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Bio-efficacy of egg parasitoid, *Trichogramma chilonis* (Ishii) against spotted stem borer, *Chilo partellus* (Swinhoe) in *Kharif* maize

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Maize stem borer, *Chilo partellus,* is a serious pest of maize in India. Insecticides are not the right choice as larvae feed internally. Moreover, the indiscriminate use of pesticides increases the cost and accounts for health and environmental hazards. Adopting non-chemical methods such as biological control is an important strategy for effective suppression of the pest population. Biological control agents such as egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) substantially reduce the pest population and are widely used against lepidopteran insect-pests. We studied the efficacy of egg parasitoid, *T. chilonis* against *C. partellus* in *Kharif* maize and recorded maximum egg parasitism by *T. chilonis* when released twice at higher rates (*i.e.* 1,25,000 and 1,00,000 parasitized eggs ha^{-1}). However, treatments with low release rates (one and two releases @ 75,000 parasitized eggs ha^{-1}, one release @ 1,00,000 parasitized eggs ha^{-1}) experienced significantly high plant damage and did not provide satisfactory monetary returns. In contrast, treatments with higher release rates (*i.e. T. chilonis* @ 1,25,000 parasitized eggs ha^{-1} at 7 and 14 DAG; *T. chilonis* @ 1,00,000 parasitized eggs ha^{-1} at 7 and 14 DAG) effectively suppressed plant infestation, dead heart formation and leaf injury by *C. partellus.* These treatments were statistically comparable with chemical control (dimethoate @ 660ml ha^{-1} at 7 DAG). It can be concluded that one release of *T. chilonis* is insufficient, and two releases are required for effective management of *C. partellus*. *T. chilonis* provides the best result when released twice @ 1,25,000 parasitized eggs ha^{-1} at 7 and 14 DAG (B:C ratio=1.42) which is at par with two releases @ 1,00,000 parasitized eggs ha^{-1} (B:C ratio=1.41). Although pesticide-treated plots (Dimethoate 30 EC @ 660 ml/ha at 7 DAG) provide a satisfactory monetary return, they are not environmentally compatible and ecologically viable for the long run.

Keywords: Biological control, *Chilo partellus,* Net economic return, Parasitization, *Trichogramma chilonis*

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Maize (*Zea mays* L.) is a staple food crop and important dietary component, especially in several African countries[1]. Maize has the highest production among cereals followed by rice and wheat[2]. It is majorly used for livestock feeding, ethanol production, starch and oil extraction, and human consumption. Maize has a wider genetic base and an extraordinary level of genotypic diversity which enables it to thrive in variable sets of the environment in more than 168 countries[2]. Globally, maize is cultivated over 186.82 million ha with an annual production of 1147.62 million MT and productivity of 5.92 t/ha[2]. India ensures a total production of 27.82 million MT over an area of 9.20 million ha[2-3]. Although maize cultivation is increasing in India, its productivity is still lower (3.02 t/ha) compared to the world (5.92 t/ha)[2-3].

Maize productivity is hampered by various abiotic and biotic factors and among them, spotted stem borer, *C. partellus* (Swinhoe) (Lepidoptera: Crambidae) is a serious pest. It is more damaging in north India during the *Kharif* season and poses a serious problem in maize and sorghum-based cropping systems[4-6]. However, it also feeds on paddy, sugarcane and millets[7-9]. The infestation starts from 1-2 weeks after germination when newly emerged larva enters the plant whorl, scraps-off the chlorophyll content and bores into the leaf sheath, where it feeds on the growing stem of young plants[10,11]. Leaf scrapping, window formation, pinhole and dead heart

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formation, stem tunnelling, and stunted growth are the characteristics damaging symptoms[10]. It causes up to 80.4 per cent yield reduction in maize in different agro-climatic zones of India[12]. Therefore, it is imperative to effectively suppress the pest population below economic threshold levels.

