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Influence of Egg Parasitoid *Trichogramma chilonis* Ishii on Sugarcane Stem Borer (*Chilo infuscatellus* Snellen) in Pakistan

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ABSTRACT

Sugarcane is one of the major cash crops in Pakistan, which is attacked by borers. Among all sugarcane stem borer, *Chilo infuscatellus* Snellen (Lepidoptera: Pyralidae) is a major pest of cane while *Trichogramma chilonis* Ishii. (Hymenoptera: Trichogrammatidae) is well known egg parasitoid used as management tool for the control of borers in IPM. The study was conducted on the efficacy of different release rates of *T. chilonis* against sugarcane stem borer and to check the infestation level of sugarcane stem borer and their management by release of *T. chilonis* egg cards. Correlation of abiotic factors, percent infestation and reduction were recorded on low and high numbers of egg parasitoids. *T. chilonis* cards were installed monthly while percent infestation was calculated after 4, 8 and 12 days interval. Results revealed that all the treated plots significantly reduced borers infestation compared to check plots. Among low to high treatments minimum infestation (3.45±0.13%) was recorded in highest parasitized egg cards treated plot T_5 (75,000 parasitized eggs/acre). Reduction in infestation was release-rate dependent. T_5 showed maximum reduction 5.37%, 4.40% and 4.38% in the months of July, August and September, respectively. Results indicated that application of *T. chilonis* egg cards is effective, cheap and environment friendly substitute for the chemical control in managing the sugarcane borers.

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MRK and SA conceived the idea and performed the experiments. MS analyzed the data. MS and SSM wrote the article.

Key words
Biological control, *Trichogramma chilonis*, Sugarcane, *Chilo infuscatellus*.

INTRODUCTION

Sugarcane (*Sacharum officinerum* L.) being one of the most important and highly water intensive cash crops of Pakistan, occupies a significant position in agriculture economy. Pakistan is world's 5th largest producer of sugarcane in terms of area under sugarcane cultivation, 11th by production and 60th in the yield (Shahid *et al*., 2007). It provides not only sugar, gur and other products for the consumption and utilization by the masses but also provides employment. Biotic and abiotic factors correlate with the production and play a vital role that affect various levels of quality and quantity of cane yield from sowing to harvesting. In dry weather, production is decreased and the juice of sugarcane becomes thick, which directly affect the quality (Rafique *et al*., 2007). In low temperature areas sugarcane cannot be cultivated there whereas if temperature rise more than 40°C in month of June and relative humidity below than 40% that direct hit on borer population (Talpur *et al*., 2002). For good production and better growth of sugarcane with healthy soil favourable physical conditions like sufficient quantity of rain, humidity and warm temperature is required (Smith, 1996). Climatic conditions have direct effect on infestation of borers, in favourable conditions pest multiply rapidly and attack is on peak, it results in serious outbreaks of different pests (Kumar *et al*., 2007).

Sugarcane stem borer, *Chilo infuscatellus* Snellen, 1890 (Lepidoptera: Pyralidae) is the most notorious, predominant and destructive pest of sugarcane and causes major damage to crop which is up to 36.51% (Ashraf *et al*., 1993; Aheer *et al*., 1994). The borer is being controlled mostly by different chemicals (Qayyum, 1975; Dent, 1991). Borers make tunnels in stubbles and internodes where approach of different sprays is not possible. Food supply to aerial parts of stem and leaves stopped leading to 80-85% loss in yield (Sharma *et al*., 1997). Damage to 3rd and 4th brood of cane by sugarcane borers may result in more than 25% reduction in weight (Gupta and Singh 1997). The stem borer is active from March to November and passes the winter as full grown larvae in the stubble, while parasitoids and predators are present in cane fields (William, 1983; White and Regan, 1999). Borers prefer relatively low temperatures, high humidity and their microbial enemies which have been investigated elsewhere but not in Pakistan (Ashraf *et al*., 1999). The average yield of sugarcane crop in Pakistan is relatively low compared to other cane growing countries.

