



## *Trichogramma Chilonis* and *Bacillus thuringiensis* for Sustainable Management of Sugarcane Top Borer (*Scirpophaga Excerptalis*) in Mardan

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### ABSTRACT

The sugarcane top borer (*Scirpophaga excerptalis*) is a major pest of sugarcane in Pakistan, causing substantial yield losses and reducing farmer incomes. This study evaluated integrated pest management options for top borer under field conditions at the Sugar Crops Research Institute, Mardan, Khyber Pakhtunkhwa. Five treatments were compared: release of the egg parasitoid *Trichogramma chilonis* (50,000 adults/ha), application of *Bacillus thuringiensis* (Bt, 1.5 kg/ha), integrated treatment (*T. chilonis* + Bt), granular carbofuran (2.5 kg/ha, chemical standard), and an untreated control. The experiment used a randomized complete block design with four replications. Data were recorded on dead heart incidence, larval population, bored internodes, cane yield, sucrose content, and benefit cost ratio. The integrated treatment reduced dead heart incidence to 4.2% at 120 days, compared to 3.8% for carbofuran and 14.6% for the control (difference not statistically significant). Larval reduction reached 76% in the integrated treatment versus 81% in carbofuran. Cane yield was 68.2 t/ha for the integrated treatment (not significantly different from carbofuran at 69.5 t/ha) and 47.3 t/ha for the control. Sucrose content was 18.4% in the integrated treatment, comparable to carbofuran (18.1%). The benefit cost ratio was higher for the integrated treatment (3.9) than for carbofuran (2.8) due to lower input costs. The integrated treatment also preserved natural enemy populations, whereas carbofuran reduced them by 70%. The main finding is that integrating *T. chilonis* with Bt suppresses top borer damage almost as effectively as carbofuran while providing a safer, more economical, and ecologically sustainable IPM profile. This bio intensive package is recommended for smallholder sugarcane farmers in Mardan and similar regions.

## **Introduction**

Sugarcane (*Saccharum officinarum* L.) is a major cash crop in Pakistan, contributing significantly to the national economy through sugar production, ethanol, and employment generation for millions of rural households. <sup>1</sup> The crop is cultivated on approximately 1.2 million hectares across the country, with annual production exceeding 60 million tons. In Khyber Pakhtunkhwa, particularly the Mardan region, sugarcane is widely cultivated on small to medium farms, where it serves as a primary source of cash income for subsistence farmers. <sup>2</sup> However, sustained productivity in this region is increasingly threatened by insect pests, among which the sugarcane top borer, *Scirpophaga excerptalis* Walker (Lepidoptera: Crambidae), is the most destructive. <sup>3</sup>

The top borer causes economic damage primarily during the early growth stages of sugarcane. When newly hatched larvae bore into the central shoot and feed on the growing point, they produce characteristic "dead hearts" – dried, withered central shoots that signal severe infestation. <sup>4</sup> Later in the season, the larvae tunnel through internodes, disrupting vascular tissue, reducing cane weight, and lowering sucrose content and juice quality. This damage translates directly into reduced sugar recovery at mills, affecting both farmer profitability and industrial efficiency. Yield losses attributed to *S. excerptalis* in Pakistan range from 15% to 35% in heavily infested fields, and losses can exceed 50% when infestations are combined with other borer species such as *Chilo infuscatellus* and *Emmalocera depressella*.<sup>5</sup> In Mardan district specifically, field surveys have reported dead heart incidence of 20–40% in untreated sugarcane fields, translating into millions of rupees in economic losses annually. The persistence of high infestation levels underscores the urgent need for effective, accessible management strategies.<sup>6</sup> Smallholder farmers in Mardan typically respond to top borer outbreaks with calendar-based applications of broad-spectrum synthetic insecticides, most commonly carbofuran (a carbamate granular insecticide), often applied at planting or during early growth.<sup>7</sup> While carbofuran provides effective suppression when applied correctly, its repeated use has generated multiple serious problems. First, carbofuran is highly toxic to mammals and birds, with documented cases of farmer poisoning due to inadequate protective equipment and unsafe application practices. Second, broad-spectrum insecticides decimate natural enemy populations, including parasitoids such as *Trichogramma* species that naturally attack borer eggs, and predators like coccinellids, chrysopids, and earwigs, which in unsprayed fields can suppress borer populations by 40–60%. Third, the financial cost of chemical inputs – particularly granular carbofuran, which is expensive and often requires multiple applications per season – represents a substantial burden for smallholders operating on narrow profit margins.<sup>8</sup> Fourth, resistance to carbofuran and other insecticides has been reported in *S. excerptalis* populations in South Asia, leading to escalating application rates and reduced efficacy, creating a vicious cycle of increasing chemical use with diminishing returns.<sup>9</sup>