Various new and old generation insecticides are recommended against *C. partellus,* however, insecticides may not be the right choice due to internal feeding behaviour of *C. partellus*. Moreover, unwarranted use of insecticides directly increases the cost of cultivation, and account for health and environmental hazards. Besides, maize is used as a fodder and feed crop, therefore, insecticide residues in the crop are deleterious to livestock[13]. Adopting non-chemical methods of pest management such as biological control can be important in such crops. Biological control agents such as egg parasitoid, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) substantially reduce the pest population and are widely used against many lepidopteran insect-pests[14]. *Trichogramma* includes the most widely produced and applied natural enemies which have been studied in more than 50 countries and are commercially released on nearly 32 million ha of agricultural fields worldwide[15-16]. *Trichogramma chilonis* can effectively suppress *C. partellus* population and provide satisfactory monetary returns to farmers[13,17-19]. It is reportedly more effective during early whorl stage and can naturally parasitize *C. partellus* eggs[18,20]. Although egg parasitism by *T. chilonis* has been studied in other states of India, very little is known about its efficacy against *C. partellus* in Haryana. We aimed to utilize this research gap and assess the bio-efficacy of *T. chilonis* at differential releases against *C. partellus.* We hypothesized that inundative releases of *T. chilonis* can substantially increase egg parasitism and decrease plant infestation, and ultimately decreases crop losses. It enables us to standardize the application or release rate and help inform effective management tactics against *C. partellus* in maize which can provide monetary returns comparable to chemical control.

Methodology

This study was conducted at the research area of CCS Haryana Agricultural University, Regional Research Station, Karnal (Haryana) during *Kharif*, 2017. Maize hybrid, HM 10 was sown in the last week of June (26th SMW) by adopting 75 x 20 cm spacing. The crop was raised as per the standard practices, recommended by CCS Haryana Agricultural University, except chemical control. Each field area was divided into nine plots (50 m^2 each) representing nine treatments (details are given below). Treatments were replicated three times in randomized block design (RBD) and isolated by 3 m distance to avoid movement of *T. chilonis* adults between the treatments[20]. *T. chilonis* was released as tricho-cards containing *T. chilonis* parasitized eggs of *Corcyra cephalonica,* just before their emergence *i.e.,* roughly one week after parasitization. Tricho-cards were stapled uniformly to the underside of the central whorl leaves in two central rows of each plot during evening hours. Chemical control comprised of dimethoate 30 EC application which is recommended and widely used against *C. partellus* and other insect-pests in maize.

Treatments	Details	Time of release/ application
T_1	*T. chilonis* parasitized eggs @ 75,000/ha	7 DAG
T_2	*T. chilonis* parasitized eggs @ 1,00,000/ha	7 DAG
T_3	*T. chilonis* parasitized eggs @ 1,25,000/ha	7 DAG
T_4	*T. chilonis* parasitized eggs @ 75,000/ha	7 DAG, 14 DAG
T_5	*T. chilonis* parasitized eggs @ 1,00,000/ha	7 DAG, 14 DAG
T_6	*T. chilonis* parasitized eggs @ 1,25,000/ha	7 DAG, 14 DAG
T_7	Dimethoate 30EC @ 660 ml/ha*	7 DAG
T_8	Dimethoate 30EC @ 660ml/ha*	14 DAG
T_9	Untreated Control	-

DAG: Days after germination; * spray volume: 500 L ha^{-1}

We recorded egg parasitization by using laboratory-reared eggs of *C. partellus.* After 24 hours of the release of *T. chilonis* parasitized eggs, one egg card containing 50 *C. partellus* eggs was placed in the whorl of the central row plant in each plot and collected back after 24 hours to examine under a microscope for parasitization[18]. Additionally, we tagged 100 plants per replicate to recorded plant infestation (PI), dead heart formation (DH) and leaf injury rating (LIR) at the weekly interval and pooled as average. LIR was recorded on 1-9 scale[21]. We also recorded the average grain yield per plot (converted to q ha^{-1}) after sun drying. We worked out the total variable cost and gross economic return to calculate the incremental cost: benefit ratio (ICBR) and net economic return from each treatment. The data on egg parasitism, plant infestation and dead heart formation was subjected to analysis of variance (ANOVA) by R-software[22]. Different treatment means were separated by Fisher's least significant difference (LSD) test at α=0.05[23].