To avoid the chance of pest attack, to overcome

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the resistance problem for producing good quality production and to meet the demand of international market it is necessary to apply integrated pest management (IPM) strategies *i.e.,* cultural, mechanical, biological, chemical and physical control methods individually and in combination. *Trichogramma chilonis* (Ishii) (Hymeoptera: Trichogrammatidae) is the typical gregarious egg parasitoid and is widely distributed throughout the world. These wasps are tiny (0.5 mm long) parasitoids which attack eggs of more than 200 species of lepidopterous pests (Farmanullah *et al*., 2011). Parasitoids complete their life cycle in borer's egg and after 7-8 days a tiny wasp emerges out and again starts searching for borer eggs for oviposition. The life cycle starts in cane field, where parasitoids multiply in field and control the pest population. In favourable conditions 70-80% parasitism was recorded on borer's egg, which break down the pest population to less than 5%. This moth-egg parasitoid *Trichogramma* species is reared in laboratories and released in the field as a bio-control agent against lepidopterous pests (Hussain *et al*., 2007). In Pakistan it has been recorded from *Chilo infuscatellus* Snellen, *Chilo partellus* Swinhoe, *Helicoverpa armigera* Hübner, *Agrotis ipsilon* Hufnagel, *Spodoptera litura* Fabricius and *Bissetia steniellus* Hampson. In many cases successful bio-control applications have been done to control borers. Crop plants treated with *T. chilonis* has wide chance to spread its generation on sugarcane, which play a vital role to suppress the population of borers (Smith, 1996; Soula *et al*., 2003). Present study was conducted to evaluate the effectiveness of different doses of egg parasitoid *Trichogramma chilonis* (Ishii) for management of sugarcane stem borer *Chilo infuscatellus* Snellen (Lepidoptera: Pyralidae).

MATERIALS AND METHODS

A field area of 1.944 hectare, in which sugarcane variety HSF-242 sown in fifty four equal plots (each having 0.036 hectares) in Randomized Complete Block Design (RCBD) was used in this study. The five treatments were given as follows: one plot was control (T_0), 35,000 parasitized eggs/ acre (T_1), 45,000 parasitized eggs/acre (T_2), 55,000 parasitized eggs/acre (T_3), 65,000 parasitized eggs/acre (T_4) and 75,000 parasitized eggs/acre (T_5). The experiment was replicated thrice. Data were recorded on the basis of percent infestation of borers by comparing the control plot with biological controlled plots from April to September 2013, while cane yield (tons/ ha) was recorded at the time of harvest.

All standard agronomic practices including irrigation and recommended doses of fertilizer were applied according to the requirement of crop. Pesticides were not used in cropped area. The infestation level of stem borer was calculated by taking the infested cane plants through random sampling method from July to October and was monitored after intervals of 4, 8 and 12 days which are in A, B and C block, respectively. To calculate the infestation five locations were randomly selected in each plot. In each location having an area of 10 square meters total number of plants and the plants injured by *Chilo infuscatellus* Snellen (Lepidoptera: Pyralidae) were counted to calculate the percent infestation of each location and average percent infestation of each plot.

To minimize the infestation level of sugarcane stem borer, *Trichogramma chilonis* (Ishii) (Hymeoptera: Trichogrammatidae) was reared on a fictitious host, stored grain pest Angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) in the laboratory of Ayub Agriculture Research Institute, Faisalabad, under controlled temperature 25±2°C and relative humidity 60-70% and released in the field as bio-control agent. For release of millions of *T. chilonis* at different intervals in the field it is necessary to rear this egg parasite in bulk.

