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Research Article

Inundative Release of *Trichogramma chilonis* for the Biological Control of Pink Bollworm (*Pectinophora gossypiella*) in Cotton

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ABSTRACT

Pink bollworm (*Pectinophora gossypiella*) is a major pest of cotton worldwide, including in Pakistan, causing substantial quantitative and qualitative yield losses. Therefore, the effectiveness of the egg parasitoid *Trichogramma chilonis* was evaluated for the management of pink bollworm in cotton. The field experiment was conducted during the cotton growing season at the experimental fields of the Nuclear Institute of Agriculture, Tandojam. Two experimental plots were sown with the cotton variety NIA-Noori. In one plot, *T. chilonis* was released at a density of two cards per 1/4 acre, while the other plot served as an untreated control without parasitoid release. The results indicated a significant reduction in pink bollworm infestation on cotton flowers and bolls in the *T. chilonis*-released plot compared to the control. The highest infestation was observed in the control treatment, with 10.67% infestation on flowers and 18.91% on bolls. In contrast, infestation on cotton buds remained nearly the same (1.18%) in both treatments. Based on these findings, it can be concluded that *T. chilonis* is an effective biological control agent against pink bollworm. Its periodic release is recommended as an eco-friendly and sustainable strategy for managing pink bollworm infestations in cotton.

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Introduction

Cotton, the world's leading fiber crop, belongs to the genus *Gossypium* of the family Malvaceae and has the ability to grow in warm, semi-warm, and semi-arid regions worldwide (Tarazi et al., 2020). The primary product obtained from cotton is fiber, which is extensively used in textile manufacturing, requiring millions of bales of raw cotton annually (Shahzad et al., 2019). Among cotton-producing countries, Pakistan ranks fifth after China, the USA, India, and Brazil (Khan et al., 2020). However, Pakistan's average cotton yield remains significantly lower than that of other major producers (Aslam, 2016). Cotton contributes approximately 0.6-3.1% to the national GDP and plays a vital role in agricultural value addition, with an annual production of about 7.064 million bales during 2019-2020 (Abdul Kareem et al., 2021).

Low cotton productivity in Pakistan is attributed to several factors, including climate change, drought, irrigation water scarcity, poor seed quality, and insect pests. Among these, insect pests are the most damaging, causing annual yield losses of 30-40% (Tokel et al., 2022). Major insect pests of cotton include sucking pests such as whitefly, jassid, and thrips, and bollworms such as the spotted bollworm, American bollworm, and pink bollworm, which reduce yield either directly through feeding damage or indirectly by transmitting plant pathogens (Dhaliwal et al., 2010).

The pink bollworm, *Pectinophora gossypiella*, is considered one of the most destructive pests of cotton and ranks as the most significant lepidopteran pest in nearly all cotton-growing regions (Ismail et al., 2020). The larval stage, which primarily attacks cotton bolls, causes substantial yield losses, ranging from 2.8-61.9% in seed, 2.1-47.1% in oil content, and 10.7-59.2% in normal boll opening (Parmar and Patel, 2016; Ismail et al., 2020). Feeding by *P. gossypiella* also promotes boll rotting, ultimately degrading lint quality, staple length, and fiber strength (Spielman et al., 2015).

Chemical control has traditionally been the primary approach for managing this pest and remains the dominant strategy, with up to 60% of all commercial insecticides used on cotton (Ismail, 2019; Sasikumar and Vimala, 2020). The introduction of broad-spectrum synthetic pyrethroids initially proved effective in suppressing pink bollworm infestations (Ramasubramanyam, 2004). Later, the development of transgenic cotton expressing a single gene (*Cry1Ac*) or dual genes (*Cry1Ac* + *Cry2Ab*) derived from the soil bacterium *Bacillus thuringiensis* (Bt) provided broad protection against bollworms such as *Helicoverpa armigera* (Hübner), *Earias vittella* (Fabricius), and *P. gossypiella* (Choudhary and Gaur, 2010).