Results and Discussion

Egg parasitization

Inundative releases supplement *T. chilonis* natural population and increase egg parasitism in release rate-dependent manner ($F_{8,18}$=311.7; p<0.001; LSD=3.89) (Supplementary Table S1). Egg parasitism was significantly higher in treatments with two releases of *T. chilonis* parasitized eggs as compared to treatments with single release and controls (Fig. 1a and Table S1). Single release treatments were significantly better than chemical and untreated control (Fig. 1a and Table S1), however, the pest population re-established after a couple of weeks, suggesting that one release is not sufficient for effective pest suppression. In contrast, treatment with two releases of *T. chilonis* parasitized eggs @ 1,25,000 ha^{-1} recorded maximum egg parasitization (62.4%) which was statistically at par with two releases of *T. chilonis* parasitized eggs @ 1,00,000 ha^{-1} (60%) but differed significantly from all other treatments ($F_{8,18}$= 311.7; p<0.001; LSD=3.89) (Fig. 1a and Table S1). Chemical treatment involving single spray of dimethoate 30 EC @ 660 ml ha^{-1} at 7 DAG recorded minimum eggs parasitization (3.5%) which was at par with one spray of dimethoate 30EC @ 660 ml ha^{-1} at 14 DAG (5.3%) (Fig. 1a and Table S1). Inundative releases of *T. chilonis* are reportedly effective against *C. partellus*[13,24]. A similar level of egg parasitization by *T. chilonis* has been reported in different parts of India[18,25-26]. *T. chilonis* parasitized 44.8% of *C. partellus* eggs as compared to chemical treatment (deltamethrin 2.8 EC @ 200 ml ha^{-1}) (1.00%) and untreated control (4.4%)[27]. Biocontrol treatments involving *T. chilonis* recorded higher egg parasitism (31.18%) compared to farmers practise (2.30%) and untreated control (7.67%)[17]. Egg parasitism was low in chemical controls as compared to untreated control in the early stages. However, it was not significantly different (Table S1). This might be due to increased parasitism during later stages of crop. In contrast to our findings, some studies have shown the effectiveness of single release of *T. chilonis* @ 1,00,000 parasitized eggs ha^{-1} on 13-day-old maize crop[28]. Deviation in results of our study may be due to different agro-climatic conditions of the region, level of pest infestation and, timing and rate of *T. chilonis* release.

Plant infestation and dead heart formation

Plant showing injury symptoms such as pinholes, shot holes, leaf scrapping, window formation, stem tunnelling and boreholes were considered as infested. *T. chilonis* application significantly reduced plant infestation and dead heart formation as compared to untreated control ($F_{8,18}$= 46.5; p<0.001; LSD=3.73; and $F_{8,18}$= 52.22; p<0.001; LSD=1.82) (Fig. 1b, 1c and Table S1). Plant infestation and dead heat incidence were significantly lower with two releases of *T. chilonis* at all doses (75,000; 1,00,000; 1,25,000 parasitized eggs ha^{-1}) as compared to one release (Table S1). However, chemical control with dimethoate at 7 DAG suffered minimum plant damage (13.1%), comparable to treatments with two releases of *T. chilonis* @ 1,25,000 parasitized eggs ha^{-1} (13.6%) and 1,00,000 parasitized eggs ha^{-1} (16.6%, LSD=3.89), respectively (Table S1). Chemical control with dimethoate 30 EC @ 660ml ha^{-1} at 7 DAG, was least affected in terms of dead heart formation (3.0%). It was statistically at par with biocontrol treatment having two releases of *T. chilonis* @ 1,25,000 parasitized eggs ha^{-1} (3.8%) but differed significantly from treatment with two releases of *T. chilonis* @ 1,00,000 parasitized eggs ha^{-1} (5.0%, LSD=1.82) (Fig. 1c and Table S1). *T. chilonis* suppressed plant infestation and dead heart formation when released twice at higher rates (*i.e.* 1,25,000 and 1,00,000 parasitized eggs ha^{-1}). However, treatments with low release rates (one and two releases of *T. chilonis* @ 75,000 parasitized eggs ha^{-1}, one release @ 1,00,000 parasitized eggs ha^{-1}) experienced significantly higher plant damage and dead heart incidence and did not provide satisfactory control (Figure 1b and 1c, respectively). Nevertheless, timely release of *T. chilonis* @ 1,25,000 or 1,00,000 parasitized eggs ha^{-1} at 7 and 14 DAG, can substantially reduce *C. partellus* infestation. Treatment with two release of *T. chilonis* @ 1,25,000 parasitized eggs/ha had lower dead hearts as compared to other biocontrol treatments and untreated control[13]. Our findings are well supported by investigations in other parts of India[13,17,29,30]. In contrast to our results, a single release of *T. chilonis* @ 1,00,00 parasitized eggs ha^{-1} significantly reduced plant infestation (14.3%) as compared to untreated control (33.0%)[27].

