Journal of Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Research Article

Parasitism Potential of Egg Parasitoid, *Trichogramma chilonis* against *Spodoptera frugiperda* on Maize

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ABSTRACT
The fall armyworm (FAW), *Spodoptera frugiperda*, is highly destructive polyphagous and invasive insect pest of maize, causing considerable yield losses and is becoming increasingly difficult to manage due to over-reliance on chemical insecticides and the development of resistance. Egg parasitoids such as *Trichogramma chilonis* offer a promising approach for the biological control of this pest through sustainable integrated pest management (IPM). Although *Trichogramma spp.* are recognized as effective parasitoids of lepidopteran pests, there is limited local data on their efficacy, host preference, age-specific performance, and field application of *T. chilonis* against *S. frugiperda* on maize under Pakistani conditions. In this study, both laboratory and field experiments were conducted to evaluate the efficacy of *T. chilonis* against FAW. Laboratory tests assessed parasitism and emergence at different of FAW eggs (20-100 eggs), host preference between *S. frugiperda* and *Sitotroga cerealella*, the effect of parasitoid female age (24 h vs. 48 h), and the influence of host egg age. Field trials compared different frequencies and numbers of *Trichogramma* card applications in maize plots. Results indicated that parasitism rates increased with egg density with a maximum parasitism of 46.1 observed at 100 eggs per female. *T. chilonis* exhibited a preference for *S. cerealella* eggs over FAW eggs and demonstrated higher parasitism on fresh FAW eggs compared to older eggs, while 48-hour-old female parasitoids outperformed 24-hour-old females. In field trials, deploying two *Trichogramma* cards per week resulted in the greatest reduction in FAW larval populations and the least amount of plant damage. These findings highlight that *T. chilonis* is a promising biocontrol agent against FAW and can be effectively integrated into IPM programs through frequent releases of *Trichogramma* cards.

Keywords: Biological control, Integrated pest management, Egg parasitism, Host preference.

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Article History
Received: November 03, 2024
Accepted: April 02, 2025
Published: April 23, 2025

INTRODUCTION
Fall armyworm (FAW), *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) has various host plants belonging to seventy-six different families (Capinera, 2002; Montezano *et al*., 2018). *S. frugiperda* prefers maize as a host, and it usually results in 15–73 percent reduction of yield (Sun *et al*., 2021; Ahmad *et al*., 2025). Heavy losses have led farmers to rely on chemical insecticides like organophosphates, carbamates, pyrethroids, and diamides to manage this pest (Deshmukh *et al.,* 2020). Unfortunately, *S. frugiperda* has a history of evolving resistance to multiple insecticide groups (Ngegba *et al.,* 2025). To counteract this, farmers increase doses and spray more frequently, which leads to excessive pesticide use (Bird *et al.,* 2022). This malpractice is not only causing environmental pollution but also posing risks to human health (Kumar *et al.,* 2025).

The use of biocontrol agents (BAs) is an important part of Integrated Pest Management (IPM) that plays a significant role in maintaining the pest population through feeding and parasitism by predators and parasitoids, respectively (Gandhi *et al*., 2005). Among parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are egg parasitized of about four hundred species belonging to seven insect orders (Chan and Chou, 2000). There are 28 caterpillar species including cotton bollworms, maize and sugarcane borers (Nadeem and Hamed, 2011) that are suppressed by augmentation of *Trichogramma*. Globally, *Trichogramma* species are being mass-reared commercially and released extensively for the management of lepidopterous pests (Wang *et al*., 2016). These tiny parasitoid wasps are said to have been introduced in 30 countries on 80 million acres of farm area and forest land per year (Khan *et al*., 2015). Native parasitoids are highly suitable to natural surroundings, the effectiveness of biological control programs is dependent on the extensive use of native egg parasitoids to control pest populations in specific ecosystems (Hassan *et al*., 1998).

Among Trichogrammatids, *Trichogramma chilonis* Ishii adults lay their eggs within the eggs of lepidopteran pests and ultimately kill the host eggs (Usman et al., 2012). The ease in mass production under laboratory conditions and their flexibility in destroying egg stages before pests begin to damage crops gives them an edge over all other BAs (Ulrichs and Mewis, 2004). Moreover, *T. chilonis* also has potential to search and parasitize the egg masses of *S. frugiperda* in maize fields (Elibariki *et al*., 2020).