Rearing of host Sitotroga cerealella

Seeds were cleaned and given hot water treatment. Grains were dried in sunlight for three days, weighed and placed in 5Kg capacity glass jars and were inoculated with 2g of fresh eggs of *S. cerealella*. Eggs hatch within 2-4 days, and pupate within 22-28 days. The newly emerged adults collected with the help of vacuum cleaner were shifted to transparent plastic jars for egg laying. Pre-oviposition period is about 3-5 h. To make process of egg collection easy lower parts of the plastic jars were removed with cutter and attached with nylon net of 40 meshes, pasted with Samad® bond. Eggs collected on daily basis were sieved with 60 meshes steel net to remove body parts of moths and other particles, used for two (i) preparing *Trichogramma* egg cards and to produce the second generation, (ii) mass production of host culture.

Preparation of egg cards

For this purpose, fresh eggs of fictitious host *S. cerealella* sprinkled on semi-hard paper card strips 3x1.5 inches pasted with glue (Kekar gum) with the help of forceps, sponge attached at the tip manually with the help of small transparent plastic vial (4 inches in length and 2.5 cm in width) covered with nylon net lid. Each egg card prepared with more than 500 eggs of *S. cerealella* were stored in refrigerator for 15-20 days at the 6-7°C in vegetable box, before use.

Rearing of Trichogramma chilonis

Prepared egg cards of *S. cerealella* were kept for 24 h in 5 Kg the glass jars of with white muslin cloth piece tightened with bands bents. Honey solution (10%) was applied on the back side of paper card strips as diet of *T. chilonis.* These egg cards were replaced with fresh egg cards of *S. cerealella* daily for mass culturing. Parasitoids card strips were placed in plastic trays for 3-5 days at laboratory temperature 25±2ºC as they mature. Card strips change their colour from orangish brown to brown and then dark brown and then blackish brown. After 6-8 days tiny wasp of *T. chilonis* emerged from the mature eggs.

After an hour of emergence this tiny female wasps are ready to copulate and search fresh eggs of *S. cerealella* for oviposition. Single female wasp laid 3-4 eggs in each egg of the *S. cerealella.* The eggs lay inside the *S. cerealella* eggs hatched within 24-48 h. Hatched larvae completed different larval and pupal stages of life inside the host eggs. Development was completed within the period of 5-7 days. As they mature the colour of parasitized eggs changed from golden brown to orange than dark orange to brown and finally dark brown.

Preservation of parasitoid cards

Parasitoid egg cards collected from glass jars were kept in plastic envelops and placed in refrigerator vegetable box at 6-7°C. These egg cards were stored up to 15-30 days. As the duration was long the emergence percent was low compare to starting days. On demand of the farmer or at the time of application, these mature egg cards can be taken out of refrigerator for 2-4 h before use.

Field application of T. chilonis *egg cards*

All parasitized egg cards were carefully packed in transparent plastic bags and placed in a container with ice cubes and shifted to Manga Mundi Lahore (Geographic coordinates of Lahore, Pakistan is latitude: 31º32'58" N, longitude: 74º20'36" E, elevation above the sea level: 224m=734 ft) and placed in a container with ice cubes.

Egg cards were stapled to the under side of sugar cane leaves in the infested plots in the morning or evening (30±2ºC and relative humidity 60-80% and wind speed 10-15 km/h). Each card has almost 500 *S. cerealella* parasitoid eggs which produced 1500-1800 *Tichogramma* adult wasps. Newly emerged tiny *T. chilonis* wasp start searching their mate and in the field the chain of parasitoid life cycle starts again. To avoid shortage of egg parasites, fortnightly releases were made till harvesting of crop.

To evaluate the performance of *T. chilonis* in the treated plot mean infestation of sugar cane stem borer was compared with the control plot. Percent reduction in the stem borer after the releases of *T. chilonis* was determined.

RESULTS AND DISCUSSION

There was significant effect of release of natural enemies on the infestation of sugarcane stem borer throughout the study period. The maximum and minimum infestation was recorded in the lowest and highest release rates of *Trichogramma chilonis* with 12 and 4 days interval, respectively. There was a significant difference observed in infestation between control and treated blocks.

