



Biological parameters of tomato fruit borer *Helicoverpa armigera* (Hubner) fed on cherry and bayberry tomato lines under controlled conditions and efficacy of *Trichogramma chilonis* (ishii), (*trichogrammatidae*: hymenoptera)

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A study of *H. armigera* fed on resistant cherry tomato line (18004) and susceptible bayberry tomato line (0010) showed that its biological parameters were significantly affected with resistant and susceptible tomato lines. Egg incubation period, larval duration, pupae duration, total duration, male adult longevity and female adult longevity were significantly longer on resistant line as compared to susceptible line. Whereas the oviposition rate, no. of eggs laid per day, % egg hatchability, % larval survival and pupal viability were high on susceptible tomato line. The study of biology and parasitic potential of *T. chilonis* showed it to be significantly effective against *H. armigera* with high % parasitism 65.08%, short duration (8.5 days) from parasitism to adult emergence and 75% adult emergence. While the adult longevity of *T. chilonis* was 10.03 days after emergence from *H. armigera* parasitized eggs. Therefore, it is recommended to grow the tomato line 18004 along with the use of *T. chilonis* cards under field conditions for integrated pest management of *H. armigera*.

Keywords: *H. armigera*, biological parameters, *T. chilonis*, tomato lines, IPM

INTRODUCTION

Tomato (*Lycopersicon esculentum* Miller) is one of the most economically important vegetable crop cultivated worldwide for its fruit and is significantly profitable and nutritive (Kevanay et al. 2008). Tomato yield is greatly affected by many factors such as diseases as well some insect pests which reduce the crop yield significantly. Among the pests attacking tomato, tomato fruit borer *Helicoverpa armigera* (Hübner) is the most destructive pest. Its larvae on initial stages feed on foliage, flowers and buds, while mature larval stages feed on the fruits. It causes severe economic losses as one caterpillar of *H. armigera* can damage up to 12 fruits (Hussain and Bilal, 2007). In Pakistan, *H. armigera* has been found to affect the tomato crop yield up to 70% (Abbas et al. 2015). Globally, *H. armigera* causes an economic loss of about US \$5 billion annually (Sharma, 2001).

Due to emerging problems of high levels of resistance

against insecticides, alternative methods have been considered among which host plant resistance and biological control are very important (Shanower et al. 1997; Ali et al. 2023). Rapid development and mobility of *H. armigera* limits the effectiveness of many natural enemies (Fitt, 1989). Thus better biocontrol agents against *Helicoverpa/Heliothis* spp. are egg parasitoids such as *Trichogramma* (Hymenoptera: Trichogrammatidae) that have short generation time and can also be mass-produced easily. These parasitoids have been successfully used as biological control agents against *Heliothis/Helicoverpa* spp. in cotton (King et al., 1986; Romeis & Shanower, 1996). Many countries of the world are working to control *H. armigera* by introducing natural enemies such as *Trichogramma* and *Chrysoperla carnea*. In Khyber Pakhtunkhwa, Inayatullah (2007) reported that yield loss of tomato due to *H. armigera* was decreased by introducing *Trichogramma* alone or in combination with *C.*

carnea.

T. chilonis is a tiny wasp from the family Trichogrammatidae. It has a wide host range and attacks different stages of insect pests particularly Lepidopteron pests. Lepidopteron eggs and their larvae attacking the field crops, fruit crops and forests are parasitized by *T. chilonis* (Zucchi et al. 2010). The parasitized eggs turn black due to internal development of the parasitoid and adult wasp eventually emerge from these parasitized eggs (Bigler et al. 1997).

In biological control programs, the *Trichogramma* species is the most commonly used egg parasitoids against the lepidopterous pests. Entomologists recognized the ability of the *Trichogramma* species as a biological control operator and began producing *Trichogramma* species on mass scale in the mid-1900s in pest control programmes. *T. chilonis* are well known to control *H. armigera* in tomato due to their efficiency and multiplication ability (Parra and Zucchi, 2004). Today, the *Trichogramma* species has become a common enemy of important insect pests of economically significant crops because they are easy to reproduce and attack many important insect pests (Ayvaz et al. 2008).

MATERIALS AND METHODS

Establishment of Stock Culture of *H. armigera*

The larval rearing containers were kept under the laboratory conditions of (24±1 °C) and 60±5% relative humidity in Insectary at National Agricultural Research Centre, Islamabad.

