette and kept into a beaker to make the volume 100 ml with distilled water [31, 32].

Whereas, the wasps of *T. chilonis* were reared on the eggs of *Sitotroga cerealella* (Olivier) (Gelechiidae: Lepidoptera), collected from Trichogramma Lab, Entomological Research Institute, Faisalabad [33].

**2.1 Field trials:** Five acres were selected in rural areas near Faisalabad city to check the infestation of SPB, that were monitored on a weekly basis during April-May, 2014-16, as described by Wright, Hoffmann [34]. Four acres were inoculated with different egg cards, i.e. 300, 400, 500 and 600 eggs card$^{-1}$, separately and the fifth acre was kept as controlled. Trials were conducted in fifteen blocks according to Randomized Complete Block Design (RCBD) with six repeats of plots size 50×10 ft. All egg cards were installed into a field at the distance of 500 ft according to the description of Smith [21]. On the other hand, Lufenuron (Match) insecticide was sprayed two weeks prior to egg release, to decrease the pressure of other insects from the field as per Butter, Singh [35]. Then, after the first week of *T. chilonis* inoculation, fruit infestation by *E. insulana* was recorded by counting infested and healthy fruits from 50 randomly selected plants per plot at weekly intervals. Whereas, one acre Okra field was kept controlled (CK) with no application of chemical insecticide and *Trichogramma*, and divided into three blocks along with six replications. Fruit infestation was recorded from six different places of each block via observing 50 plants.

**2.2 Insecticidal susceptibility test:** The present experiment was performed according to Complete Randomized Design (CRD) having ten treatments with three repeats, and the concentration of these insecticides was same as in the field (Table 1). The trials were conducted in the laboratory at Entomological Department, Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan, during 2015-2016, to assess the toxicity of insecticides against *T. chilonis* by using the following method [18].

**2.3 Egg card exposure test:** The parasitized *S. cerealella* egg cards were kept in an incubator for seven days under the conditions of 25±2 ºC temperature and 65±5% relative humidity (R.H). Each card had a hundred eggs. After seven days, egg cards were dipped into the insecticide solution for one minute and then put back into the incubator by modifying the description of Ahmad, Arif [36]. Then adult emergence data were recorded after the passage of 24 hrs and 48 hrs under the microscope. Adult emergence was also observed in controlled conditions with same replications.

**2.4 Statistical analysis:** One-way Analysis of Variance (ANOVA) was performed to find out f-value of treatments and controlled observations, separately. While, the t-test was performed to calculate the mean variation among effectiveness of different egg cards by using statistical program IBM-SPSS, at a significance level of $p<0.05$ [37].

## 3. Results

**3.1 Field trial:** The egg parasitism was ranged from 0.6% to 75% in inoculated plots during the entire period of the trial. The occurrence of egg parasitism was more prominent in plots with the higher number of eggs per card than the plots of fewer eggs per card. The effectiveness of egg cards was irrelevant following the first week of egg release in the field by 300-500 eggs card$^{-1}$ during the entire time of trial. Whereas, the release of 600 eggs card$^{-1}$ shown much potential to parasitize the eggs of *E. insulana*, and effectiveness was come to up to 20.93±0.62% and 14.78±1.31% during 2014 and 2016, respectively. Although, fruit infestation in a controlled (CK) field was recorded up to 55.67±1.15% (Fig. 1). Significant efficacy was seen after second, third and fourth inoculation of eggs in the field.

In like manner, the same pattern of low efficiency was recorded in the release of fewer egg numbers/card after the fourth week during the season of 2014. While extensive effectiveness was recorded in the plots of 500 and 600 eggs for each card, which controlled infestation up to 36.59±0.59 and 43.09±0.51 percent, respectively.

