CHILO INFUSCATELLUS SNELLEN'S (LEPIDOPTERA: PYRALIDAE) BIOLOGY AND ITS MANAGEMENT

S. H. Shah[1,2*], S. Sattar[1], J. Khan[2], I. Khan[3], M. U. Din[1], A. Jan[4], S. Hussain[5], M. Jamil[6*]

[1]Department of Plant Protection, Faculty of Crop Protection Sciences, The University of Agriculture Peshawar
[2]Insect pest Management Program, Institute of Plant and Environmental Protection, National Agriculture Research Centre, Islamabad, Pakistan
[3]PARC-Adaptive Research cum Demonstration Institute Miranshah, Pakistan
[4]Department of Entomology, Faculty of Crop Protection Sciences, The University of Agriculture, Peshawar, Pakistan
[5]Department of Entomology, Faculty of Crop and Food Sciences Pir Mehar Ali Shah Arid Agriculture University Rawalpindi, Pakistan
[6]PARC Arid Zone Research Centre, Dera Ismail Khan, Pakistan
[*]Corresponding authors: jamilmatrah@parc.gov.pk, Hussainshah1421@aup.edu.com

ABSTRACT: SUGARCANE stem borer, *Chilo infuscatellus* is the most devastating pest, causing huge economic losses in the sub-tropics and therefore requiring effective management strategies. The current research was conducted to study the biology and management of *C. infuscatellus*. Moreover, we checked the efficacy of *Trichogramma chilonis* and granular insecticides against this key pest. The incubation period for *C. infuscatellus* was 2.30 days, with a total developing time of 23.30 days for the larvae and 5.90 days for the pupa. Male and female *C. infuscatellus* had a 36.80-day and 38.00-day total life span, respectively, with the female living longer (4.8 days) than the male (3.90 days). The total female fecundity was 315.90 eggs with a 90.87 egg hatchability rate. The mean percent parasitism (87.01%) and percentage of adult emergence (76.11%) of *T. chilonis* on *C. infuscatellus* were both very high, with a total developmental time of 8.74 days in laboratory environment. Additionally, there were notable differences between the findings on the effectiveness of granular pesticides and *T. chilonis* in the field. The plot treated with Fipronil had the least mean percent infestations (3.58%), followed by Carbofuran (4.26%), and *T. chilonis* (5.63%). The control plot had the highest mean incidence of infestations (13.34%). Future IPM initiatives should incorporate the introduction of Trichogramma or the utilization of Fipronil @ 16 kg ha-1 for the treatment of *C. infuscatellus*. This practice will play a critical role in environmental protection and natural resource conservation against insecticides.

Key Point: *Chilo infuscatellus*, *Trichogramma chilonis*, Granular insecticides, Biological control, Sugarcane

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INTRODUCTION

Saccharum officinarum L., popularly known as sugarcane, is the most significant crop for producing sugar worldwide. It is widely grown in tropical and subtropical areas, with an estimated 1.6 billion tons of sugarcane produced a year worldwide (Krishnan *et al*. 2010; Raza 2010). However, because these pests cause damage throughout the sugarcane growing season, the existence of numerous different sugarcane borers poses a danger to the productivity of sugarcane (Li and Yang 2015; De *et al*. 2013).

*Chilo infuscatell*us (snellen), often known as the sugarcane stem borer (Lepidoptera: Pyralidae), is an important insect that has an impact on the stem's apical dominance. As a result, the sugar level tends to diminish, and the stem produces numerous lateral shoots. Additionally, it penetrates the mature stem throughout the middle and late phases, harming the tissue and impairing stem elongation, which reduces sugar content and yield in the end (Goebel *et al*. 2014). Sugarcane seedlings are destroyed when *C. infuscatellus* larvae feed on the seedlings' growth point, which results in the seedlings' withering and death. According to reports, a single species of this insect can, in rare instances, result in yield losses of ≥ 40%, but it often only causes losses of 10-15% (Arvinth *et al*. 2010). The larvae create holes in the sugarcane shoots or stalks, which causes a major economic loss because the quantity and quality of the sugarcane are both reduced (Goebel *et al*., 2011; Goebel *et al*., 2010). The center whorl of the leaf dries out and begins to wilt because of the larvae cutting the plant's growth point while eating in the stem (Anwar *et al*., 2004). Almost 30-70% decline in sugarcane production may be resulted from this attack. The plant won't grow in these conditions, but its buds might continue to grow and sprout new shoots (Shahid *et al*., 2007). Larvae that consume the cane from the inside out have a difficult