To maintain the stock culture of *H. armigera*, tomato plants were grown under glass house conditions. Infested tomatoes from NARC farm fields were shifted in glass houses as well as in plastic rearing containers. The *H. armigera* larvae were provided with fresh tomatoes after 5-7 days to maintain the culture successfully. The infested tomatoes were kept in plastic rearing containers with wet sand for pupal formation until adult emergence. Upon adult emergence, the adults were shifted in rearing cages and provided with seedling and tomato fruit for egg laying. The adults were also provided with 10% honey solution as food. The eggs laid on the seedling inside the cage were collected daily and were kept for larval emergence. The process was continued throughout the experimental duration.

Establishment of *T. chilonis* stock culture

The stock culture of *T. chilonis* was maintained on *Sitotroga cerealella* eggs. In the adult rearing chamber, the grain moths of *S. cerealella* were reared. The trays (36x30x5cm) were filled with 5kg wheat grains and eggs of *S. cerealella* were released in these trays. The larvae were fed on wheat grains upon emergence and the adults emerged after 25-30 days. The adults were shifted directly into the lower end of chamber containing oviposition jar. The oviposition jars having sufficient number of adults

were replaced daily with new jars. Plastic plates with starch were kept in oviposition jars for adult to lay eggs. Sieves (90 mesh size) were used for the collection of eggs from starch. A known number of eggs of *S. cerealella* were collected from the stock and were stuck with glue on ivory card. The cards were then placed in the transparent glass jars containing *Trichogramma* adults. The *Trichogramma* adults parasitized the eggs and the eggs turned black after parasitization.

Experiment 1:-

Developmental time, survival rate and reproductive potential of *H. armigera* under controlled conditions

To check the biology and survival rate of *H. armigera* a total of 100 newly laid fresh eggs of *H. armigera* were collected from the stock culture. The eggs batches laid on infested leaves of cherry and bayberry tomato plants were kept in rearing jars (10×7) with five replicates having 20 eggs in each jar under lab conditions of 26±1 C and R.H 65%. The eggs were checked regularly for hatching. Upon hatching, the newly emerged larvae were provided with fresh Bayberry tomato fruits in rearing containers along with old infested tomatoes for efficient growth of larvae. After 5-7 days the total number of eggs hatched were calculated under binocular microscope. The process was continued till all the larvae entered into the pupal condition. For pupation the containers were provided with pet mass and sandy soil. The pupae were kept under the same laboratory conditions for adult emergence. The total no. of male and female adults emerged was counted and were kept in adult rearing cages for breeding. The data was collected by using Complete Block Design (CRD) with 5 replications on the following parameters.

Total No. of eggs and No. of eggs laid per female/day

To determine the total no. of eggs and no. of eggs laid/female, ten pairs of newly emerged adults were collected from the culture and were kept in 5 rearing jars (having 2 pair in each jar) containing 20 % honey solution provided as food. The males/female pairs were identified from morphological characters and were allowed for mating and egg laying. The deposited eggs were counted regularly till the whole life span of adults.

Eggs incubation time and percent hatchability:

Total ten eggs were collected from the culture and were kept in Petri dishes under controlled conditions and till hatching the eggs incubation period was recorded in days. This procedure was replicated 5 times.

Larval duration (days) and percent larval survival:

Ten newly emerged larvae were collected from the culture in 5 replications and were provided with fresh bayberry tomato fruits as food. The food was replaced on each alternative day till pupation.

Developmental time of pupae (days):

Ten pupae in five replications were collected from the same culture and were kept in same laboratory conditions. Pupae were observed daily to determine their developmental time till the emergence of adults.

Duration from egg to adult emergence (days):

Total duration from eggs to adult emergence was counted by collecting freshly laid 50 eggs from the established culture and were monitored till hatching. Upon hatching the fresh tomato fruits were provided until larvae entered into pupation. The pupae were observed daily till the adult's emergence. The total duration from eggs to adult emergence were recorded in days.

Adult longevity (days):

Total 20 newly emerged adults were collected and 20% of honey solution was provided as food on cotton swab. These adults were monitored daily till their death and the total longevity was recorded in days.

Sex ratio (%):

Sex ratio was calculated by using the following formula,

$$\text{Female (\%)} = \frac{\text{total no. of females}}{\text{total no. of females} + \text{total no. of males}} \times 100$$

Statistical Analysis

Developmental duration for different stages was analyzed using ANOVA and the data was subjected to analysis of variance using Statistics 8.1 package. Means was compared using LSD test at 5% level of significance.