The objective of the study was to determine the potential parasitism and the hatching of *T. chilonis* on eggs of *S. frugiperda* at different egg densities. It also measured parasitoid longevity, parasitism by 24 and 48 hours-old females, parasitism among egg ages, host preference in *S. frugiperda* and *Sitotroga cerealella* and impact of various schedules of applying *Trichogramma* card in the field. The study hypothesis was *T. chironis* would show measurable potential of biological control of fall armyworm and could be integrated into IPM programs on maize.

MATERIALS AND METHODS

Rearing of Host

The eggs of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) were used as host of *T. chilonis*. *S. cerealella* was reared on wheat grains as it was cheap source of food and easily available round the year (Hassan, 1997). Prior to infestation by *S. cerealella*, wheat grains were heat-sterilized and placed into a glass jar so that they became soft and disinfected from other insect pests of wheat and mites. After sterilization 1.5 Kg wheat was filled in glass jars (length 20.5cm and Diameter 55.8cm). *Sitotroga* eggs used to infest wheat to start culture were obtained from the laboratory of Pakistan Agriculture Research Council (PARC) Bahauddin Zakariya University Multan Station. Eggs were sprinkled in a glass jar covered with muslin cloth and tightened with a rubber band to prevent the escape of adults. *S. cerealella* culture was kept at 70 ± 10% relative humidity and 25 ± 2 ˚C temperature. Adults were emerged after twenty-five days and collected in another plastic jar named as ovipositor jar by an electric suction machine. The jar with dislodged moths was placed in a plastic plate having “wheat starch” as egg laying media. The eggs were collected after sieving the starch. Eggs of *S. cerealella* were sieved with 60-size mesh (U.S Standard Sieve). These eggs were used for making egg cards for *T. chilonis.*

Rearing of *T. chilonis*

Eggs of *S. cerealella*, which were parasitized by *T. chilonis*, were obtained from PARC and kept in a glass jar to obtain parasitoid emergence. After emergence, parasitoid adults were provided with fresh eggs of *S. cerealella* glued onto the thick white cards so that parasitism could take place. The jar was covered with muslin cloth and tightened with a rubber band to prevent the escape of adults. Eggs color changed to black after parasitism. A few drops of honey solution were provided to *T. chilonis* as an adult diet. The same procedure was repeated to get more cards. The population of the parasitoids was maintained under the same climatic conditions as mentioned above.

Parasitism by *T. chilonis* on *S. frugiperda* Eggs

The eggs of *S. frugiperda* and *S. cerealella* were collected from the rearing culture. The eggs of each host were glued on hard cards by using gum. The eggs were handled with camel hair brush. The eggs cards were kept in plastic glass in which egg parasitoids of *T. chilonis* were released. A droplet of five percent honey solution on cotton wool was kept in plastic glass for *T. chilonis* adult feeding. Two sets of females were used in treatments in such a way that one set of 24h aged of *T. chilonis* female was parasitoid and other 48 aged. Twenty eggs of *S. frugiperda* were kept in each treatment. The parasitism of eggs was noted by changing color from whitish to black under microscope. There were two treatments and twenty replications in this experiment.

Parasitism of *T. chilonis* on eggs of *S. frugiperda*

Eggs from the 1st, 2nd, and 3rd days of *S. frugiperda* were used in this experiment. The eggs of the same developmental stage were used in each treatment. One pair of parasitoid eggs (near about to hatch) was introduced in a plastic glass with a droplet of honey solution on a cotton swab. There were three treatments and twenty replications in this experiment.

Host Preference of *T. chilonis*

Two egg cards of *S. cerealella* and *S. frugiperda* were placed in a plastic jar, and one pair of parasitoid eggs of *T. chilonis* were introduced in this jar with a few droplets of honey solution on a cotton swab. After emergence female was moved towards the egg card based on touching and odor. There were twenty replications in this experiment. Host preference was noted by counting the number of parasitoid eggs. Lab condition was controlled at 25±2ºc temperature and 70±10 percent relative humidity.

Longevity of *T. chilonis*

To evaluate the longevity, twenty individuals of *T. chilonis* (<24 h old) were selected randomly from the colony of each host. These were kept separately in transparent plastic cups, provided with honey solution on a cotton swab as food, and covered with baby liner.