During the season of 2015, the okra fruit infestation with *E. insulana* was low and ranged from 32.17±0.83 to 37.05±1.12 percent in controlled (CK) conditions (Fig. 2). Again the same pattern of effectiveness was observed like an earlier year. In this manner, after the fourth week of inoculation, infestation decline was sensible in all plots, and most extreme abatements were seen in the plots of 600 eggs card$^{-1}$, which showed 25.65±1.59% efficiency.

So also, the infestation by *E. insulana* was likewise low amid 2016 (Fig. 3), ranging from 35.33±0.88 to 38.67±2.04% of fruits. In treated plots, the infestation was decreased significantly in the second week to around 29.46±1.75%. After the fourth week of egg release, the same pattern of efficiency was observed like past two years. By the third week of egg inoculation, the effectiveness of the parasitoid was high (38.67±2.04%) than fourth-week inoculation (37.05±2.13%) in the fields of 600 eggs card$^{-1}$.

**3.2 Insecticidal susceptibility test:** The susceptibility of the parasitoids to various insecticides was conducted in the laboratory, during 2015 and 2016 (Table 1). Six out of ten isecticticides were extremely poisonous to parasitoids while other four insecticides (Triflumuron, Emamectin, Spinosad and Imidacloprid) were minimum dangerous. Remaining four insecticides caused just 10-24% and 12-44% more mortality than in controlled conditions after 24 and 48 hrs. While insignificant differences were observed after 72 hrs. Six different insecticides including Carbosulfan, Chlorpyrifos, Indoxacarb, Lufenuron, Bifenthrin and Abamectin were found to be more harmful, and significantly less survival was recorded. It was observed that Emamectin was safest during entire trial of insecticidal susceptibility for *Trichogramma*.

## 4. Discussion

For better control of vegetables, it is important to merge diverse strategies like cultural practices, chemical and biological measures in Integrated Pest Management to minimize the harm created by insects. Thus, *Trichogramma* is a biological specialist in charge of management and control of pests, particularly lepidopterans, which relies on other natural enemies, chemical management, pest species, nature and status of interactions between these segments. Thus, introducing *Trichogramma* with an IPM program alone is not adequate to achieve fruitful control of pests. In this manner, it must be complemented with sufficient examination for both prior and after an outbreak of the insect in the field. Here, we concentrated on the effectiveness of *Trichogramma* along with particular prescribed insecticides as reported by some researchers [23, 38].

The egg parasitoid, *T. chilonis* is described as a natural enemy of SPB, which is the major target pest in the Okra field [39]. Combinations of *T. chilonis* and both insecticides were more effective against SPB [40]. The effectiveness *T. chilonis* increased with the passage of time along with each release up to one month. Due to more than a single release infestation of insect was boosted again in the fourth week. The results described by Mohamed, Bashir [41] were consistent with our insecticide treatments, that insecticides are extremely effective against SPB in okra fields. Similarly, a combination of *T. chilonis* and insecticides caused 95% mortality of SPB in the laboratory conditions within 24 hrs [42], which has supported the present results.

Several insecticides were found to cause less than 50% mortality of *T. chilonis*: Triflumuron, Emamectin benzoate (Proclaim), Spinosad, and imidacloprid. But, Hewa-Kapuge, McDougall [43] reported more than 95% mortality of *T. chilonis* with the direct insecticidal application, and in residual application up to 70% mortality [44-46].

A spiny bollworm is a multivoltine and polyphagous pest of Okra crop, which destroys the whole plant, including foliage, buds and flowers while older larvae also feed on the fruits. Okra crop is managed through different strategies like chemical management, biological management, and cultural management along with quarantine measures.

For three years, we conducted a research program in the field to check the efficiency of *T. chilonis* and insecticides against SPB. We released *T. chilonis* eggs in the field for one month at weekly intervals, followed by insecticidal applications after the first release of eggs. We observed that pest infestations decreased after the application of eggs and insecticides, but if there was no further release of parasitoid eggs in the field, then the pest population increased again in the fourth week, and the whole crop was destroyed. On the other hand, if egg numbers per each release were high, then the pest infestation declined by more than 70-80% with the passage of time.