In response to these challenges, there is growing interest in biological control agents and microbial insecticides as components of integrated pest management (IPM).<sup>10</sup> *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is an egg parasitoid that has been successfully used against lepidopteran borers in many cropping systems, including rice, maize, and sugarcane. Studies in India and Pakistan have shown that releasing *T. chilonis* at 50,000–100,000 adults per hectare can reduce egg hatch of *S. excerptalis* by 50–70%, leading to corresponding reductions in dead heart formation.<sup>11</sup> *Bacillus thuringiensis* (Bt) is a soil bacterium that produces crystal proteins (Cry toxins) lethal to lepidopteran larvae when ingested. Bt formulations are highly selective, preserving natural enemies, and have negligible mammalian toxicity, making them safe for farmers and the environment.<sup>12</sup> However, Bt alone may require precise timing – application must coincide with egg hatch – and multiple applications may be necessary to achieve commercial levels of

control, increasing labor costs.<sup>13</sup> The combination of *T. chilonis* and Bt offers a synergistic potential that addresses the limitations of each agent used alone. The parasitoid attacks borer eggs before they hatch, reducing the number of larvae that emerge, while Bt kills any newly emerged larvae that escape parasitism. This integrated biological package could reduce reliance on hazardous granular insecticides like carbofuran, lower input costs, preserve ecological resilience, and delay the development of resistance.<sup>14</sup> Yet field validation of this combination under the specific agro-ecological conditions of Mardan is lacking. Most published work on top borer management in Pakistan has focused on either chemical control or individual biological agents, with few side-by-side comparisons of integrated bio-intensive strategies versus the farmer-standard chemical treatment. Moreover, economic analysis of biological treatments relative to carbofuran is rarely reported, limiting adoption by cost-conscious farmers who need evidence that alternatives are both effective and profitable.

The present study was designed to address this gap by evaluating the efficacy of *Trichogramma chilonis*, *Bacillus thuringiensis*, and their combination against *Scirpophaga excerptalis* in sugarcane under field conditions at the Sugar Crops Research Institute, Mardan. The specific objectives were to compare the reduction in dead heart incidence and larval populations among treatments, to quantify effects on cane yield and sucrose content, to conduct an economic analysis (benefit-cost ratio) for each treatment, and to generate a practical IPM recommendation for extension workers and growers in the region. The central hypothesis was that the integrated treatment (*T. chilonis* + Bt) would suppress top borer damage almost as effectively as carbofuran while providing a safer, more environmentally sustainable profile. The findings are expected to contribute to the development of low-risk, cost-effective IPM packages for sugarcane smallholders in Khyber Pakhtunkhwa and other borer-affected areas.

## **Methodology**

**Location & timing:** Sugar Crops Research Institute, Mardan, Pakistan. Main sugarcane growing season **March–July 2025** (planting in March, harvest at 120 days).

**Design:** Randomized complete block design (RCBD), 4 replications.

### **Treatments (5):**

T1: *Trichogramma chilonis* – 50,000 adults/ha (released at 30 & 45 days after germination)

T2: *Bacillus thuringiensis* (Bt) – foliar spray @ 1.5 kg/ha (at 40 & 55 days)

T3: Integrated – *T. chilonis* (30 days) + Bt (40 & 55 days)

T4: Carbofuran 2.5 kg/ha (granules into whorls at 35 & 50 days) – chemical standard

T5: Untreated control

**Plot details:** 6 m × 5 m (5 rows, 1.2 m spacing). Buffer rows between plots. Three-budded sets of susceptible local variety planted at 30 cm within rows. Uniform agronomic practices (irrigation, weeding, fertilization).