time being repaired by insecticides. Additionally, the widespread use of pesticides contributes to problems including environmental degradation, secondary pest outbreaks, and initial pest recurrence. With the use of specialized culture techniques and biological control agents like Trichogramma parasitoids, there has been some progress. These efforts, however, have been modest (Jalali *et al*. 2006; Viswanathan and Samiyappan 2001).

Trichogramma species are used in biological control programs as defense tools against a number of lepidopteran pests (Zang *et al*. 2021). About 650 species of this egg parasitoid genus are known to attack the eggs of different agricultural pests, and about 200 of these species have been mass produced and dispersed to combat lepidopterous pests of different crops. About 200 of these species are known to target the eggs of different crop pests, and about 70 of them have been mass-produced and dispersed (Du *et al*. 2017; El-Arnaouty *et al*. 2014; Chailleux *et al*. 2013a; Yuan *et al*. 2012; Tabone *et al*. 2010; Desneux *et al*. 2010). There have been substantial economic and biological benefits because of the periodical release of these Trichogramma parasitoids in certain areas over the course of several years (Wang *et al*. 2014). The essential tactic for developing mass production systems and successful biological control programs is to keep Trichogramma spp. in good condition (Zhang *et al*., 2018). In natural field environments in Asia, *Trichogramma chilonis* (Ishii) (Hymeoptera: Trichogrammatidae) was successful in controlling the sugarcane early shoot borer, cotton boll worm, and sugarcane stem borer (*Chilo infuscatell*us). This was achieved by utilizing this bug (Ahmed *et al*., 2012; Shahid *et al*., 2007; Bhut *et al*., 2004; Bharati *et al*., 2002). The infestation of sugarcane stem borer was declined by about 83% as a result of the release of 60,000 eggs of the parasitoid *T. chilonis* in sugarcane crops (Rafique *et al*., 2007). For the management of *C. infuscatellus*, trials were carried out by several co-researchers, including (Saljoqi and Walyat, 2013; Ullah *et al*., 2012; Aajoud *et al*., 2003; Chanton *et al*., 2001). *T. chilonis* was introduced alongside granular pesticides in sugarcane fields to manage *Chilo infuscatellus* more effectively. However, further investigation is needed to examine how it should be managed in relation to *C. infuscatellus*.

The present study was therefore conducted to further explore the biological attributes and management strategies of *C. infuscatellus*. The incubation period (2.30 days), larval developmental duration (23.30 days), and pupal duration (5.90 days) of *C. infuscatellus* were first investigated, along with the total life cycle of *C. infuscatellus* and its total female fecundity with its egg hatchability rate. The percentage of adult emergence (76.11%) and mean percent parasitism (87.01%) of *T. chilonis* on *C. infuscatellus* were both evaluated significantly. The minimum and maximum mean percent infestation were also recorded in the plots treated with different pesticides. Among them, Fipronil @ 16 kg/ha or the release of *Trichogramma* for the management of *C. infuscatellus* in future IPM programs were recommended for use. Moreover, this practice may have a critical role in environmental protection and natural resource conservation against insecticides.

MATERIALS AND METHODS

Research on the biological characteristics of the host *Chilo infuscatellus* and the parasitoid *Trichogramma chilonis* was conducted at Bio-control laboratory, National Agriculture Research Center (NARC), Islamabad in 2020. Field experiments to evaluate the efficiency of 2 granular insecticides (Fipronil 0.4%G and Furadan 3G) and *T. chilonis* the egg parasitoid against *C. infuscatellus* were conducted on a sugarcane variety (CP-77/400) at Sugar Crops Research Institute (SCRI), Mardan, during the same year 2020.