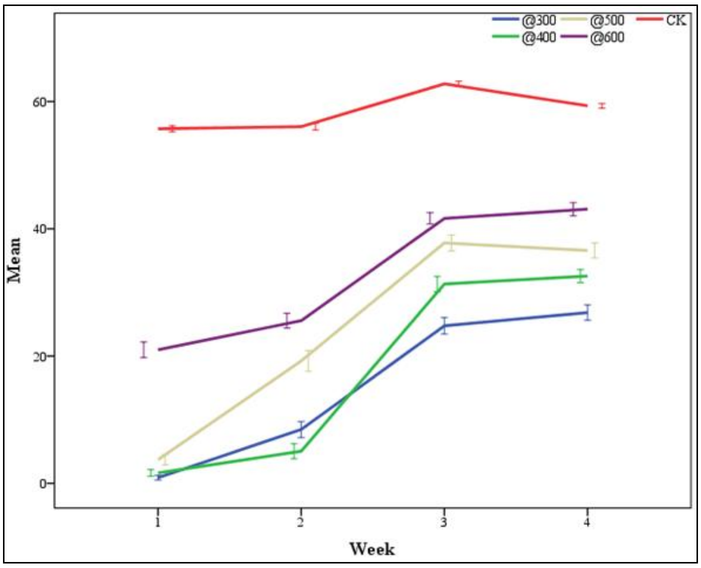


**Fig 1:** Percent effectiveness of *T. chilonis* against *E. insulana* during 2014

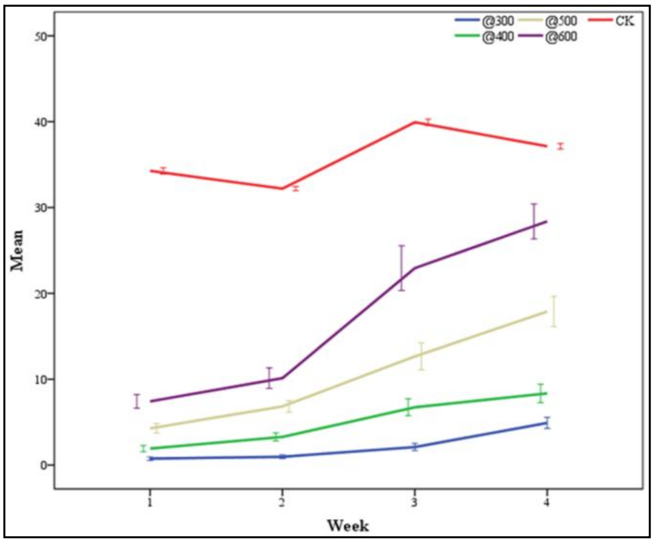


**Fig 2:** Percent effectiveness of *T. chilonis* against *E. insulana* during 2015

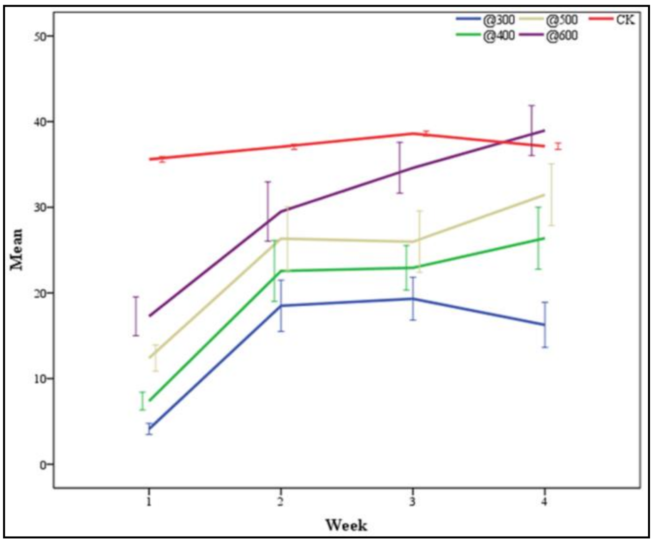


**Fig 3:** Percent effectiveness of *T. chilonis* against *E. insulana* during 2016

**Table 1:** Percent adult emergence of *T. chilonis* from insecticide treated eggs after 24, 48 and 72 hours during 2015-16