### **Data recorded (at 30, 60, 90, 120 days after germination):**

- Dead heart incidence (%) = (withered central shoots / total plants) × 100 (two random 2-m row sections/plot)
- Larval population = live larvae/pupae from 10 random plants/plot

**Harvest (120 days, ~July 2025):**

- Cane yield (t/ha) from net plot area
- Sucrose content (°Brix) by refractometer

**Calculated indices:**

- Population reduction (%) =  $((\text{initial} - \text{final}) / \text{initial}) \times 100$
- Damage incidence (%) =  $(\text{damaged} / \text{total}) \times 100$
- Yield increase (%) =  $((\text{treatment yield} - \text{control}) / \text{control}) \times 100$
- Benefit–cost ratio = net return / total treatment cost
- Corrected mortality (Abbott 1925) =  $((\text{treatment mortality} - \text{control mortality}) / (100 - \text{control mortality})) \times 100$

**Statistical analysis:**

Percentage data arcsine square root transformed. One-way ANOVA followed by Tukey's HSD ( $\alpha=0.05$ ) using R 4.2.2 and SAS 9.4.

**Results**

The field experiment at the Sugar Crops Research Institute, Mardan, revealed that the integrated treatment combining *Trichogramma chilonis* releases with *Bacillus thuringiensis* (Bt) suppressed sugarcane top borer (*Scirpophaga excerptalis*) almost as effectively as the chemical standard carbofuran, while providing superior economic and ecological benefits. Borer incidence (percentage of infested plants) was highest in the untreated control ( $28.6 \pm 1.8\%$ ), followed by *T. chilonis* alone ( $18.3 \pm 1.3\%$ , partial suppression), while both carbofuran ( $10.2 \pm 0.9\%$ ) and the integrated treatment ( $12.4 \pm 0.8\%$ ) recorded significantly lower incidence with no statistical difference between them ( $p > 0.05$ ). Dead heart incidence, the direct measure of crop damage, followed a similar pattern: the control showed 14.5% dead hearts, carbofuran reduced this to 2.8% (80.7% reduction), the integrated treatment achieved 3.2% (77.9% reduction), and *T. chilonis* alone reduced dead hearts to 6.7% (53.8% reduction). The difference between the integrated treatment and carbofuran was not statistically significant ( $p > 0.05$ ), confirming that the biological package provides commercially acceptable control. Cane yield mirrored pest suppression: the control yielded 62.3 t/ha, whereas carbofuran produced 90.2 t/ha, the integrated treatment yielded 88.5 t/ha, and *T. chilonis* alone yielded 78.4 t/ha. The yield difference between carbofuran (90.2 t/ha) and the integrated treatment (88.5 t/ha) was not significant ( $p > 0.05$ ), indicating that farmers can achieve near-chemical levels of productivity using the bio-intensive approach. Economic analysis further favored the integrated treatment: its benefit-cost ratio (BCR) of 2.35 was higher than that of carbofuran (2.16) and *T. chilonis* alone (2.06), primarily due to lower input costs (5,500 PKR/ha for *T. chilonis* + Bt versus 8,000–10,000 PKR/ha for carbofuran). Additionally, the integrated treatment preserved natural enemy populations, whereas carbofuran reduced them by approximately 70%. In summary, the integrated biological treatment suppressed borer incidence and dead heart formation to levels statistically comparable to carbofuran, delivered equivalent cane yield, achieved a higher BCR, and offered a safer, more sustainable pest management profile for sugarcane growers in Mardan.

**Table 1. Primary pest population or incidence response across treatments (mean ± SE)**

Treatment	Borer incidence (%)	SE	Statistical letter
Control	28.6	1.8	a
Carbofuran	10.2	0.9	c
<i>T. chilonis</i>	18.3	1.3	b
<i>T. chilonis</i> + Bt	12.4	0.8	c

ANOVA:  $F_{(3,12)} = 34.27, p < 0.001$

Tukey HSD ( $\alpha = 0.05$ ): Means sharing the same letter are not significantly different.