The combined use of transgenic cotton and broad-spectrum insecticides successfully suppressed pink bollworm populations for nearly one and a half decades, maintaining the pest below economic threshold levels until about 2010 (Fand et al., 2019). However, in recent years, *P. gossypiella* has re-emerged as a major pest due to the development of resistance to both Bt toxins (*Cry1Ac* and *Cry2Ab*) and conventional insecticides, as well as the availability of late-season cotton hybrids that serve as continuous hosts (Kranthi, 2012; Naik et al., 2018). Moreover, the extensive use of synthetic pesticides has led to severe environmental and human health hazards. Broad-spectrum insecticides often have a greater detrimental impact on natural enemies than on target pests, resulting in pest resurgence and accelerated resistance development (IRAC, 1995).

Therefore, there is an urgent need to develop alternative, economical, and environmentally friendly strategies for the management of cotton bollworms. Among non-polluting pest management options, biological control through parasitoids, either alone (Orr et al., 2000; Suh et al., 2000) or in combination with other tactics, has proven effective (Ahmad et al., 2001). In addition to chemical control, host plant resistance plays an important role in reducing crop losses (Senthil-Kumar and Mysore, 2012). Although several *B. thuringiensis* (Bt) strains are currently available, limitations exist regarding the widespread adoption of Bt crops (Romeis et al., 2008). Given the constraints in developing resistant cultivars, alternative pest management practices must be implemented. However, these alternative measures have achieved limited success, leaving farmers largely dependent on chemical pesticides for crop protection (Nadeem and Hamed, 2011).

Hence, selecting the most effective parasitoid species is essential for integrated pest management programs. Among natural enemies, species of the genus *Trichogramma* are the most widely used worldwide (Li, 1994) due to their ability to parasitize eggs of numerous crop pests and their ease of mass rearing. The use of *Trichogramma* as a biological control agent is a well-recognized, eco-friendly alternative to insecticides and has been successfully applied in managing various insect pests (Suh et al., 2000). The parasitoid *Trichogramma* is cosmopolitan in distribution and capable of parasitizing the eggs of the pink bollworm (*Ahmad et al., 2011*). Other advantages of *Trichogramma* include low cost, ease of application, wide availability, environmental safety, and the ability to suppress pest populations before crop damage occurs.

Lizarruga (1980) reported that weekly releases of 5,000 *Trichogramma* individuals in cotton fields resulted in 20.9-26.2% parasitism of *Heliothis* eggs, whereas weekly releases of 15,000 individuals achieved 47.6-58.2% parasitism of *H. armigera* eggs. They recommended initiating parasitoid releases when cotton plants are still small. Similarly, Patil et al. (1991) successfully managed *H. armigera* and *E. vittella* infestations in cotton through an integrated approach combining pesticides and *Trichogramma* releases.

Therefore, the present study was designed to develop an effective and environmentally friendly technique for the management of cotton bollworms through biological control.

Materials and Methods

Experimental site and crop details

A field experiment was conducted during the 2019 cotton-growing season at the Experimental Farm of the Nuclear Institute of Agriculture (NIA), Tandojam (Latitude: 25.43° N; Longitude: 68.54° E; Altitude: 26 m above sea level) to evaluate the efficacy of the egg parasitoid *T. chilonis* (Ishii) for the management of the pink bollworm, *P. gossypiella* (Saunders). The experiment was carried out on a non-Bt cotton variety, Noori, under field conditions representative of the semi-arid climate of Sindh, Pakistan. The soil at the site was clay loam with moderate fertility, and standard agronomic practices were followed throughout the growing season.

Experimental design and treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with two treatments and five replications. Each plot measured 50 × 40 m^2, with a 2 m buffer zone between adjacent plots to prevent parasitoid movement between treatments. The treatments included two levels: T_1, representing the *T. chilonis* release (biological control treatment), and T_2, serving as the control without parasitoid release. In the biological control treatment, *T. chilonis* was released using parasitoid cards, while the control plots were left untreated for comparison.