The reduction in infestation was release-rate dependent. However, there was no significant difference in infestation at the highest release rates of 65,000 and 75,000 parasitized eggs/acre. Table I shows highly significant results ($P<0.01$) of infestation of stem borers. In the month of July data was recorded after fourth day, in the first week the maximum infestation percent was observed in T_0 8.50±0.14 followed by T_1 7.53±0.15. Whereas in the last week of July highest infestation percent was recorded in T_0 8.82±0.04 followed by T_1 8.13±0.03 and lowest infestation percent was recorded in T_5 3.45±0.13. In the month of August data was recorded after seven days, maximum infestation was recorded in last week T_0 9.03±0.03 followed by T_1 8.42±0.06 and minimum infestation was recorded in T_5 4.63±0.03. In the month of September, percent infestation data was recorded after twelve days, maximum infestation was recorded in the first week T_0 8.95±0.03, whereas minimum infestation was recorded in T_5 3.05±0.03.

Results of Table I also show the percent reduction in infestation (mean±S.E.). In the first week of July maximum reduction (5.78±0.03) in infestation was recorded in T_5 whereas minimum percent reduction (0.68±0.06) was recorded during fourth week of July in T_1. In the month of August the highest percent reduction was recorded during first week. Maximum reduction was recorded in T_5 followed by T_4. In September, lowest percent reduction was recorded in control T_1 0.60±0.03 whereas highest percent was recorded in T_5 5.90±0.00. However, there was no significant difference in reduction of infestation of pest in release rates.

Correlations between percent infestation of sugarcane borer and environmental variables, temperature (minimum and maximum) relative humidity and rainfall for different treatments are shown in Table II. The correlation between percent reduction of sugarcane borer *Chilo infuscatellus* and environmental factors for different treatment doses is shown in Table II. In Pakistan

Table I. Percent infestation (Mean±SE) and percent reduction in infestation of stem borer in different treatments after release of *T. chilonis* in July and August in sugarcane crop.

Treatments*	July 7	July 9	July 13	July 17	August 8	August 16	August 24	September 12	September 24
Infestation (%)									
T_0	8.50±0.14a	8.52±0.11a	8.60±0.06a	8.82±0.04a	9.10±0.06a	9.08±0.04a	9.03±0.03a	8.95±0.03a	8.90±0.03a
T_1	7.53±0.15b	7.58±0.19b	7.80±0.15b	8.13±0.03b	8.27±0.03b	8.32±0.04b	8.42±0.06b	8.30±0.03b	8.30±0.03b
T_2	6.68±0.09c	6.83±0.27c	7.05±0.13c	7.77±0.15c	7.83±0.03c	7.87±0.04c	7.92±0.04c	7.10±0.06c	7.28±0.11c
T_3	4.75±0.09d	5.37±0.07d	6.20±0.15d	6.72±0.06d	6.80±0.03d	6.82±0.03d	6.92±0.04d	4.85±0.03d	6.43±0.12d
T_4	2.97±0.09e	2.98±0.06e	3.18±0.09e	3.50±0.06e	3.88±0.13e	4.15±0.08e	4.83±0.09e	3.17±0.04e	4.62±0.07e
T_5	2.72±0.12e	2.77±0.03e	3.18±0.04e	3.45±0.13e	3.75±0.18e	4.07±0.03e	4.63±0.03f	3.05±0.03e	4.52±0.09e
Reduction in infestation (%)									
T_1	0.97±0.02d	0.93±0.08d	0.80±0.10d	0.68±0.06d	0.83±0.09d	0.77±0.08d	0.62±0.06d	0.65±0.05e	0.60±0.03d
T_2	1.82±0.22c	1.68±0.16c	1.55±0.08c	1.05±0.16c	1.27±0.07c	1.22±0.02c	1.12±0.06c	1.85±0.05d	1.62±0.11c
T_3	3.75±0.06b	3.15±0.12b	2.40±0.21b	2.10±0.08b	2.30±0.03b	2.27±0.02b	2.12±0.07b	4.10±0.03c	2.47±0.13b
T_4	5.53±0.06a	5.53±0.07a	5.42±0.06a	5.32±0.02a	5.22±0.07a	4.93±0.04a	4.20±0.10a	5.78±0.02b	4.28±0.07a
T_5	5.78±0.03a	5.75±0.13a	5.42±0.06a	5.37±0.08a	5.35±0.13a	5.02±0.02a	4.40±0.06a	5.90±0.00a	4.38±0.07a