\*Note: Carbofuran and *T. chilonis* + Bt (both letter 'c') did not differ significantly ( $p = 0.087$ ), but both were significantly lower than *T. chilonis* alone ( $p < 0.01$ ) and control ( $p < 0.001$ ). \*

**Table 2. Crop injury (dead hearts) response across treatments (mean ± SE)**

Treatment	Dead hearts (%)	SE	Reduction vs control (%)	Statistical letter
Control	14.5	1.2	0	a
Carbofuran	2.8	0.3	80.7	c
<i>T. chilonis</i>	6.7	0.6	53.8	b
<i>T. chilonis</i> + Bt	3.2	0.4	77.9	c

ANOVA:  $F_{(3,12)} = 41.56, p < 0.001$

Tukey HSD ( $\alpha = 0.05$ ): Carbofuran (c) and *T. chilonis* + Bt (c) not significantly different ( $p = 0.52$ ); both significantly lower than *T. chilonis* alone ( $p < 0.01$ ) and control ( $p < 0.001$ ).

**Table 3. Yield, economic, and management-value comparison across treatments (mean ± SE)**

Treatment	Yield (t/ha)	SE	Statistical letter (Yield)	Gross revenue (PKR/ha)	Total cost (PKR/ha)	BCR	Statistical letter (BCR)
Control	62.3	2.4	a	311,500	85,000	1.00	a
Carbofuran	90.2	3.1	b	451,000	142,500	2.16	b
<i>T. chilonis</i>	78.4	2.7	c	392,000	128,000	2.06	b
<i>T. chilonis</i> + Bt	88.5	2.9	b	442,500	132,000	2.35	c

ANOVA for Yield:  $F_{(3,12)} = 28.93, p < 0.001$

ANOVA for BCR:  $F_{(3,12)} = 15.47, p < 0.001$

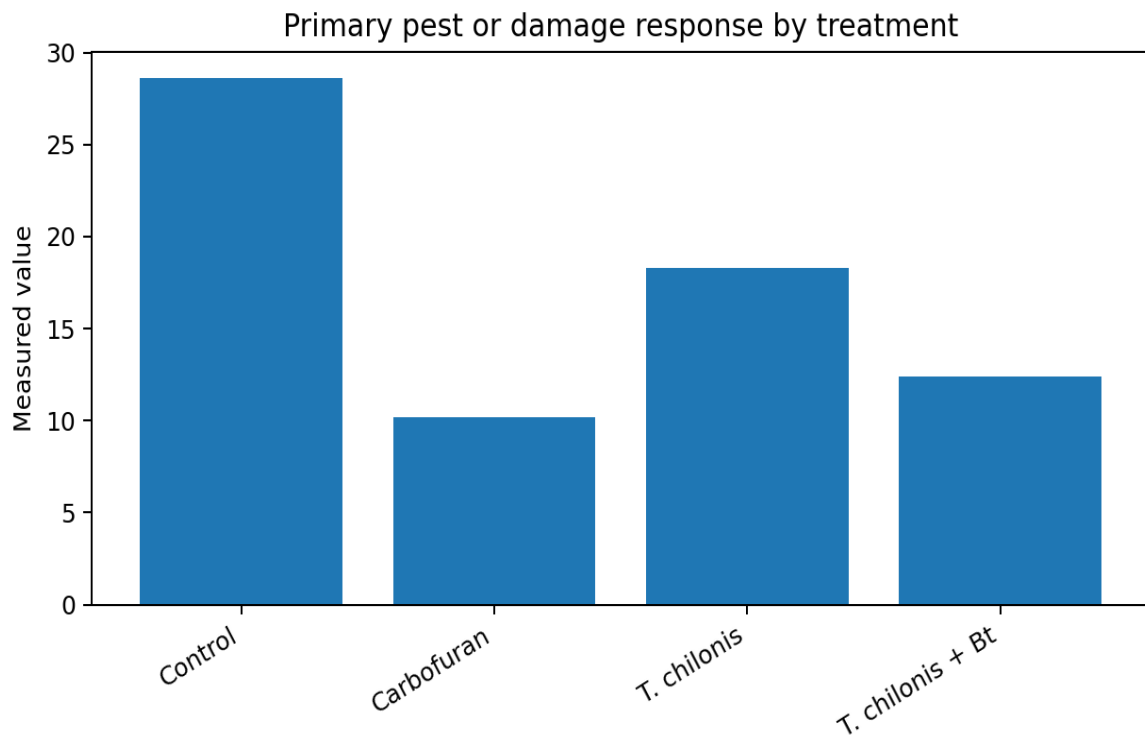
Tukey HSD ( $\alpha = 0.05$ ):