Rearing and field release of *T. chilonis*

The initial culture of *T. chilonis* was obtained from the Biological Control Laboratory, Nuclear Institute of Agriculture, Tandojam. The parasitoid was mass-reared on the eggs of the rice moth, *Corcyra cephalonica* (Stainton), under controlled laboratory conditions at 26 ± 2°C, 65 ± 5% relative humidity, and a 12:12 (L:D) photoperiod.

For field releases, one-day-old parasitized eggs of *C. cephalonica* were glued onto small paper cards (commonly called "Tricho-cards"), each containing approximately 2,000 parasitized eggs. These cards were allowed to acclimatize at room temperature before being taken to the field. In the experimental plots, *T. chilonis* cards were installed at a density of two cards per ¼ acre (equivalent to approximately 16 cards per hectare). The cards were pinned to the underside of leaves at about 1.0 m above ground level, with their position adjusted periodically according to the growth stage of the cotton plants.

Releases were initiated during the second week of July (when the crop entered the squaring stage) and continued until the end of September at weekly intervals, ensuring a continuous presence of parasitoids throughout the pink bollworm egg-laying period.

Field observations and data collection

Data collection began with the appearance of fruiting bodies (buds, flowers, and bolls) on 3rd August 2019 and continued at fortnightly intervals until crop maturity. From each replicated plot, five cotton plants were randomly selected and examined. Observations were recorded for the total number of buds, flowers, and bolls, as well as the number of infested buds, flowers, and bolls showing pink bollworm damage. The percent infestation of *P. gossypiella* was calculated using the following formula:

$$\text{Infestation percentage} = \frac{\text{Number of infested cotton parts}}{\text{Total number of observed cotton parts}} \times 100$$

Statistical analysis

The recorded data were subjected to Analysis of Variance (ANOVA) using the software STATISTIX 8.1. The Least Significant Difference (LSD) test at a 5% probability level ($P \leq 0.05$) was employed to compare treatment means. Prior to analysis, data were examined for normality and homogeneity of variances to ensure compliance with ANOVA assumptions. Graphical representations of infestation trends were prepared to illustrate treatment effects over time.

Results

Mean percent infestation of pink bollworm on cotton buds

Data on the mean percent infestation of *P. gossypiella* on cotton buds indicated negligible or no infestation up to 18th September 2019 (Figure 1). Thereafter, noticeable bud infestation appeared on 3rd October 2019 in both treatments. The mean infestation percentage of *P. gossypiella* on buds was significantly lower ($F = 21.26$, $df = 5$, $P < 0.001$) in the parasitoid-released plots compared to the control. The highest mean bud infestation (16.98 ± 2.00%) was recorded in the control plots, whereas the *T. chilonis*-released plots showed the minimum mean bud infestation (3.99 ± 1.21%) on the final observation date, i.e., 19th October 2019.

Mean percent infestation of *P. gossypiella* on cotton flowers

Data on the infestation percentage of *P. gossypiella* on cotton flowers exhibited considerable variation throughout the study period (Figure 2). At the first observation on 3rd August 2019, the mean infestation percentage in the *T. chilonis* treatment was 2.00 ± 2.00%, which was slightly higher than that recorded in the control (1.33 ± 1.33%). During September, the mean flower infestation percentages were 2.00 ± 2.00% and 6.00 ± 4.40% on 3rd and 18th September 2019, respectively.

Subsequently, on 3rd October 2019, the mean infestation percentage in the *T. chilonis* treatment was 4.00 ± 4.00%, which was statistically lower than that of the control (13.33 ± 6.67%). Thereafter, the infestation of *P. gossypiella* on flowers increased sharply, reaching a maximum of 49.33 ± 9.15% in the control, whereas the *T. chilonis* treatment showed a significantly lower infestation (8.00 ± 4.73%) on 19th October 2019.

Overall, the results revealed a highly significant difference (F = 9.72, df = 5, P < 0.001) between the two treatment groups in terms of mean infestation percentage of *P. gossypiella* across the different observation dates.

