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RESEARCH ARTICLE

Refuge in bag: A smart strategy for outpacing bollworm resistance in Bt cotton

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Abstract

Transgenic crops expressing *Bacillus thuringiensis* (Bt) toxins effectively control major insect pests; however, the emergence of pest resistance can diminish their long-term effectiveness. The predominant strategy for delaying the progression of pest resistance to Bt crops boosts the survival of susceptible insects through "refuges" of host plants that do not produce cry toxins. Ideally, most of the resistant insects emerging from Bt crops will mate with the more abundant susceptible insects from nearby refuges. As Bt cotton has been widely adopted by Indian farmers since 2002, a proactive strategy was introduced to delay resistance to Bt proteins by planting 20 % of the field area with non-Bt cotton as a structured refuge. But to increase yields, farmers forego refuge planting. This reluctance resulted in short-term gains through increased yields but led the pink bollworm (PBW) to develop resistance to single-stacked gene Bt cotton by 2010 and to BGII by 2015. Thus, an extensive study assessing and comparing different refugia-in-bag (RIB) patterns for the bollworm complex, with special emphasis on PBW was conducted at Agricultural Research Station (ARS), Dharwad. Isogenic lines of Bt cotton hybrid (KCH-14K59 BG II) and its non-Bt were planted, following recommended agronomic practices. The pooled results indicated that the commercial RIB, fixed 5 % RIB, and fixed 10 % RIB treatments were statistically at par in terms of good boll opening (GBO), bad boll opening (BBO), and locule damage across all treatments. However, the highest seed cotton yield was recorded in T_1 (13.45 q/ha), followed by T_5 (12.37 q/ha) and T_4 (11.78 q/ha), while the lowest yield was observed in T_2 (6.62 q/ha). There was no infestation of *Helicoverpa* on Bt plants across the different treatments; however, PBW incidence and damage were predominant in all the blocks of both Bt and non-Bt plants.

Keywords: *Bacillus thuringiensis*; *Helicoverpa;* pink bollworm; refuge in bag; structured refuge

Introduction

Cotton (*Gossypium hirsutum* L.), a member of the Malvaceae family, is a vital crop for both the agricultural and industrial sectors of the Indian subcontinent. It significantly contributes to India's economy as the primary source of natural fibre, commonly known as vegetable wool. Cultivated in approximately 111 countries, cotton accounts for nearly 44 % if the world's total fibre production and contributes to 10 % of global edible oil production (1). India leads the world in cotton cultivation, covering over 112.30 lakh hectares and producing 307 lakh bales annually, with a mean productivity of 571 kg/ha. In Karnataka alone, cotton is grown on 6.75 lakh hectares, yielding 22.67 lakh bales with a productivity of 571 kg/ha (2).

Seed blends contained a higher percentage of transgenic Bt cotton seed mixed with a smaller percentage of non–Bt cotton isoline seed (3), to ensure that a refuge would be a proactive measure to prevent advancement of resistance to Bt protein(s). In India, the Genetic Engineering Appraisal Committee (GEAC) approved the first transgenic single-gene Bt cotton hybrids in 2002, followed by next-generation Bt cotton with stacked genes (Bollgard II®) in 2006. Initially, farmers were asked to plant 20 % of their fields with non-Bt cotton as a structured refuge. However, this practice became voluntary over time, leading many farmers to forego refuge planting to maximize yields. Structured refuge compliance is limited due to farmers' lack of understanding of its importance, along with other challenges like non-isogenic 'refugia' seeds and asynchronous agronomy between main and refuge crops. Though the refugia-in-bag (RIB) concept was studied in 2012, the government introduced 'RIB', which mandates 5–10 % non-Bt seeds blended with 90–95 % Bt seeds in every BG-II hybrid seed packet sold in the market from June 2020.

In the production of genetically modified Bt cotton (Bt for *Bacillus thuringiensis*), the sowing of refuge crops (refugia) serves as the primary insect resistance management (IRM) strategy adopted worldwide to delay the evolution of lepidopteran insects to becoming resistant to the cry toxin produced by the Bt crop. Thus, it has become the prevalent policy measure recommended by seed producers and authorities. However, since lepidopteran (i. e., pink bollworm, PBW) pest infestations have recently returned in several cotton-producing states in India, the planting of these refugia has become the "Achilles" heel of Bt cotton in the country (4). While the pest had recently been declared eradicated in the USA (5) and had been successfully suppressed in China (5-8), widespread resistance to the Bt cotton target pest has been reported in central and southern Indian cotton producing states, such as Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, and Telangana (9-12). In India, the refuge policy measure was introduced parallel to the Bt cotton technology itself when the GEAC under the Ministry of Environment, Forest and Climate Change (MoEFCC) concurrently stipulated refuge.