Pest culture and Plant Maintenance: To preserve *C. infuscatellus* culture, sugarcane genotype CP-77/400 was acquired from SCRI Mardan. To rear the pest on mass scale the acquired sugarcane genotype was cultivated under controlled environments of greenhouses in the close locality of bio-control laboratories at NARC. Regular agronomic procedures were followed as and when required to carry out the investigations. The leaf portion of sugarcane with the egg cluster of *C. infuscatellus* was scissor incised from the cane field at SCRI. The collected clusters of eggs were kept in tiny plastic containers of (15×10× 6 cm^3) size. The egg clusters were then shifted to insectary and stapled to the leaves of sugarcane plant grown in greenhouse. The eggs were constantly observed till the larval and adult development. After the laying of eggs by these adults on sugarcane leaves in the greenhouse, the egg cluster containing leaf portions were incised and transported to laboratory for further investigations.

Laboratory study of *C. infuscatellus* life cycle: The experiment was conducted in a controlled environment (32°F, 65°R, and CR Design) using the identical methodologies as Kumar *et al.* (2019), with just a few minor adjustments. To investigate the life cycle of *C. infuscatellus*, we utilized the procedure of collecting egg clusters from the greenhouse by following the method that had been described earlier. In a laboratory environment, ten clusters with varying egg counts were collected and placed in little plastic boxes measuring (10x6x2.5) $inch^3$ in volume. After the eggs hatched, the amount of time the eggs needed to be incubated and the hatchability rate were calculated. The features, including the number of egg clusters, the number of eggs in each cluster, the number of eggs carried by a female, the length of the incubation period, and the percentage of

eggs that hatched, were investigated. First-instar larvae were reared individually in larval rearing boxes (10x6x2.5) $inch^3$ and then released on fresh succulent stems of sugarcane (variety CP-77/400). A new supply of stems was provided every other day, and the larvae were always observed closely. The statistic for the overall duration of the larval stage was noted. To monitor the transition from the pupal stage to the adult stage, the pupae were collected following the completion of the final larval instar and placed in separate cages measuring (35x20x35) cm^3.

The period of longevity and oviposition of adult: The adult moths were raised in a cage (two pairs), and each was Penta replicated after their first emergence. Males and females could be distinguished from one another based on their physical and morphological traits (Bhavani 2013). Out of the total 10% of the cage's volume was filled with a honey solution. The sugarcane leaves were preserved by being put in a vial next to a cotton swab that had been drenched in water, ensuring that the leaves maintain their moisture. The vial holding the leaves was kept inside the cage, where the adult females rested, mated, and laid their eggs. The eggs were counted and added up every day. The adults confined in the cage died one by one till the end of the experiment. Daily observations on the pre-oviposition, the oviposition, the post-oviposition stages, the adult male and female life lengths, and the sex ratio were made.

***Trichogramma chilonis*' effectiveness against *Chilo infuscatellus* eggs in a lab:** Fresh eggs of *S. cerealella* were collected from a culture that had already been produced to test the efficacy of *T. chilonis* against *C. infuscatellus*. These eggs were then transferred to a smaller, mesh-topped vial, and uniformly spread across a sheet of sticky card. These egg cards were put inside the glass jar containing the adults of the *T. chilonis* species for the aim of parasitization. The eggs were taken out of the jars once they had become black and stored at a temperature of 5 degrees Celsius (Iqbal *et al*., 2020).

One hundred eggs of the *Chilo infuscatellus* species were also delivered to the lab along with the leaf piece that was taken from the greenhouse. Each of these eggs from each cluster was put onto a laboratory dish that was 150x15 mm^2 in size. Four pupae with parasites that were about to emerge were also put in the same petri dish. The *C. infuscatellus* eggs were taken out of the petri dish after five days and examined to see how much parasitization they had endured. After they had all died, the male and female Trichogramma adults were divided into their respective sexes under a microscope. The following formula was used to calculate the parasitism rate: (Saljoqi and Walayat, 2013).

$$\% \text{ Parasitism} = \frac{\text{Total number of parasitiod eggs}}{\text{Total number of eggs}} \times 100$$

The biological characteristics of *T. chilonis*, such as the amount of time it takes for it to develop from a parasitoid into an adult, the amount of time adults live, and how effective they are, were investigated.