Efficacy of *T. chilonis* on *S. frugiperda* Eggs

Egg parasitoid adult female was introduced in plastic jars with *S. frugiperda* eggs cards containing 20, 40, 60, 80, and 100. There were five treatments and ten replications in this experiment. A droplet of honey solution on cotton wool was kept in each for adult feeding. One pair of parasitoid eggs of *T. chilonis* (near about hatch) was released to parasitoid the eggs of *S. frugiperda*. The color of parasitoid eggs was changed from white to blackish. The parasitism of adult females was noted by counting the parasitoid eggs of each treatment.

Field Experiment

A field trial was carried out at the agricultural farm of MNS-University of Agriculture, Multan. The experiment was designed in Randomized Complete Block Design (RCBD). This study evaluated five treatments with three replications for pest control using Trichogramma cards (Tricho-cards). The plot size was 10 square meters. The first treatment (T_0) is a control with no cards. The second treatment (T_1) applied 1 Tricho-card per plot every fortnight, and the third treatment (T_2) used 1 Tricho-card per plot weekly. The fourth treatment (T_3) applied 2 Tricho-cards per plot every fortnight, while the fifth treatment (T_4) used 2 Tricho-cards per plot weekly. The objective is to assess how different numbers and frequencies of Tricho-cards impact pest control. Tricho-cards were installed in maize fields throughout the season. *Trichogramma* cards were applied in different plots with respect to time intervals. Standard agronomic practices were applied as per recommendations for maize crops for each treatment. Tricho-cards were stapled underside of leaves of maize plants. The objective was to assess how different numbers and frequencies of Tricho-cards impact pest control.

Statistical Analysis

Data recorded was subjected to statistical analyses by using computer operated statistical software "Statistic 8.1 and XL Statistics".

RESULTS

Efficacy of *T. chilonis* on *S. frugiperda* eggs

Analysis of variance (ANOVA) on the effect of *T. chilonis* on egg density of FAW for parasitism percentage and emergence is presented in Table 1. The results revealed that egg density had a significant effect on both parasitism and emergence of *T. chilonis* ($F = 198$; $df = 4$; $P < 0.001$) for parasitism and $F = 250$; $df = 4$; $P < 0.001$) for emergence. A total of 50 observations were recorded to assess the effect of egg density on parasitism and emergence of *T. chilonis*. Minimum parasitism was 9.0, maximum was 51.0% while the average parasitism and emergence were 30.44% and 25.02% respectively. Mean comparisons for the effect of *T. chilonis* on egg density of FAW for Parasitism percentage and emergence were performed using Tukey's HSD test. Results of all-Pairwise Comparisons at P=0.05 revealed that parasitism of FAW eggs were differed significantly among treatments, ranging 10.70% to 46.10% across different egg densities (20, 40, 60, 80, 100 eggs). Parasitism was highest at an egg density of 100 (46.10%), followed by 80 eggs (40.80%) while it was lowest 20 eggs (10.70%), followed by 40 eggs (21.40%). The median parasitism was observed at eggs 60 (33.20%) (Table 2). Similarly, mean comparisons for emergence of *T. chilonis* showed significant differences among egg densities, ranging from 8.00% to 38.40%. The emergence was highest at 100 eggs (38.40%), followed by

80 eggs (33.30%), and lowest at 20 eggs (8.00 %), followed by 40 eggs (17.60%), with median emergence observed at 60 eggs (27.80%) (Table 2).

Table 1. Analysis of variance for the effect of *Trichogramma chilonis* on egg density of *Spodoptera frugiperda* for parasitism percentage and emergence

Source	DF	F-value (Emergence)	P-value (Emergence)	F-value (Parasitism)	P-value (Parasitism)
Egg density	4	198	<0.001	250.0	<0.001
Host preference	1	50.3	<0.001	53.5	<0.001
Female age	1	51.2	<0.001	101.0	<0.001
Egg age	2	225.0	<0.001	370.0	<0.001
Error	45	--	--	--	--
Total	49	--	--	--	--

df = degrees of freedom; F = F-statistic; P < 0.05 indicates statistical significance

Table 2. Tukey's HSD all-pairwise comparisons of parasitism (%) and emergence (%) of *T. chilonis* at different egg densities of *S. frugiperda*