Leaf injury rating (LIR)

Leaf injury rating (0-9) is a pre-defined scale for *C. partellus* infestation in maize[21]. Treatments with two inundative releases of *T. chilonis* effectively reduced the leaf injury and initial damage by *C. partellus* as compared to untreated control ($F_{8,18}$=11.82; p<0.001; LSD=0.90) (Fig. 1d and

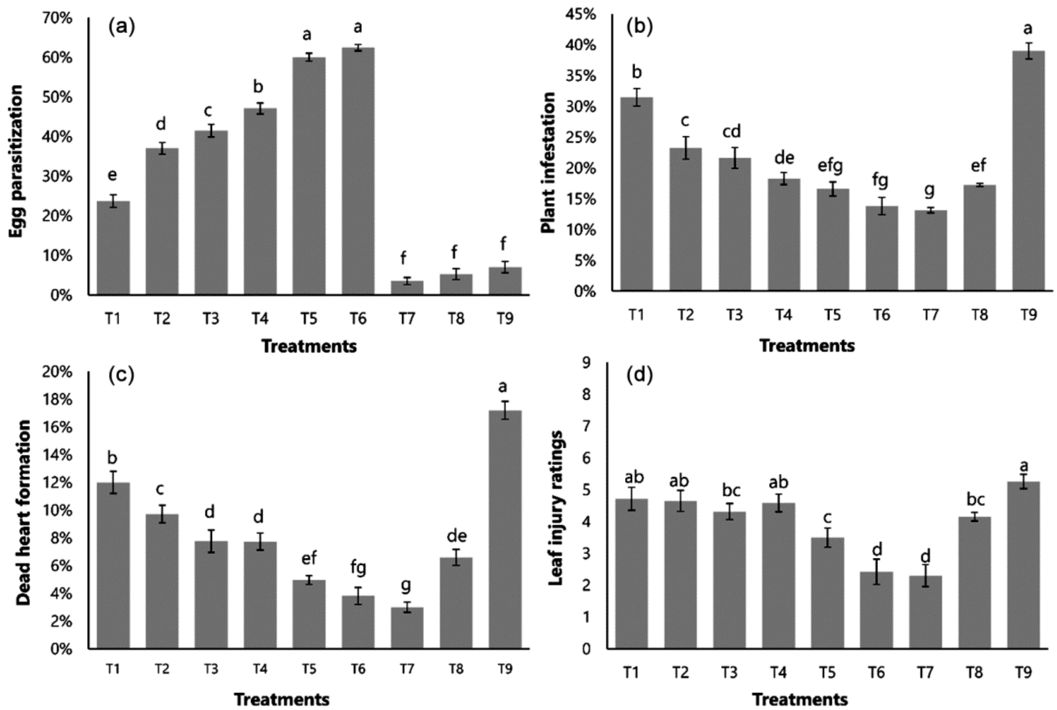

Fig. 1 — The extent of *C. partellus* egg parasitization by *T. chilonis* (a) and plant infestation (b), dead heart formation (c) and leaf damage (d) due to *C. partellus* infestation under different treatments. The columns represent the mean values and error bars denote the standard error (mean ±SE). Bars with a different letter indicates that the treatments are significantly different based on Fisher's LSD at α=0.05.

Table S1). However, one inundative release of *T. chilonis* parasitized eggs (@ 75,000, 1,00,000 and 1,25,000 ha^{-1}) did not substantially reduce the leaf injury as compared to untreated control ($F_{8,18}$= 11.82; p<0.001; LSD=0.90) (Fig. 1d and Table S1). Leaf injury was minimum with one spray of dimethoate @ 660ml ha^{-1} at 7 DAG (LIR=2.30) and it was statistically similar to treatment with two inundative releases of *T. chilonis* @ 1,25,000 parasitized eggs ha^{-1} (LIR=2.4; LSD=0.90) (Fig. 1d). Comparatively, leaf injury was slightly higher with two releases of *T. chilonis* @ 1,00,000 parasitized eggs ha^{-1} at 7 and 14 DAG (LIR=3.5; LSD=0.90), however, it was significantly lower compared to all other treatments (Fig. 1d). Therefore, two inundative release of *T. chilonis* (either 1,00,000 or 1,25,000 parasitized eggs ha^{-1} at 7 and 14 DAG) are required to significantly reduce leaf injury by *C. partellus*[13].