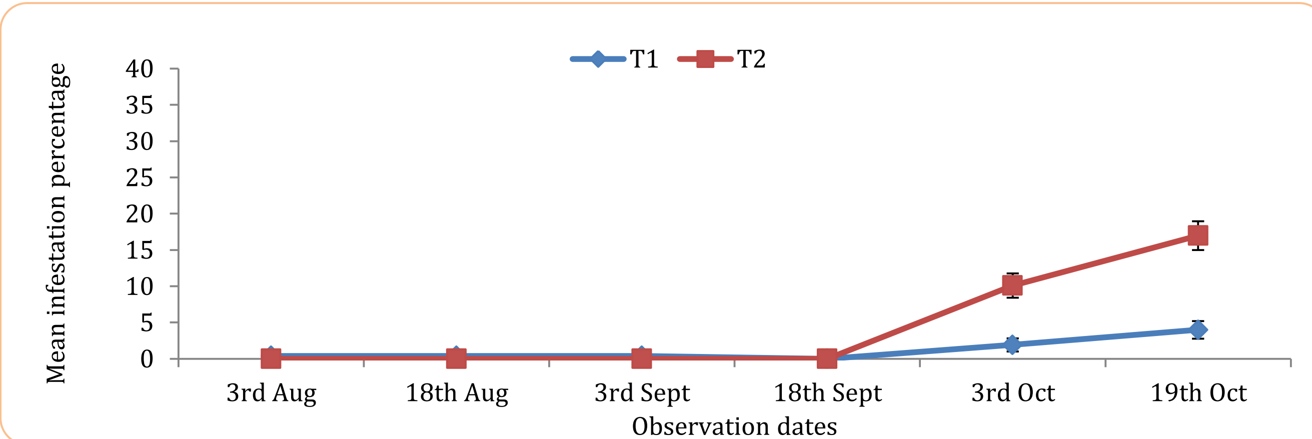

Figure 1. Mean infestation percentage of *P. gossypiella* on cotton buds in *T. chilonis*-released and control (unreleased) cotton fields (LSD = 0.0249, P < 0.05). T1 = *T. chilonis* release; T2 = control.

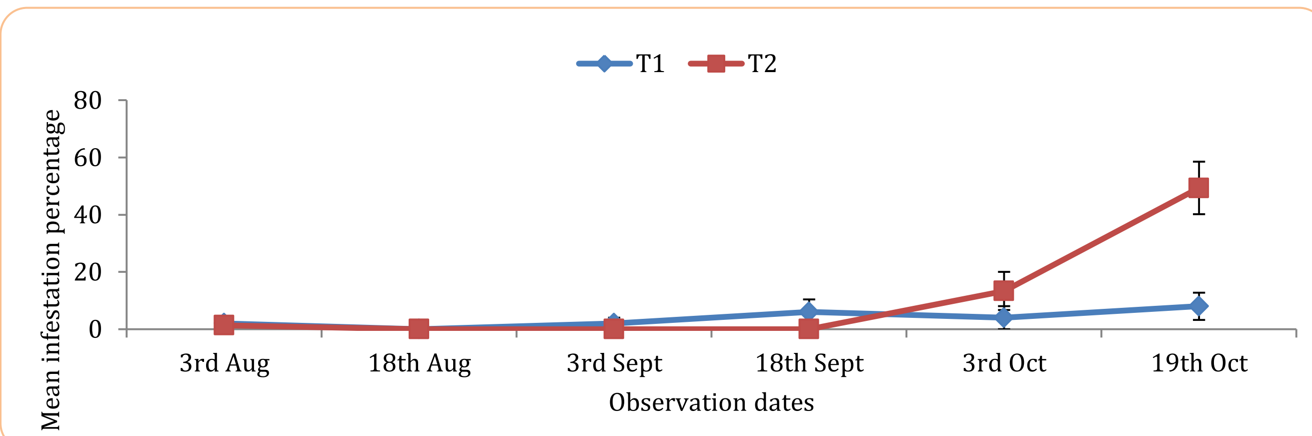

Figure 2. Mean infestation percentage of *P. gossypiella* on cotton flowers in *T. chilonis*-released and unreleased (control) fields (LSD = 0.0453; P < 0.05). T1 = *T. chilonis* release; T2 = control.