Treatment (Egg density)	Parasitism (%)	Emergence (%)
100 eggs	46.10 $\pm$ -A	38.40 $\pm$ -A
80 eggs	40.80 $\pm$ -B	33.30 $\pm$ -B
60 eggs	33.20 $\pm$ -C	27.80 $\pm$ -C
40 eggs	21.40 $\pm$ -D	17.60 $\pm$ -D
20 eggs	10.70 $\pm$ -E	8.00 $\pm$ -E

Host Preference

Analysis of variance (ANOVA) for the effect of *T. chilonis* on eggs of *S. cerealella* and *S. frugiperda* is presented in Table 1. Results showed that *T. chilonis* has significant effect on eggs of *S. cerealella* and *S. frugiperda* for host preference (F =53.5; df=1; P < 0.001) for parasitism and F =50.3; df=1; P < 0.000) for emergence). A total of 40 observations were recorded to evaluate host preference. Parasitism ranged from 9.00% and 24.0%, with mean parasitism 16.40% while mean emergence was 12.80%. Mean comparisons using by using Tukey's HSD test (P$\leq$ 0.05) indicated significant differences between the two host species. Parasitism was significantly higher on *S. cerealella* eggs (19.50%) compared to *S. frugiperda* eggs (13.30%) (Table 3). Similarly, emergence differed significantly between hosts, with higher emergence recorded from *S. cerealella* (15.70%) than from *S. frugiperda* (9.90%) (Table 3). These results indicate a clear preference of *T. chilonis* for *S. cerealella* over *S. frugiperda.*

Table 3. Tukey's HSD all-pairwise comparisons of parasitism (%) and emergence (%) of *Trichogramma chilonis* on eggs of different host species

Treatment (Host species)	Parasitism (%)	Emergence (%)
S. cerealella (T_2)	19.50 A	15.70 A
S. frugiperda (T_1)	13.30 B	9.90 B

Mean followed by different letters within a column are significantly different at P $\leq$ 0.05 (Tukey's HSD test)

Parasitism by *T. chilonis* (24 h and 48 h old females) on fall armyworm's eggs

Analysis of variance regarding the parasitism capacity of 24 h and 48 h old females of *T. chilonis* on eggs of *Spodoptera frugiperda* is given in Table 1. Results revealed that female age had a significant effect on both parasitism and emergence (F =101; df =1; P < 0.001 for parasitism and F =51.2; df =1; P < 0.001 for emergence). A total of 40 observations were recorded to assess the effect of female age on parasitism and emergence. Parasitism ranged from 3.00% to18.0% with a mean parasitism was 9.20%. Emergence ranged from 2.00% to 14.00%, with a mean emergence of 6.60%. Mean comparisons using Tukey's HSD test (P $\leq$ 0.05) indicated significant difference between the two age groups. Parasitism was significantly higher in 48 h old females (9.10%) than in 24 h old females (4.10%) (Table 4). These results indicate that older females (48 h) of *T. chilonis* exhibit greater parasitism efficiency and emergence success compared to younger females (24 h) (Table 4).

Table 4. Tukey's HSD all-pairwise comparison of parasitism (%) and emergence (%) of *T. chilonis* at different female ages.

Treatment (Females age)	Parasitism (%)	Emergence (%)
48 h (T2)	12.40 A	9.1000 A
24 h (T1)	6.00 B	4.1000 B

Means followed by different letters within a column are significantly different at $P \leq 0.05$ (Tukey's HSD test)

Effect of egg age on parasitism of *T. chilonis* on *S. frugiperda*

Analysis of variance (ANOVA) for the effect of fresh, 2nd day and 3rd day old eggs of fall armyworm on parasitism of *T. chilonis* and emergence is presented in Table 1. Results revealed that the parasitism on different aged eggs of FAW has significant effect on parasitism and emergence of *T. chilonis* and (F =370; df=2; P= 0.001) for parasitism and F =255; df=2; P= 0.001) for emergence. A total of 60 observations were recorded to assess the effect of egg age on parasitism and emergence. Parasitism ranged from 0.00% to 15.0 with mean parasitism of 6.83%.

Mean comparison using Tukey's HSD test ($P \leq 0.05$) showed significant differences among egg ages groups. Parasitism was highest on fresh eggs (11.50%), followed by 2-days-old eggs (8.80%), while it was negligible on 3-day old eggs (0.20%) (Table 5). Similarly, emergence was highest from fresh eggs (8.90%), followed by 2-day-old eggs (6.45%), and was minimum from 3-day-old eggs (0.050%) (Table 5). These results indicate that *T. chilonis* strongly prefer freshly laid eggs, and parasitism success declines sharply with increasing egg age.