Experiment 2:-**Biological parameters and parasitic potential of *T. chilonis* against *H. armigera***

Total of 100 freshly laid eggs of *H. armigera* along with the leaf portion were collected from the stock culture. Ten eggs were placed in each of the ten glass tube (1inch diameter x5inch length), separately. Ten *Trichogramma* females were provided to parasitize these *H. armigera* eggs for 24 hrs. After 24 hrs, *Trichogramma* females were removed and parasitized *H. armigera* eggs were kept under controlled conditions 24±1°C, R.H 65±5, L:D 14:10. The treatment was replicated 10 times following completely Randomized Design (CRD). Data was recorded on the following parameters:

Percent parasitism of *H. armigera* eggs:

Number of parasitized eggs was counted (eggs turned black) and % parasitism was calculated by the following formula;

$$\text{Percent parasitism} = \frac{\text{number of parasitized eggs}}{\text{total number of observed eggs}} \times 100$$

Table 1: Developmental time, survival rate and reproductive potential of *H. armigera* reared on resistant and susceptible lines under controlled conditions 26±1 °C

Developmental stage	Tomato lines	LSD
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Percent adult emergence:

Total Number of *T. chilonis* adults emerged from the parasitized eggs was counted and recorded. % adult emergence of *T. chilonis* was calculated by the following formula.

$$\text{Percent emergence} = \frac{\text{number of eggs with emergence holes}}{\text{total number of parasitized eggs}} \times 100$$

Developmental time:

Development time of *T. chilonis* on *H. armigera* eggs was determined by counting the number of days from parasitism till emergence of adult wasps.

Adult longevity (days):

Parasitoid longevity was determined by counting the number of days from adult wasps emergence till death.

Statistical Analysis

The data was analyzed using ANOVA and was subjected to analysis of variance by using Statistics 8.1 package. Means were compared using LSD test at 5% level of significance.

RESULTS AND DISCUSSION

The developmental time, survival rate and reproductive potential of *H. armigera* reared on resistant (18004) and susceptible (0010) tomato lines under controlled conditions revealed that the biology of *H. armigera* varied significantly on resistant and susceptible tomato lines. Egg incubation period, larval duration, pupae duration, total duration from egg to adult emergence, male adult longevity and female adult longevity were found to be shorter on susceptible line as compared to resistant line. Results showed egg incubation period as 2.80 and 3.69 days, larval duration 19.40 and 22.34 days, pupal duration 7.90 and 10.0, total duration 31.08 and 36.17, male longevity 6.90 and 7.96 while female longevity was found to be 9.20 and 10.57 days in susceptible and resistant tomato lines respectively. Whereas the oviposition rate, no. of eggs laid per day, % egg hatchability, percent larval survival and pupal viability were high on susceptible line as compared to resistant line. Susceptible lines showed oviposition rate as 273 days, no. of eggs laid/ day as 24.60, % egg hatchability 87.00%, % larval survival 90.00% pupal viability 80.00 and sex ratio % 1.1, while resistant lines showed oviposition rate as 160 days, no. of eggs laid/ day as 16.60, % egg hatchability 73.60%, % larval survival 75.00% pupal viability 74.00 and sex ratio 1.09 (Table 1).

	Resistant (18004)	Susceptible (0010)	
Egg Incubation period (days)	3.69 a	2.80 b	0.2824
Larva duration(days)	22.34 a	19.40 b	1.3543
Pupae duration (days)	10 a	7.90 b	1.6306
Total Development Time (days)	36.17 a	31.08 b	1.5605
Adult male longevity	7.96 a	6.90 b	0.9961
Female longevity	10.57 a	9.20 b	1.0875
Oviposition rate	160 b	273 a	32.936
No of eggs /day	16.60 b	24.60 a	1.6629
% egg Hatchability	73.60 b	87.00 a	5.3388
Percent Larval survival	75.00 b	90.00 a	5.2077
Pupae viability	74.00 b	80.00 a	4.8918
Sex ratio %	1:09	1:1	

Mean followed by different letters are significantly different @ P value = ≤ 0.05

These findings are in line with those of Devi et al. (2019) who found out that the resistant chickpea genotypes affected the biological parameters of *H. armigera* and thus exhibited lower larval and pupal survival while higher larval, pupal duration and adult longevity against *H. armigera*. Srivastava and Srivastava (1990), who reported 77% *H. armigera* larval survival on resistant cultivar, while susceptible cultivar showed 90 % larval survival in chickpea. Ali et al. (2016) also reported 73% *H. armigera* larval survival in resistant cultivar as compared to 93% in susceptible cultivar, similarly larval development period was also found to be shortest 16.41 days on susceptible and longest 22.71 days on resistant cultivars. Naseri et al. (2009) also reported that the total development time of *H. armigera* larvae ranged from 17.30-26.20 days on different soybean cultivars.