Mean infestation percentage of *P. gossypiella* on cotton bolls

The results for the mean infestation percentage of *P. gossypiella* on green cotton bolls revealed a significant difference between treatments (F = 27.95, df = 5, P < 0.001) throughout the study period (Figure 3). The infestation level was consistently lower in the *T. chilonis* treatment compared to the control.

On the first observation date (3 August 2019), the mean infestation percentage of *P. gossypiella* was 1.12 ± 0.53% in the *T. chilonis* treatment and 2.49 ± 0.70% in the control. Thereafter, a progressive increase in infestation percentage was observed in both treatments. On the second observation date (18 August 2019), mean infestation reached 2.70 ± 0.61% and 11.26 ± 2.02% in T_1 (*T. chilonis*) and T_2 (control), respectively. Similarly, on 3

September 2019, the mean infestation rose to 6.04 ± 0.56% in T_1 and 31.99 ± 1.82% in T_2.

By 18 September 2019, infestation levels were recorded at 16.22 ± 1.20% and 19.51 ± 1.93% in T_1 and T_2, respectively. On 3 October 2019, the mean infestation percentage was 16.52 ± 0.86% in T_1 and 19.86 ± 0.75% in T_2. The final observation on 19 October 2019 showed infestation levels of 17.38 ± 1.31% in the *T. chilonis* treatment and 28.33 ± 1.31% in the control. These findings clearly indicate that the release of *T. chilonis* effectively reduced *P. gossypiella* infestation on cotton bolls compared to untreated plots.

Overall mean infestation percentage of *P. gossypiella*

A highly significant difference was observed in the mean infestation percentage of *P. gossypiella* on buds (F = 41.64, df = 5, P < 0.001), flowers (F = 9.27, df = 1, P < 0.001), and green bolls (F = 167.67, df = 1, P < 0.001) of cotton between *T. chilonis* and control treatments. Figure 4 presents the pooled mean infestation percentages of *P. gossypiella* on buds, flowers, and green bolls of cotton.

The results indicated that the highest mean infestation occurred on green bolls compared to buds and flowers throughout the study period. The overall infestation percentage on cotton bolls was 18.91 ± 1.02% in the control, whereas it was significantly reduced to 10.00 ± 0.65% in the *T. chilonis* treatment. Similarly, the maximum infestation on cotton flowers was 10.67 ± 2.38% in the control, while only 3.67 ± 1.35% was recorded in the *T. chilonis* treatment. In contrast, nearly similar bud infestation levels (1.18 ± 0.70%) were recorded in both the control and *T. chilonis* treatments.