Means sharing same letters in a column are statistically non-significant (P>0.05)
*To control, T_1, 35,000 parasitized egg/acre; T_2, 45,000 parasitized egg/acre; T_3, 55,000 parasitized egg/acre; T_4, 65,000 parasitized egg/acre;; T_5, 75,000 parasitized egg/acre.

sugarcane production has increased over time. For the enhancement of yield it is essential that crop be treated with bio-control agents. Sugarcane production could never be improved until and unless resistant varieties and modern technologies are adopted on large scale (Glaz, 2000). Bohinc *et al.* (2014) found the natural resistance of cabbage against pests. Antioxidative potential and level of injury caused by insect pests reduced through different genotypes (Markovic *et al.*, 2014). Correlation between percent infestation and reduction of sugarcane borer for different treatments were highly significant (P<0.01) that describes the correlation among all environmental variables.

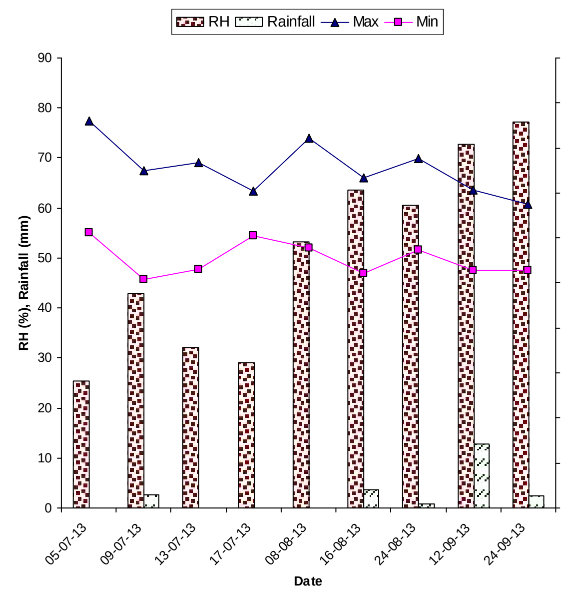

Fig. 1. Meterological data during study.

Figure 1 shows the meteorological data recorded during the study. Damage due to 3rd and 4th brood of sugarcane borers may result more than 25% reduction in weight (Gupta and Singh, 1997). Balanced use of fertilizers to sugarcane crop help in the control of borers (Madan, 2001). Effect of minimum temperature on percent infestation on *C. infuscatellus* is shown in Figure 2A. The population of pest is at peak in control and T_5 indicates the lowest infestation. The reduction in percent infestation of *C. infuscatellus* was minimum when 35,000 *Trichogramma* parasitoid eggs were applied. As the range of parasitoid eggs increased, the percent infestation decreased, infestation fluctuated at maximum temperature (Fig. 2B). Inundative releases of bio-control agents applied to overcome the pest problem and suppress the lepidopterous pests are being practiced in more than 32

million hectares each year around the world (Saroj and Jaipal, 2000).

Table II. Correlation between percent infestation and % reduction of infestation of sugarcane borer and environmental factors for different treatments.