Table 5. Tukey's HSD all-pairwise comparison of parasitism (%) and emergence (%) of *T. chilonis* on *S. frugiperda* eggs of different ages

Treatment (Egg age)	Parasitism (%)	Emergence (%)
Fresh eggs (T1)	11.50 A	8.95 A
2-day-old eggs (T2)	8.80 B	6.45 B
3-day-old eggs (T3)	0.20 C	0.05 C

Means followed by different letters within a column are significantly different at $P \leq 0.05$ (Tukey's HSD test)

Longevity of *T. chilonis*

A total of 20 observations were recorded to assess the longevity (life span) of adults of *T. chilonis*. Adult longevity ranged from 5.0 to 9.0 days, with mean life span of 7.70 days. Minimum longevity (5 days) was recorded for only one insect, followed by 6 days for two individuals and 7 days for three individuals. The majority of insects survived for 8 days (10 individuals), while the maximum longevity (9 days) was recorded for four individuals (Fig. 1, Table 6).

Descriptive statistics indicated that the median longevity was 8.00 days, with the first and third quartiles at 7.00 and 8.00 days, respectively. The variance and standard deviation were 1.168 and 1.081, respectively, indicating relatively low variability in adult longevity.

Table. 6. Descriptive Statistics for longevity (days) of adult, *T. chilonis*

Statistic	Longevity (days)
No. of observations (n)	20
Minimum	5.00
Maximum	9.00
1st Quartile (Q1)	7.00
Median	8.00
3rd Quartile (Q3)	8.00
Mean	7.70
Variance (n-1)	1.168
Standard deviation (n-1)	1.081

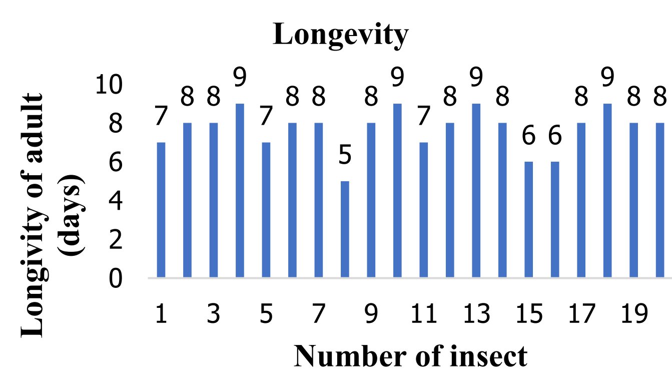

Figure 1. Longevity (life span in days) of adults *T. chilonis*

DISCUSSION

Results demonstrated that egg density of fall armyworm has significant effect on parasitism and emergence of *T. chilonis*. Our findings of current study are supported to the research findings of (Figueiredo *et al*., 2015) who concluded that use of *T. chilonis* highly manage the population of FAW at egg stage and yield of the crop was enhanced. *Palexorista zonata* was essential parasitoid with 12.5 percent parasitism of FAW eggs (Sisay *et al*., 2018). *T. pretiosum* showed 29.23 percent parasitism potential of *S. frugiperda* (Jaraleño *et al*., 2020).

Results demonstrated that *T. chilonis* has significant effect on eggs of *S. cerealella* and *S. frugiperda* for host preference. Our findings of current study are supported to the research findings of (Zahid *et al*., 2007) who reported that *T. chilonis* parasitoid the maximum eggs of *S. cerealella* and mass production of *T. chilonis* was mainly done on *S. cerealella* eggs. The parasitism of FAW by *T. pretiosum* was about 71.64% of egg masses (Varshney *et al*., 2021). One of the reasons why there is low parasitism against *S. frugiperda* could be attributed to the egg-mass structure of FAW. Fall armyworm normally places eggs in groups which are usually covered by scales of the female body. This kind of covering may serve as physical interference with host recognition, antennal inspection and ovipositor insertion by Trichogramma females. This could be the reason why there was less host preference and parasitism amongst FAW compared to the factitious host.