Average grain yield

Treatments with a single release of *T. chilonis* experienced lower average yield which did not differ significantly from untreated control ($F_{8,18}$= 3.90; p=0.008; LSD=9.10) (Fig. 2 and Table S1). In contrast, treatment with two release of *T. chilonis* @ 1,00,000 parasitized eggs ha^{-1} recorded higher yield

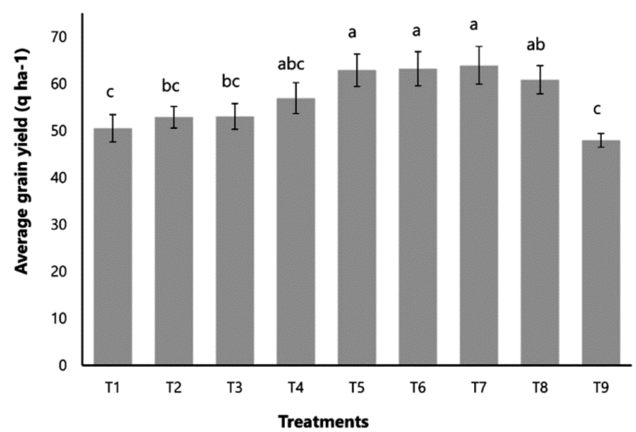

Fig. 2 — Average grain yield obtained from different treatments (converted in q ha^{-1}). The columns represent the mean values and error bars denote the standard error (mean ±SE). Bars with a different letter indicates that the treatments are significantly different based on Fisher's LSD at α=0.05.

(62.89 q ha^{-1}). However, average grain yield was maximum with one spray of dimethoate 30 EC @ 660 ml ha^{-1} (63.91 q ha^{-1}), which was statistically comparable with two release of *T. chilonis* @ 1,25,000 parasitized eggs ha^{-1} (63.20 q ha^{-1}, LSD=9.10) (Fig. 2). Thus, *T. chilonis* can be a good alternative to harmful insecticides and can provide satisfactory yield returns. Similarly, the higher yield

Table 1 — Benefit cost analysis of different biocontrol treatments and comparison with chemical controls

Treatments	Average yield (q ha^{-1})	Gross economic Return (₹ ha^{-1}) #	Return over control (₹ ha^{-1})	Cost of treatment (₹ ha^{-1}) #	Net profit from treatment (₹ ha^{-1}) #	Incremental cost benefit ratio (ICBR)	Total variable cost (₹ ha^{-1}) #	Net economic Return (₹ ha^{-1})	Cost: benefit Ratio (C:B)
T1	50.49	78245	3891	287.5	3604	1:12.54	68039	10206	1:1.15
T2	52.87	81862	7508	350	7158	1:20.45	68143	13719	1:1.20
T3	53.02	82090	7736	412.5	7324	1:17.76	68214	13876	1:1.20
T4	56.92	88018	13664	575	13089	1:22.76	68451	19567	1:1.29
T5	62.89	97093	22739	700	22039	1:31.48	68678	28415	1:1.41
T6	63.20	97564	23210	825	22385	1:27.13	68820	28744	1:1.42
T7	63.91	98643	24289	863	23426	1:27.14	68873	29770	1:1.43
T8	60.85	93992	19638	863	18775	1:21.76	68827	25165	1:1.37
T9	47.93	74354	-	-	-	-	67684	6670	1:1.10

For more details, see Table S2 (Supplementary)

was recorded with biocontrol treatment (*T. chilonis* @ 1,00,000 /ha) as compared to untreated control[19]. Average yield was also higher with two releases of *T. chilonis* @ 1,00,000 ha^{-1} at a weekly interval as compared to untreated control[28].