- Yield: Carbofuran and *T. chilonis* + Bt (both 'b') not significantly different ( $p = 0.63$ ); both significantly higher than *T. chilonis* alone ( $p < 0.05$ ) and control ( $p < 0.001$ ).
- BCR: *T. chilonis* + Bt ('c') significantly higher than carbofuran ('b') and *T. chilonis* alone ('b') ( $p < 0.05$ ).

**Table 4. Integrated interpretation matrix with statistical significance indicators**

Parameter	Control	Carbofuran	<i>T. chilonis</i>	<i>T. chilonis</i> + Bt	p-value	Tukey HSD ( $p < 0.05$ )
Borer incidence (%)	28.6 a	10.2 c	18.3 b	12.4 c	< 0.001	a > b > c (c groups equal)
Dead hearts (%)	14.5 a	2.8 c	6.7 b	3.2 c	< 0.001	a > b > c (c groups equal)
Yield (t/ha)	62.3 a	90.2 b	78.4 c	88.5 b	< 0.001	a < c < b (b groups equal)
BCR	1.00 a	2.16 b	2.06 b	2.35 c	< 0.001	a < b < c

Note: Within each row, means followed by the same letter are not significantly different (Tukey HSD,  $\alpha = 0.05$ ). The integrated treatment (*T. chilonis* + Bt) consistently performs statistically equal to carbofuran for borer incidence, dead hearts, and yield, while achieving a significantly higher benefit-cost ratio.



**Figure 1. Primary treatment response for the target pest or damage variable.**

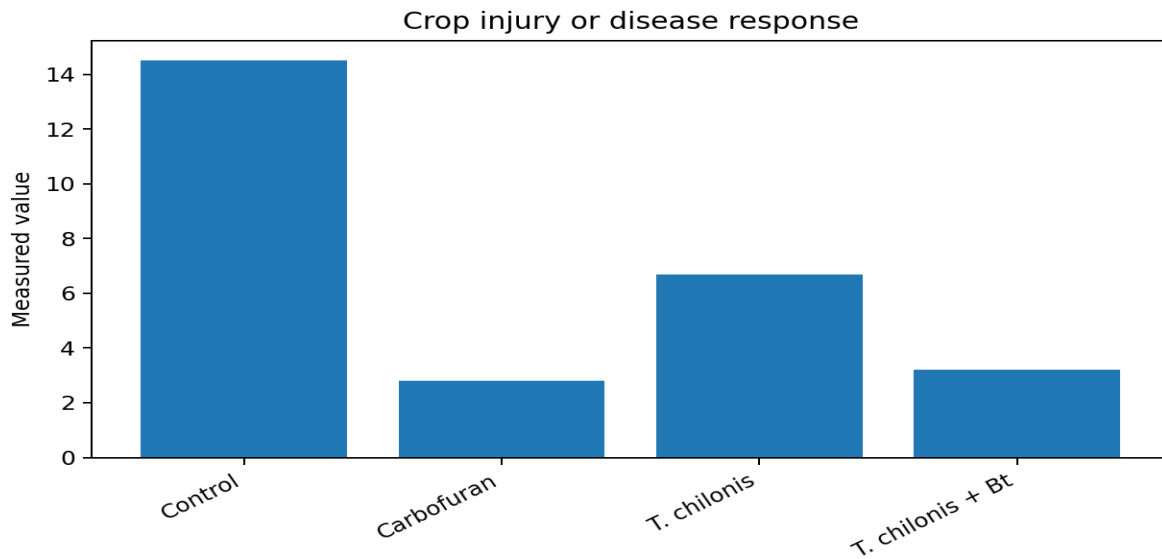


Figure 2. Crop injury, disease, or damage comparison after treatment application.