Effectiveness of certain pesticides (Fipronil 0.4%, Carbofuran 3G, and *Trichogramma chilonis*) against *C. infuscatellus* in field: An experiment was carried out at SCRI Mardan with the objective of determining the degree to which two granular insecticides, carbofuran and fipronil, as well as *T. chilonis*, were effective under field conditions. The experiment consisted of four different treatments, each of which was carried out three times and organized according to an RCB layout. In September of 2019, the sugarcane variety CP-77/400 was grown in a plot of 57.6x20.7 m^2. The land was subdivided into smaller portions, each measuring 18x6 m^2. Each individual plot consisted of nine rows, with a total of twenty plants in each row. The distance between plants was 15 cm, while the distance between rows was 60cm, (Jabran *et al*., 2011). To prevent spreading to other areas, a separation distance of 1.2 m was left between each sub plot and each side. Both pesticides were administered at the rates (32 and 16 kg per hectare) that were recommended for their use. Trichogramma cards were also stapled onto the leaf, and this was done while maintaining 40 m^2 from chemically treated plots that were the same size (18x6) m^2. Trichogramma cards were distributed at a rate of 12 cards ha^{-1} with a gap of 15 days between each release. A comparison was made between these applications and control plots, which had none of the pesticides applied. From March to October, the treatment was administered monthly during the entire time. Observing the symptoms (holes in stem and dead heart) induced by the *C. infuscatellus*, on a biweekly basis, allowed for the collection of the necessary data. The formula that was utilized by Saljoqi *et al*. (2015) to compute the mean percentage of infestation for each month was found below.

$$\% \text{ Infestation} = \frac{\text{Total number infested plants}}{\text{Total number of observed plants}} \times 100$$

Data Analysis: All of the data that were collected were analyzed with the statistical program Statistix 8.1, and the means were compared with using the Least Significant Difference (LSD) test at a significance level of 5%. CRD was used in laboratory and RCBD was used in field conditions (Steel and Torrie, 1980).

RESULT AND DISCUSSION

***C. infuscatellus* Larval and Pupal Developmental Duration (Days) in Lab:** This research was carried out to investigate the *C. infuscatellus's* biology by rearing them on sugarcane genotype (CP-77/ 400) in lab environment. The immature stages development period

(days) of *C. infuscatellus* during 2020 is described in (Table-1). The data in table-1 reveals that the incubation duration of the eggs, larvae, pre-pupa and pupa of *C. infuscatellus* was 2.30±0.152, 22.30±0.395 1.60±0.163 and 5.90±0.23 days, respectively, while the time for five instars ranged from 2.70±0.15 days for 1st instar and 6.00±0.210 days for 5th instar, respectively. Our results indicated that the incubation period, total larval, and pupal duration of *C. infuscatellus* took the minimum time. These findings are consistent with those of Kalariya *et al*. (2014) who conducted the same experiment on *C. infuscatellus* in the laboratory and reported that the incubation period, larval and pupal duration were all recorded as short as possible, lending full support to the finding of the present study. Kumar *et al*. (2019) conducted a study that is similar to this one but produced entirely different findings. They raised *C. infuscatellus* in a lab setting and found that the incubation time, larval timespan, and pupal period all took the maximum length of time. These variations may be due to using different temperatures, relative humidity as well as different host plants.

***C. infuscatellus* adult stage development (days) in Lab conditions:** Three developing durations (days) of the adult stage (pre, ovi, and post-oviposition) of *C. infuscatellus* nurtured on sugarcane genotype CP-77/400 were also recorded in this study, and are presented in Table 2. The periods of *C. infuscatellus* female's pre, post and oviposition were 0.470±0.036, 1.000±0.129 and 3.100±0.23 days, respectively. The data clearly show that the oviposition period took significantly longer than the pre- and post-oviposition periods. These findings are consistent with the finding of Kumar *et al.* (2019), and Kalariya and Radadia (2014), who reported that pre, post and oviposition period varied from 0-1, 1-2 and 2-4 days, respectively, and the oviposition period was also longer than pre- and post-oviposition periods in their studies, fully supporting the current findings. The total life cycle, longevity, and sex ratio of a male and female were also recorded in this study. The female life cycle was found to be significantly longer (38.00±1.145 days) as compared to the male life cycle (36.80±0.89 days). Similarly, the female longevity (4.800±0.249 days) was also found to be much higher than the males (3.90±0.23 days). The sex ratio of *C. infuscatellus* was 1: 2.3 (M: F), revealing that females outnumbered the male *C. infuscatellus*. The results of the total life cycle of this study are quite different than Kalariya and Radadia (2014) results (32.60 ± 4.07 days), which might be due to different diets, locations, and temperatures, while in partial agreement with the findings of Kumar *et al.* (2019), where the male and female life cycles were observed 33.23±0.76 and 35.61±0.94 days, respectively. The enchanted female endurance and sex percentage as equated to males was also acknowledged by Kumar *et al.* (2019) and Bhavani (2013), providing full support and logic to the findings of our present experiments. These results indicate that, due to their long life cycle, female *C. infuscatellus* at an adult stage are more active sexually and live a long life as compared to male *C. infuscatellus*, suggesting that the total life cycle has a greater influence on longevity and sex life.