	Max. temp.	Min. temp.	R.H.	Rainfall
Infestation (%)				
T_0 (Control)	-0.260	-0.043	0.700*	0.238
	0.499	0.912	0.036	0.537
T_1	-0.462	-0.077	0.748*	0.297
	0.211	0.844	0.020	0.438
T_2	-0.178	0.175	0.318	-0.181
	0.648	0.653	0.405	0.641
T_3	-0.201	0.057	0.186	-0.479
	0.604	0.885	0.633	0.192
T_4	-0.267	-0.033	0.624	-0.168
	0.488	0.933	0.073	0.666
T_5	-0.323	-0.059	0.620	-0.162
	0.397	0.880	0.075	0.677
Percent reduction of infestation				
T_1	0.708	0.118	-0.651	-0.328
	0.033	0.762	0.058	0.389
T_2	0.077	-0.310	0.047	0.473
	0.845	0.417	0.905	0.198
T_3	0.152	-0.080	0.007	0.637
	0.696	0.837	0.985	0.065
T_4	0.226	0.023	-0.490	0.315
	0.558	0.953	0.181	0.410
T_5	0.301	0.057	-0.493	0.308
	0.432	0.884	0.178	0.420

Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability.
* = Significant (P<0.05); ** = Highly significant (P<0.01)
For treatment details, see Table I.

Figure 2C shows relationship of percent infestation with relative humidity. Unfortunately, commercial varieties are mostly susceptible to sugarcane stem borer, so it is imperative to apply bio-control strategies for sugarcane (Hassan, 1993). Six ecology based approaches applied in combination *i.e.*, timely irrigation and urea application, mechanical removal of borer damaged plants, covering the roots with soil taken from seedbed in case when some plant roots are uncovered, release of egg parasitoid and foliar nitrogen application in sugarcane significantly reduced borers infestation and increased cane yield compared to check plots (Saroj and Jaipal, 2000).

CONCLUSION

Release of *T. chilonis* egg cards reduced the infestation level. The technology is effective and eco-friendly that control the qualitative and quantitative losses in sugar cane against stem borers.

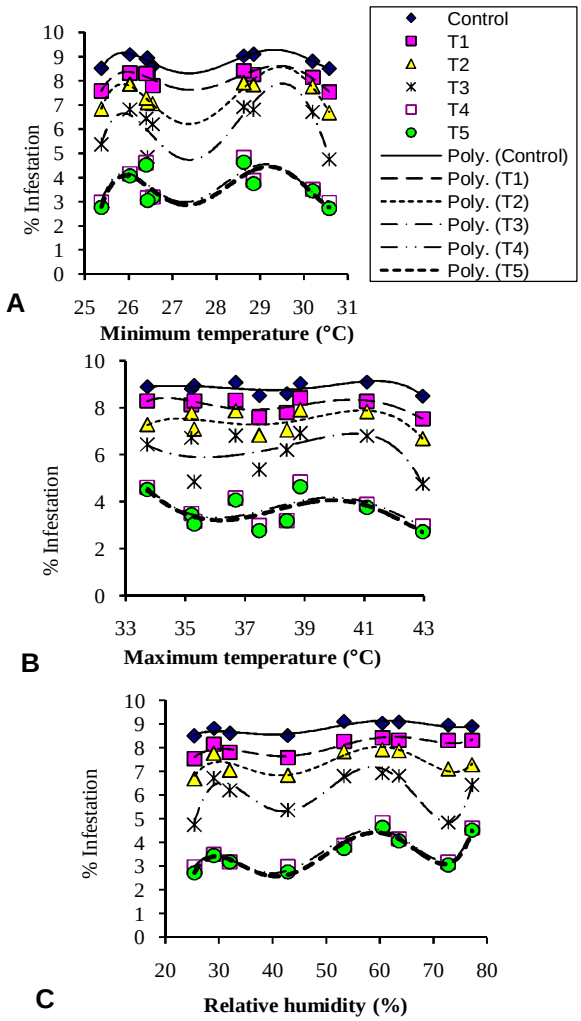

Fig. 2. Percent infestation of *Chilo infuscatellus* on sugarcane at minimum temperature (A), maximum temperature (B) and relative humidity (C).

Statement of conflict of interest

Authors have declared no conflict of interest.

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