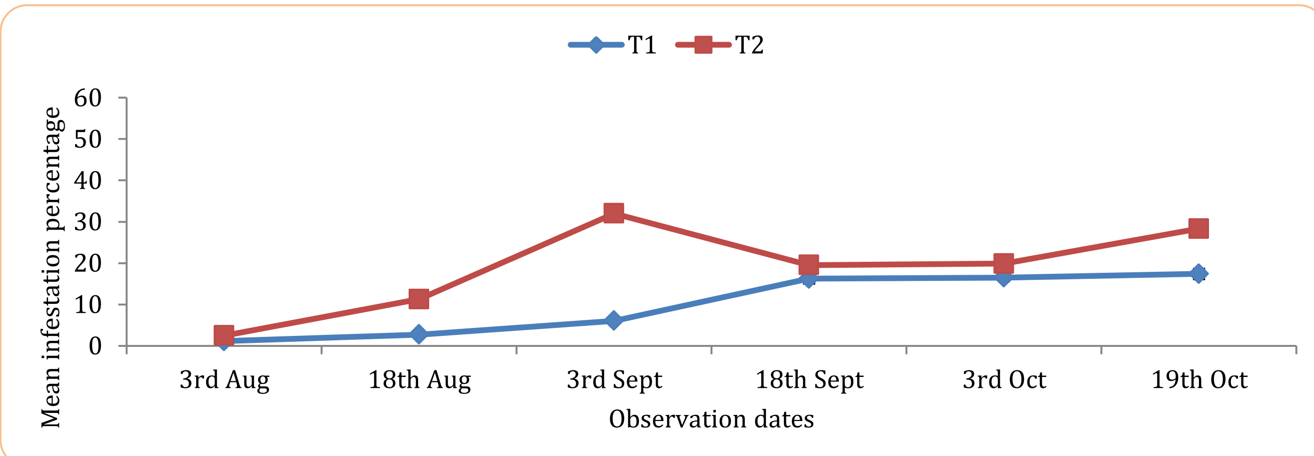

Figure 3. Mean infestation percentage of *P. gossypiella* on cotton bolls in *T. chilonis*-released and unreleased (control) cotton fields (LSD = 0.0339, P < 0.05). T_1 = T. chilonis release; T_2 = Control.

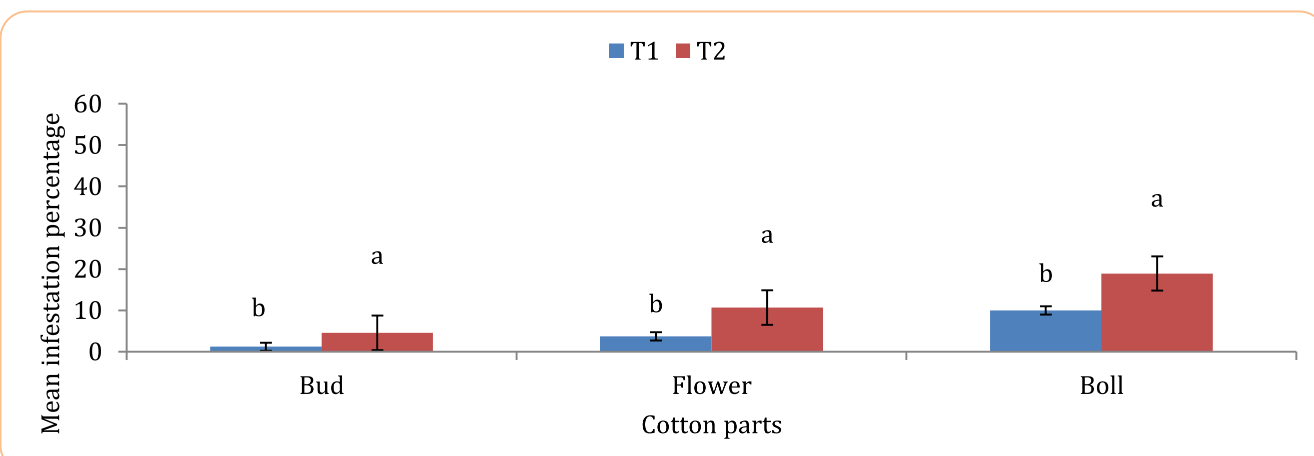

Figure 4. Overall mean infestation (%) of *P. gossypiella* on cotton buds, flowers, and bolls in parasitoid-released and unreleased fields. T1 = *T. chilonis* release; T2 = Control. Means followed by the same letters are not significantly different (LSD values: Buds = 0.010, Flowers = 0.1109, Bolls = 0.0138; P < 0.05).

Discussion

T. chilonis is one of the most important egg parasitoids used extensively for the management of several major pests in various agricultural crops, including *H. armigera*, *P. gossypiella*, and *C. suppressalis*, among others (Liu et al., 2016; Yang et al., 2016; Tang et al., 2017; Zhang et al., 2021). In the present study, *T. chilonis* also demonstrated its effectiveness in reducing the infestation of *P. gossypiella* under field conditions in cotton. A significantly lower infestation of *P. gossypiella* was observed on different fruiting bodies of cotton, including buds, flowers, and bolls.