Economic analysis of treatments

Incremental cost: benefit ratio **(**ICBR) and monetary return over control were calculated to ascertain the efficacy of different treatments in terms of benefit over incurred cost. ICBR and net economic return were higher in two release treatments as compared to a single release and untreated control (Fig. 3 and Table 1; for details see Supplementary Table S2). Highest ICBR (1:31.48) was recorded with two releases of *T. chilonis* parasitized eggs @ 1,00,000 ha^{-1} followed by treatment having one spray of dimethoate 30EC @ 660 ml ha^{-1} at 7 DAG (1:27.15), treatment with two releases of *T. chilonis* parasitized eggs @ 1,25,000/ha (1:27.13) and treatment (two releases of *T. chilonis* parasitized eggs @75,000 ha^{-1}) (1:22.76). However, treatment (one release of *T. chilonis* parasitized eggs @ 75,000 ha^{-1} at 7 DAG) resulted in the lowest ICBR ratio (1:12.54) followed by treatment (one release of *T. chilonis* parasitized eggs @ 1,25,000 ha^{-1} at 7 DAG) (1:17.76) (Table 1). Biocontrol treatments with two releases of *T. chilonis* (1,00,000 parasitized eggs ha^{-1} or 1,25,000 parasitized eggs ha^{-1}) were comparable with chemical control (dimethoate @660 ml ha^{-1}) in terms of net economic return and benefit: cost (BC) ratio (Figure 3 and Table 1). Among biocontrol treatments, lowest B: C ratio was recorded with the single release of *T. chilonis* parasitized eggs @ 75,000 ha^{-1} (1.15), followed by single release @ 1, 00,000 ha^{-1} (1.20). However, it did not differ significantly from untreated

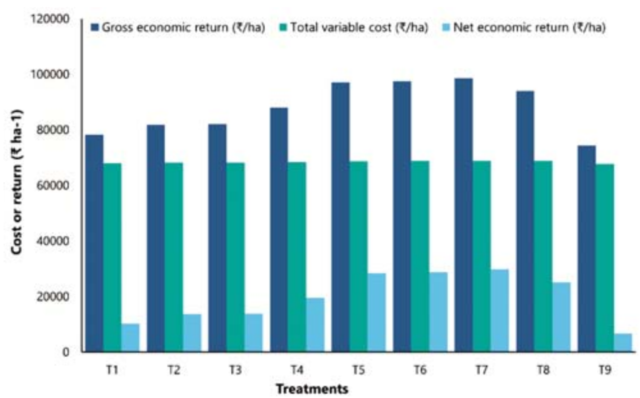

Fig. 3 — Economic analysis of different treatments. The columns represent the mean values.

control (1.10) which was the least profitable treatment among all (Table 1). Biocontrol treatments with two releases of *T. chilonis* (1,00,000 parasitized eggs ha^{-1} or 1,25,000 parasitized eggs ha^{-1}) can provide satisfactory monetary returns to the farmers and it can be a good alternate to dimethoate foliar sprays[28].

Conclusions

T. chilonis was more effective when two releases were made at weekly interval during the early stages of crop growth as compared to a single release. The efficiency of *T. chilonis* increased with an increase in the number of parasitized eggs released. Higher plant infestation was recorded with single releases of *T. chilonis* which implies that one release is insufficient, and two releases are required for satisfactory control. We conclude that *T. chilonis* provide best results when released twice @ 1,25,000 parasitized eggs /ha at 7 and 14 DAG which is at par with two releases @ 1,00,000 parasitized eggs/ha. It provides a sufficient level of control against *C. partellus,* and also minimize environmental and health hazards caused by repetitive use of pesticides. Further studies are required to access the compatibility of *T. chilonis* with other pest control methods such as microbial, botanicals, behavioural compounds and selective pesticides. *T. chilonis* can be integrated with other biocontrol agents such as larval parasitoid, *Cotesia flavipes,* which substantially reduces larval population in the mid and later stages of crop growth[11]. One better way is to release *T. chilonis* twice at early whorl stage (7 and 14 DAG) and *C. flavipes* during later stages of the crop (30 to 45 DAG). It can considerably reduce the pest population in first as well as subsequent generations and keep it below economic threshold levels.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution Statement

GS, MSJ and TV designed the experiment; GS conducted the experimental work and data analysis; GS wrote the manuscript; MSJ and TV provided the feedback on initial drafts and all the authors revised it.

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