In tomatoes, trichomes both non-glandular and glandular play a role in insect resistance (Firdaus et al. 2013). Trichomes negatively affect the oviposition of insect pests. They not only cause antixenosis effect (reduced oviposition), but glandular trichomes also result in antibiosis due to the secretion of toxic substances (Rakha et al. 2017).

Rath (1994) has also studied that the adult duration was less on resistant variety (HT 64) and more on susceptible variety (HS 173) which corroborate with present findings. The sex ratio (male: female) was also found to vary a little among the tomato lines though there was no significant difference but comparatively more no. of female adults emerged on susceptible genotypes than on other genotypes tested which may be attributable to preferred nutritional status of the high susceptible genotypes. Osiemo et al. (2012) also found out that the sex ratio was unaffected by increase in number of host eggs per patch in females.

In the present research experiment, the effect of host

insect *H. armigera* eggs on biological attributes and parasitism potential of *T. chilonis* showed significant results. It was found that parasitism rate of *T. chilonis* was 65.08 with adult emergence of 75%. *T. chilonis* took 8.5 days from the time of parasitism to adult emergence. While the adult longevity of *T. chilonis* was 10.03 days after emergence from *H. armigera* parasitized eggs (Table 2).

Table 2: Biological parameters and parasitic potential of *T. chilonis* against *H. armigera*

Parameters	Mean \pm S.E
% Parasitism	65.08 \pm 0.54
% Adult emergence	75.00 \pm 1.12
Duration from parasitism to adult emergence (days)	8.5 \pm 0.25
Adult Longevity	10.03 \pm 0.52

These results are in line with (Khan et al. 2004) who reported highest parasitism (48.25%) with 5 parasitized eggs and thus recommended 3 pairs of *T. chilonis* for 50 eggs of *S. cerealella*. Nisar et al. 2020 found out that the larval infestation of *H. armigera* varied according to the released level of the parasitoid. He observed reduction in pest infestation levels as well as minimum percent weight loss in fruits with higher yield in treatment T3 having highest no. of parasitized eggs (1200 parasitized eggs). The genetic variability of *Trichogramma* results in aggressive females with greater acceptance and ability of parasitism in postures with different physical barriers (Beserra and Parra, 2004). Zuim et al. (2017) proposed that *T. pretiosum* showed greatest performance on *H. armigera* eggs in between 20 and 25 eggs. They further revealed that Each *T. pretiosum* female was able to parasitize 70% of the pest eggs, highlighting the potential of this natural enemy for biological control of *H. armigera*.

Ballal and Singh (2003) who tested *T. chilonis* against *H. armigera* found egg 65% parasitism by *T. chilonis*. The slight difference in mean parasitism may be due to different strains, temperature, relative humidity and photoperiod.

These results are also in accordance with those of Reddy and Manjunatha (2000) who also found significant results in order to control lepidopterous pests while using *T. chilonis* alone or in combination with some synthetic pesticides or plant extracts. Shah (2008) in his conducting field experiments also determined that *Chrysoperla carnea* and *T. chilonis* are the best bio-control agents against *H. armigera* in tomato crop.

CONCLUSION

The data concludes that *H. armigera* took more time to complete its development on resistant tomato lines, less no. of eggs were laid on resistant lines with lower larval survival rate while higher no. of eggs were laid on susceptible lines with higher chances of survival as well as its development also completed faster on susceptible lines. The study also established the effectiveness of *T. chilonis* suggesting its beneficial use in biological control programmes, due to its greater parasitism rate, faster development, higher longevity and significant control. Thus the present study suggests the use of resistant tomato lines along with *T. chilonis* to control the *H. armigera* infestations in tomato.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: <https://www.isisn.org/article/10.3390/antiox12081524/s1>,

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Data Availability Statement

All of the data is included in the article/Supplementary Material.

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AUTHOR CONTRIBUTIONS

: Conceptualization, J.K. A.U. and J.R.; methodology, J.K.

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