Results demonstrated that 24h and 48h aged *T. chilonis* female has significant effect on parasitism and emergence against fall armyworm eggs. Our findings of current study are supported to the research findings of (Laurentis *et al*., 2019) who reported that parasitism percentage of 48h aged *T. pretiosum* female was high from 24h aged T. pretiosum female against *Helicoverpa armigera* eggs. For 24- and 48-hour-old *Telenomus remus* females, greater the egg density, greater the parasitism, but there was a propensity for parasitism to stabilize at 150 eggs density (Carneiro *et al*., 2010). There are also direct operational implications to female age. The increased parasitism of 48-hour-old females than of 24-hour-old females indicates the possibility that older females, perhaps due to increased egg load, increased mating status or increased host-searching behavior, are better physiologically adapted to oviposition. This implies that the release recommendations must be done based on the number of parasitoids released as well as the age composition and maturity of the released population. In fields, parasitoids released prematurely can be less efficient in immediate parasitism, whereas those released at the same time as females and at the same time as a fresh FAW egg deposition can have a greater control effect.

Results demonstrated that parasitism of fresh, 2nd day and 3rd day eggs of *S. frugiperda* has significant effect on parasitism of *T. chilonis* and emergence. The age of the host egg is of particular significance to explaining field use. Parasitizing of fresh eggs was the most successful, second-day eggs were less suitable and third-day eggs were almost not suitable. This is a biologically plausible pattern since the inner environment becomes less conducive to the development of parasitoid as host embryos develop. Eggs that are older could also hatch prior to the development of parasitoid immatures. Thus, it does not just demonstrate the preference of younger eggs, but prove the fact that the time of release is essential. To be successful with regard to suppression, the presence of parasitoids in the field should be at the beginning of oviposition, prior to the development of the FAW eggs to the most susceptible stage. Our findings of current study are supported to the research findings of (Queiroz *et al*., 2020) who reported that the parasitism of eggs by *T. pretiosum* was higher from fresh to 24h ages of eggs of *Anticarsia gemmatalis* and intermediate for 48h aged egg but no parasitism was noted on 72h aged eggs. *T. ostriniae* exhibited significant difference on 2nd and 3rd old eggs of armyworm. All the species of Trichogramma showed dissimilarity in adult emergence except *T. leucaniae* (Hou *et al*., 2018). *T. chilonis* showed higher parasitism percentage on fresh eggs than the old age eggs of stem borer (Liu et al., 2014).

Results demonstrated that Minimum longevity was 5.0 days and maximum was 9.0 days whereas average life span of adult was 7.70 days. Our findings of current study are supported to the research findings of (Miura *et al*., 1995) who reported that longevity was 7 to 8 days at 24°C and longevity decreased above this temperature. The longevity of *T. chilonis* was five to eight days depending on tichocard color and temperature (Baitha and Sinha, 2002).

This study has certain limitations that should be mentioned despite the results. First, majority of laboratory experiments occurred under controlled condition and this might not reflect the complex situation in the field where temperature, humidity, rainfall, and natural enemy interactions could affect parasitoid performance. Second, despite *S. frugiperda* being parasitized by *T. chilonis* at measurable levels, but did not exhibit as strong parasitism levels on *S. frugiperda* as on other host, *Sitotroga cerealella*, which suggests that these two hosts are not open to parasitism and that host specificity could restrict the field efficacy. Third, the research focus on parasitism, emergence, and short-term population suppression but it did not determine seasonal persistence, dispersal ability, and compatibility to other IPM strategies. Moreover, the field experiment was carried out in one place and season, and this fact might limit the ability to generalize the results to other agroecological areas. Hence, additional and multi-location and multi-season tests are required to confirm the uniformity, viability, and long-term performance of *T. chilonis* releases against fall armyworm in maize.

CONCLUSIONS

The present study demonstrates that *T. chilonis* has strong potential as a biological control agent against *S. frugiperda* in maize. Parasitism and emergence rates increased with higher host egg density, indicating improved efficiency under abundant host availability. Freshly laid eggs were more suitable for parasitism than older eggs, highlighting the importance of host age in parasitoid performance. Furthermore, 48 hours old females were more efficient than the 24 hours old females, confirming that parasitoid age influences biological efficiency. Although, *T. chilonis* showed a preference for *S. cerealella* eggs; it effectively parasitized *S. frugiperda* eggs as well. Field application of *Trichogramma* cards significantly reduced larval populations and crop damage, with the most effective control achieved using two cards per week. These findings support the inclusion of *T. chilonis* in integrated pest management (IPM) of maize.

ACKNOWLEDGEMENTS

Not applicable.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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