| Treatments | ml acre$^{-1}$ | Percentage adult emergence | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2015 | | | 2016 | | |
| | | 24 hrs | 48 hrs | 72 hrs | 24 hrs | 48 hrs | 72 hrs |
| Emamectin benzoate | 200 | 65.67±1.45 | 82.00±2.08 | 84.33±3.76 | 74.67±2.33 | 82.00±1.73 | 84.00±1.53 |
| Triflumuron | 30 | 63.00±2.31 | 82.33±2.73 | 85.33±1.76 | 63.67±1.45 | 73.67±0.88 | 76.67±0.33 |
| Spinosad | 80 | 60.33±2.03 | 83.00±0.58 | 84.00±2.65 | 62.67±0.33 | 68.00±0.58 | 71.00±1.53 |
| Imidacloprid | 125 | 58.33±4.37 | 63.67±3.71 | 72.00±2.65 | 72.00±1.73 | 81.00±1.73 | 79.33±0.88 |
| Lufenuron | 250 | 32.67±3.28 | 44.00±2.08 | 37.33±1.86 | 28.00±1.53 | 24.33±4.37 | 26.00±1.15 |
| Carbosulfan | 500 | 22.67±2.33 | 32.67±2.03 | 26.67±2.40 | 19.67±1.76 | 8.33±2.67 | 4.33±2.19 |
| Abamectin | 60 | 16.67±2.33 | 12.33±2.73 | 3.67±2.33 | 9.67±2.03 | 5.67±1.20 | 0.67±0.33 |
| Bifenthrin | 250 | 10.33±2.19 | 8.33±2.33 | 0.33±0.33 | 4.00±2.08 | 3.33±1.86 | 00 |
| Indoxacarb | 175 | 5.33±2.96 | 4.00±2.08 | 00 | 1.67±0.67 | 1.67±1.20 | 2.67±2.67 |
| Chlorpyrifos | 800 | 1.67±1.20 | 0.67±0.33 | 00 | 1.00±0.58 | 1.33±0.33 | 0.33±0.33 |
| Control | | 80.67±1.45 | 90.67±0.88 | 88.33±5.17 | 83.33±1.20 | 87.67±1.76 | 89.33±1.33 |
| f-value | | 126.63 | 269.42 | 235.85 | 448.02 | 368.55 | 836.57 |
| *p*-value | | 0.31 | 0.06 | 00 | 0.35 | 0.007 | 00 |
| d.f | | 10 | 10 | 10 | 10 | 10 | 10 |

## 5. Conclusion

Results showed that infestation was suppressed up to maximum potential after fourth-week inoculation of *Trichogramma*'s eggs in all treatments during all the three years. But, it was surprising to observe that effectiveness of 500 eggs treatment was higher in the third week durimg first year, as well as, there was no significant difference between third and fourth week. The potential was reached up to maximum level after fourth-week inoculation of *Trichogramma*'s eggs during the whole period of assessment. Thus, Okra production was increased and the cost of management was also decreased significantly. The infestation of *Earias* was observed up to the least level of 4% by cards of 600 eggs during a third-year trial. It was observed that some of the insecticides were less toxic to *Trichogramma*'s larvae, which caused less mortality than 50% of *Trichogramma* after 24 hrs exposure, such as Emamectin benzoate, Triflumuron, Spinosad and Imidacloprid. Emamectin benzoate was proved to be safer for parasitoid, which caused 18% mortality after 48 hrs exposure during laboratory assessment for both years.
So, for the management and control of vegetables and fruits pests, application of safer insecticides along with the regular release of biological agents is incredibly recommended.

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