***C. infuscatellus* female fecundity and hatchability in the lab:** Observations were made for the eggs cluster quantity per female, eggs in a cluster, fecundity of female's lifetime, and eggs hatchability percentage of *C. infuscatellus* under *in vitro* conditions and are presented in table 3. The mean quantity of egg clusters laid down by a female was recorded 3.9±0.27, whereas the mean eggs quantity in a cluster was calculated 47.3±1.73. The total amount of eggs laid down by a female in her total lifespan was 315.9±4.35, with an average hatchability of 90.87±0.4. Kumar *et al.* (2019) also stated the similar fecundity percentage of female (317.7±9.84), strongly supporting the current investigation, while the normal hatchability was 74.4±5.7 in his study, which is much lower than our findings. The disparity in egg hatchability might be due to the difference in location's temperature and humidity. However, as compared to the current study, Bhavani (2013) and Kalariya and Radadia (2014) described higher fecundity rates of *C. infuscatellus* 376.67±5.85 and 349.08 ± 70.43, respectively. The variations in fecundity may be attributed to the variations in the ambient temperature, genotypes of the specie and food availability.

Parasitism of *Trichogramma chilonis* on *C. infuscatellus* and its biological traits in lab: In general, the biocontrol potential of Trichogramma parasitoids is assessed in relation to the parasitism rate of host eggs, the appearance of offspring, the percentage of female-biased progeny, and the maturation period (Takada *et al*., 2000; Zhang *et al*., 2014; Song *et al*., 2015). We investigated the parasitic efficacy of *T. chilonis* against *C. infuscatellus* in the present experiment and found that it was effective. The results show that *T. chilonis* spends 87.01 percent of its time as a parasite on *C. infuscatellus* while only spending 76.11 percent of its time as an adult. Under laboratory conditions, emergent Trichogramma lasted for a total of 8.74 hours, and *T. chilonis* was found to be 3.55 percent efficient against pest eggs. (Song *et al*., 2015; Hou *et al*., 2018; Iqbal *et al*., 2019; Khan *et al*., 2019) investigated in the laboratory the biological parameters of different Trichogramma species, including *T. chilonis* on different pest eggs and came up with findings that were similar to those shown here. The researchers discovered that although the different Trichogramma species had different parasitism preferences, under carefully controlled laboratory settings, every species of Trichogramma was able to successfully parasitize on pest eggs. The aforementioned

studies back up the present findings, which suggest that *T. chilonis* can be successfully mass-produced on pest eggs and used as a biocontrol agent to manage these pests. These conclusions are based on earlier research that provide this support.