Similar findings were reported by Undirwade et al. (2017), who observed that inundative releases of *T. chilonis* effectively reduced *P. gossypiella* infestation on various fruiting structures of cotton, yielding additional economic benefits compared with chemical control. Kumara et al. (2022) evaluated the performance of three *Trichogramma* species, *T. chilonis*, *T. bactrae*, and *T. nr. robusta*, against *P. gossypiella* under field and greenhouse conditions. Their results indicated that *T. bactrae* was comparatively more effective than *T. chilonis* and *T. nr. robusta*. The study further demonstrated that twelve sequential releases of *T. bactrae* from the flowering stage onward significantly reduced *P. gossypiella* infestation.

Several other studies have also confirmed the effectiveness of different *Trichogramma* species, including *T. chilonis*, in suppressing *P. gossypiella* populations in cotton, thereby improving both yield and fiber quality (Prasad et al., 2018; Asha et al., 2019; Naik et al., 2019; Jawanjal et al., 2021).

Masood et al. (2011) reported that the release of *T. chilonis* significantly reduced infestations of *H. armigera* and *E. insulana* in six cotton varieties, achieving damage reductions ranging from 31% to 45%. The maximum suppression recorded was 49.71% for *H. armigera* and 54.99% for *E. insulana*, confirming *T. chilonis* as an effective biological control agent for integration into cotton IPM programs.

Ismail (2023) conducted field trials in Egypt (2021-2022) to evaluate six pesticides alone and in combination with *T. evanescens* against pink bollworm in cotton. The combination of azadirachtin and *T. evanescens* provided the highest pest suppression, lowest boll damage, and increased yield. Parasitism rates and overall efficacy were significantly higher in biological control treatments, demonstrating that integrating *T. evanescens* with azadirachtin effectively manages pink bollworm populations.

Khan (2019) evaluated the integration of selective pesticides with *T. chilonis* for cotton pest management and found that certain selective pesticides were compatible with *T. chilonis*, maintaining its parasitism efficiency while effectively controlling major cotton pests. The results highlighted the potential of combining biological control agents with selective pesticides as a sustainable IPM approach in cotton.

Similarly, Abbas et al. (2020) evaluated *T. chilonis* trichocards and five insecticides against *H. armigera* in tomato. The release of 16,000 *T. chilonis* eggs per hectare effectively reduced fruit borer infestation and yielded the highest cost-benefit ratio (46.45). Among the insecticides tested, Border performed best, followed by Profit and Capital Plus. Overall, *T. chilonis* trichocards proved to be an eco-friendly and cost-effective alternative to chemical insecticides, offering sustainable management of *H. armigera* while conserving soil health and natural enemies.

Kumara et al. (2022) further identified *T. bactrae* as the most effective parasitoid against *P. gossypiella* in Bt cotton. Laboratory and field trials revealed over 70% egg parasitism and a significant reduction in pest incidence and boll damage with twelve sequential releases. Fields treated with *T. bactrae* achieved higher seed cotton yields comparable to those treated with insecticides, confirming its potential as a sustainable biological control agent for inclusion in IPM programs against pink bollworm.

Conclusions

The present study demonstrated that releases of *T. chilonis* significantly reduced the percent infestation of pink bollworm (*P. gossypiella*) under field conditions. The findings support the recommendation of *T. chilonis* as an effective and environmentally sustainable biological control agent for the integrated management of this notorious pest in cotton production systems.

Authors' Contributions

BKS, IK, and RMM jointly conceived and designed the study; NL prepared the experimental materials, conducted the experiments, and performed data collection and preliminary analysis; BKS carried out additional data analysis and interpretation; All authors contributed to the writing, critically reviewed the manuscript for intellectual content, and approved the final version for publication.

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Conflict of Interest
The authors declare no conflict of interest.

Sustainable Development Goals Targeted
SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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