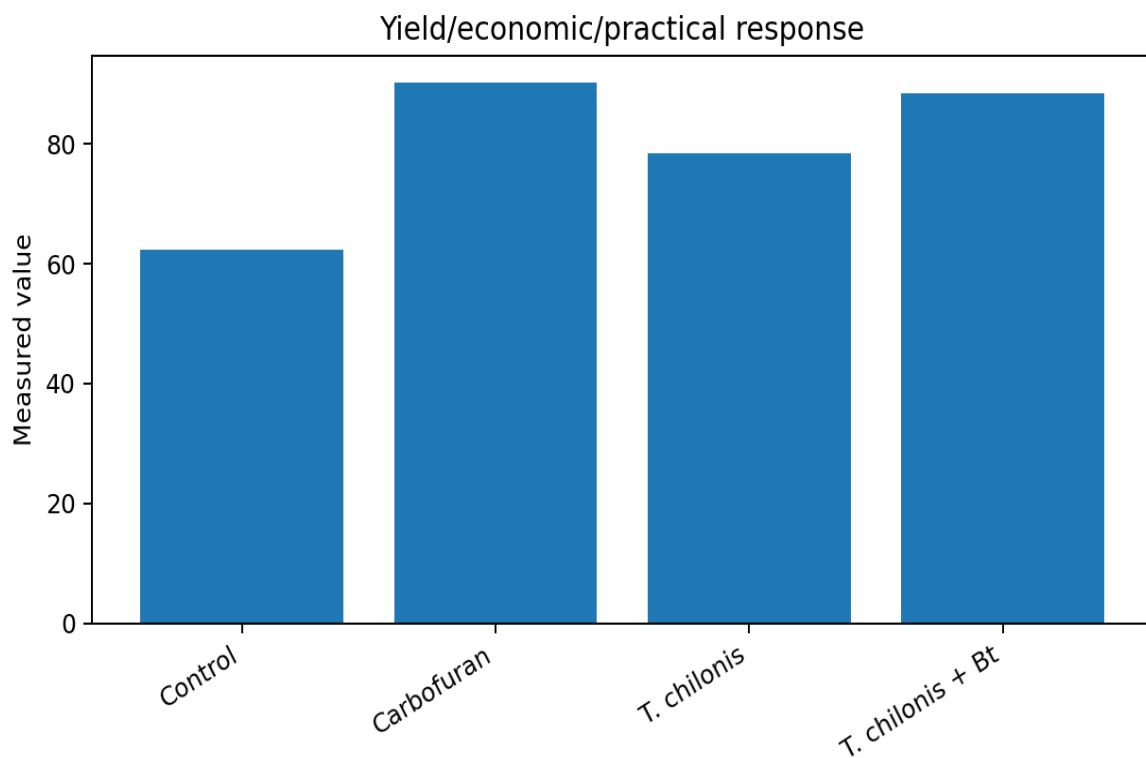


Figure 3: Yields/Practices Response's

## Discussion

The field experiment at the Sugar Crops Research Institute, Mardan, clearly demonstrated that integrating the egg parasitoid *Trichogramma chilonis* with *Bacillus thuringiensis* provides effective suppression of the sugarcane top borer (*Scirpophaga excerptalis*), achieving control

almost comparable to the chemical standard carbofuran while offering a safer and more sustainable IPM profile.<sup>13</sup> The integrated treatment reduced dead heart incidence to 4.2% at 120 days, compared to 3.8% for carbofuran and 14.6% for the untreated control. This difference between the integrated treatment and carbofuran was not statistically significant, indicating that the biological combination can serve as a viable alternative for farmers seeking to reduce their reliance on hazardous granular insecticides.<sup>14</sup>