Bio-efficacy of selected insecticides against *C. infuscatellus* under field conditions: Insect pest infestation is a major contributor to sugarcane yield loss and needs to be controlled through different insecticides. (Gul *et al*., 2008; Way *et al*., 2012; Xavier and Merlindayana, 2012). In order to address the difficult situation provided by the sugarcane stem borer, we test the bio-efficacy of numerous insecticides, including Carbofuran 3G, Fipronil 0.4%, and *Trichogramma chilonis*, against *C. infuscatellus* under field conditions in this paper. The results of the experiment, as shown in figure 1, showed that the efficiency of several insecticides in eradicating *C. infuscatellus* varied significantly. The plot that received fipronil treatment had a minimum infestation of *C. infuscatellus* of 3.58 percent; the plot that received carbofuran treatment had a minimum infestation of 4.26%. The differences between these treatments and the other treatments, particularly trichogramma, which had a percentage of 5.63 percent, were not statistically significant although they were. The control plot's 13.34 percent infection rate was the highest ever seen. The plot treated with carbofuran exhibited a significantly greater mean percentage of infestation compared to the plot treated with fipronil. These results are in line with those of Ullah *et al*. (2012), who discovered that checked plots had the greatest mean infection while checked plots had the lowest mean infestation (0.84±0.35), followed by Furadan plots (0.99±0.20), and Tricho-cards (1.08±0.72). (7.77±1.94) Our results are in line with those of Saljoqi *et al*. (2013), who examined *T. chilonis*'s ability to suppress *C. infuscatellus* using Carbofuran 3G and Thimet 5G. According to their findings, the Trichogramma released plot had the lowest mean infestation of *C. infuscatellus* (0.48%), whereas control plots had the highest mean infection (8.02%).

We also observe and investigate the infestation rate during different months, i.e., from April to October. The data shows that high infestation started in April in the control plot (15.43), reached its maximum point in June (18.13), while low infestation was recorded in the Fipronil-treated plot (3.66), reached its maximum point (3.96). However, in July, the infestation started decreasing and reached its minimum level in October in the control plot (5.33) while in Fipronil treated plots, the infestation decreased to 1.80 as shown in the figure 1. In June, a considerable infestation rate was seen in the control plot, whereas a low infestation was observed in the plot treated with Basudin and other insecticides, according to Ullah *et al*. (2012) and Nadeem and Hamed (2011).

From the current investigation, it is confirmed that the borers highly infest sugarcane from April to September. In order to control the borers effectively in this crop growth period, our findings suggest that application of Fipronil 0.4% and Carbofuran 3G against *C. infuscatellus* along with other insect pest management strategies would be a better option to excellently control the stem borer.

Table 1. Developmental duration (days) of Larval and pupal periods of *C. infuscatellus* under laboratory conditions.

Developmental stage of *C. infuscatellus*	Mean developmental duration ± SE (days)	Minimum	Maximum
Incubation period	2.30±0.152	2.000	3.000
1st instar	2.70±0.152	2.000	3.000
2nd instar	3.70±0.152	3.000	4.000
3rd instar	4.60±0.163	4.000	5.000
4th instar	5.20±0.200	4.000	6.000
5th instar	6.00±0.210	5.000	7.000
Larvae	22.30±0.39	21.00	24.00
Pre pupa	1.60±0.16	1.000	2.000
Pupa	5.90±0.23	5.000	7.000

Table 2. Adult stages duration (days) of *C. infuscatellus* under laboratory conditions

Developmental stage of *C. infuscatellus*	Mean duration (days)± SE	Minimum	Maximum
Pre-oviposition Period	0.470±0.036	0.300	0.600
Oviposition Period	3.100±0.233	2.000	4.000

Post-Oviposition Period	1.000±0.129	0.500	1.500
Male Longevity	3.900±0.233	3.000	5.000
Female Longevity	4.800±0.249	4.000	6.000
Male total life cycle	36.80±0.891	33.00	41.00
Female total life cycle	38.00±1.145	32.00	43.00
Sex ratio	Male : Female = 1: 2.3		

Table 3. Number of eggs cluster, No of eggs per cluster, No of eggs per female and % hatchability of *C. infuscatellus* under laboratory conditions.

Eggs	Mean ± SE	Minimum	Maximum
No of Eggs Cluster	3.90±0.27	3.00	5.00
No of eggs per Cluster	47.30±1.73	38.00	55.00
No of eggs per Female	315.90±4.35	295.00	334.00
Eggs Hatchability	90.87±0.40	89.24	93.75

Table 4. Mean percent parasitism and biological attributes of *Trichogramma chilonis* against *C. infuscatellus* under laboratory condition.

Parameter	Host Insect *Chilo infuscatellus* eggs
%parasitism	87.01 ± 0.70
%Adults emergence	76.11 ± 0.66
Total duration in days	8.74 ± 0.07
Adult longevity	3.55 ± 0.06

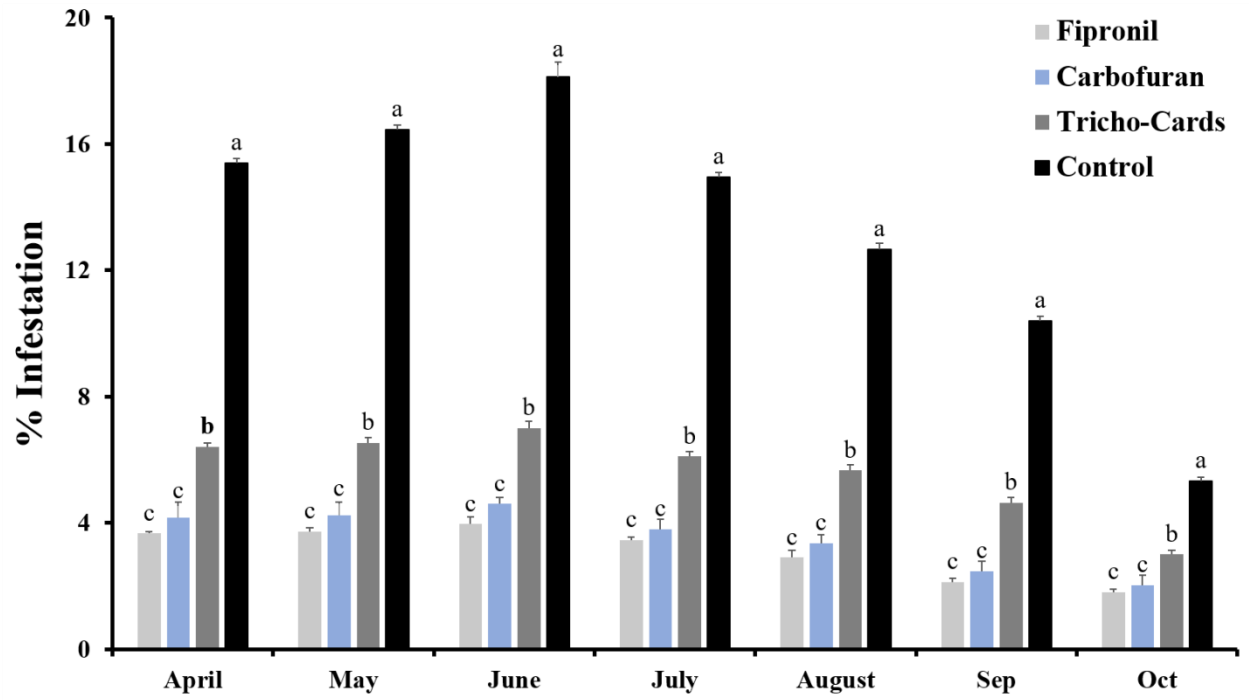

Figure 1. Impact of fipronil, carbofuran and tricho-cards against *C. infuscatellus* under field conditions

Conclusion: The amount of time it took for *C. infuscatellus* to mature from an egg stage to an adult stage was between 36.80 days for males and 38.00 days for females. *T. chilonis* had a parasitism rate of 87.01 percent and an adult emergence rate of 76.11% when hatching from *C. infuscatellus* eggs. The whole

developmental period of *T. chilonis* was 8.74 days, but the adult longevity was only 3.55 days. The plot that had been treated with fipronil (3.58), followed by the plot that had been treated with carbofuran (4.26), and then *T. chilonis*, showed the most significant difference in mean infestation (5.63). In light of the findings presented above, it has been determined that future integrated pest management (IPM) programs should include the application of fipronil at a rate of 16 kg/ha and the inundated release of *Trichogramma chilonis* for the purpose of controlling *C. infuscatellus*. Additional research is required to evaluate the efficacy of *T. chilonis* in the field for the control of lepidopteran insect pests.

Author contributions SHS conducted the experiments. SHS, SS, and JK designed the experiment. SHS, AI and IK analyzed data and wrote the manuscript. FU revised the manuscript.

Data availability Data collected from different sources during this study are included in this article.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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