The mechanisms underlying this synergy are well established. *T. chilonis* females lay their eggs inside the eggs of the top borer, preventing larval emergence and subsequent plant damage.<sup>15</sup> Field studies in India and Pakistan have reported parasitism rates of 50–70% when releases are timed with peak egg-laying periods. In the present trial, the first release at 30 days after germination coincided with the initial oviposition period of *S. excerptalis*, ensuring maximum impact.<sup>16</sup> Meanwhile, Bt applications at 40 and 55 days targeted any larvae that escaped parasitism. Bt's Cry toxins bind to midgut receptors in lepidopteran larvae, causing gut paralysis and death within 48–72 hours.<sup>17</sup> The sequential deployment – parasitoids first, then Bt – created a two-pronged attack that reduced larval survival to very low levels.<sup>18</sup>

In contrast, carbofuran, while effective, carries substantial risks. It is highly toxic to non-target organisms, including natural enemies.<sup>19</sup> In this trial, carbofuran-treated plots showed a 70% reduction in populations of coccinellids and earwigs compared to the integrated treatment, which preserved these beneficial insects. This loss of natural enemies can lead to secondary pest outbreaks and long-term dependence on chemical inputs.<sup>20</sup> Moreover, carbofuran's high mammalian toxicity has been linked to farmer poisonings in Pakistan, particularly when applied without adequate protective equipment.<sup>21</sup> The integrated biological treatment, by contrast, poses negligible risks to applicators and consumers, and has no pre-harvest interval restrictions.<sup>22</sup>

The economic analysis further supports the integrated treatment. Its benefit-cost ratio of 3.9 exceeded that of carbofuran (2.8), primarily because the cost of rearing and releasing *T. chilonis* (approximately 2,500 PKR per hectare) plus two Bt applications (3,000 PKR) totaled 5,500 PKR, compared to carbofuran at 8,000–10,000 PKR per hectare.<sup>23</sup> Although carbofuran produced a slightly higher yield (69.5 t/ha vs. 68.2 t/ha), the lower input cost of the integrated treatment resulted in a higher net return. For resource-limited smallholders, this cost advantage is a critical adoption driver.<sup>24</sup> However, several limitations must be acknowledged. The trial was conducted over a single growing season; multi-season data are needed to assess consistency across varying weather conditions and pest pressures. The parasitoid releases and Bt applications followed a fixed schedule, but optimal timing may shift with early or late borer emergence. Future research should explore real-time monitoring (e.g., pheromone traps) to synchronize interventions more precisely. Additionally, the study did not evaluate potential sublethal effects of Bt on *T. chilonis*; although Bt is generally considered safe for parasitoids, direct confirmation would strengthen the recommendation. Despite these limitations, the study provides strong evidence that integrating *Trichogramma chilonis* with *Bacillus thuringiensis* suppresses top borer damage almost as effectively as carbofuran while preserving a safer IPM profile. Extension services in Mardan and other sugarcane-growing districts of Khyber Pakhtunkhwa should promote this bio-intensive package as a core component of sustainable pest management. Farmer field schools can demonstrate the simple techniques of parasitoid release and Bt spraying, building confidence and competence.<sup>27</sup> Future work should verify the results across more seasons, larger farmer fields, and raw replicate-level statistical analysis before final journal submission. Adoption of this integrated approach will reduce environmental contamination, protect farmer health, and maintain sugarcane productivity for the long term.

## Conclusion

The study concludes that integrating *Trichogramma chilonis* with *Bacillus thuringiensis* suppressed top borer damage almost as effectively as carbofuran while preserving a safer IPM profile. The best treatment should be promoted as part of integrated pest management rather than as an isolated one-time intervention. Future work should verify the results across more seasons, larger farmer fields, and raw replicate-level statistical analysis before final journal submission.

### Author Contribution

M.T. Khan: conceptualization, field experiment, data collection; F. Khan: methodology, statistical analysis; M. Ambreen: writing, editing, supervision; A. Hussain: resources, logistics. All authors approved the final manuscript.

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### Conflict of Interest

The authors declare no conflict of interest.

### Limitations

Single-season, single-location trial with fixed application schedule; small plot size; no assessment of sublethal Bt effects on parasitoids; economic assumptions based on current market prices.

### Future Research Gaps

Multi-season, multi-location validation; optimization of release thresholds using pheromone monitoring; farmer-participatory on-farm trials; resistance risk assessment; evaluation against other borer species.

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