Materials and Methods

A large-scale experiment was conducted at ARS, Dharwad, during the kharif seasons of 2021 and 2022 to analyze and compare different proportions of RIB and structured refugia against the bollworm complex in Bt cotton, with particular reference to the PBW. The experiment was laid out in a randomized block design (RBD) design with six treatments and four replications, with a plot size of 18 × 10 sqmt.

Sowing was completed in the 2^{nd} fortnight of June in both seasons with the popular Bt hybrid Jadoo and its isogenic non-Bt version for refugia purposes. All the agronomic practices were adopted as prescribed by the university. Sowing was performed according to the treatments, with a spacing of 90 cm between rows and 60 cm between plants. To manage the sucking pests, insecticide sprays were taken after assessing the economic threshold level (ETL). Six treatments were designed for the studies, which are detailed below: T_1-100 % Bt, T_2-10 % non-Bt, T_3-20 % structured refugia, T_4- RIB-commercial (5-10 % minimal non-Bt seeds), T_5- RIB-Fixed pattern (5 % Minimal non-Bt seeds), T_6- RIB- Fixed pattern (10 % Maximum non-Bt seeds). Observations regarding infestation of cotton bolls by different bollworms in each of the blocks were recorded at 60, 80, 100, 120 and 140 along with open boll damage at the time of harvest and finally, the yield was recorded. For PBW, cotton bolls were randomly collected from the respective treatments, and destructive sampling was performed in the laboratory to count the number of larvae. All data were appropriately transformed using square root and arcsine transformations for statistical analysis.

The experimental design and RIB/structured refugia treatments followed protocols similar to those described earlier (13). The effectiveness of non-Bt refugia in delaying resistance evolution in bollworm populations is well documented (14). Pink bollworm sampling and larval enumeration methods were adapted from established Bt resistance monitoring procedures (15). Previous studies indicate that refuge quality influences larval survival, supporting our laboratory-based assessment of bolls (16).

Results

The population of American bollworm larvae at 80 days after sowing was elusive in all six treatments on the Bt cotton. In contrast, the maximum population of larvae and green boll damage was observed in non-Bt as compared to Bt cotton. In Bt, the lowest PBW larval recovery of 4.95 per ten green bolls was noticed in treatment T_1, next followed by T_4(RIB commercial), which is statistically at par with T_5 (5 % RIB) with 5.50 and 5.75 larvae per ten green bolls, respectively. While non-Bt plants have the highest recovery of larvae in T_2 and T_3 (15.38 and 13.38 larvae per ten green bolls, respectively) (Table 1).

Regarding boll damage of Bt plants due to PBW, T_3 recorded the highest damage (20.85), followed by T_6 (15.75), with T_1 showing the least damage (11.58). But non-Bt plants, T_2 has the highest boll damage (32.76), followed by T_3 (26.03), and the lowest in T_5 (19.09) and T_4 (19.91) from 50 green bolls. The lowest green boll damage in non-Bt plants (13.59 %) was recorded in T_4 block (RIB commercial), which is at par with treatments T_5 13.98 % and $T_2$with a high damage of 23.10 %.

The remarkably highest rosette flowering in Bt was perceived in T_3 (8.07 / 50 flowers), while in non-Bt the highest damage was noticed in T_2(14.19 / 50 flowers) (Table 1). No *Helicoverpa* larvae were observed on Bt plants across all treatments. In contrast, the non-Bt plants of T_2 revealed the highest larval numbers (4.34), followed by T_3 (3.73), with the lowest counts in T_5 (2.59), T_4 (2.68) and T_6 (2.78). Square damage due to American bollworm, spotted bollworm and tobacco caterpillar was highest in non-Bt T_2 (16.78) and least in T_5 (9.85). While in Bt, the highest square damage was observed in T_3 (3.84), which was significantly on par with T_4 (3.60), T_6 (3.58) and T_5 (3.55) (Table 1). Observations on 100 days after sowing, the larval recovery and boll damage % due to PBW revealed a similar trend as noticed in 80 days after sowing (Table 2). These outcomes indicated that the PBW larval recovery was increased significantly in both non-Bt and Bt plants.

Table 1. Observation on cotton bollworms and their damage in different treatments at 80 days after sowing (2021-2023)

Tr. No.	Treatments	Square damage ABW/SBW/TC		*Helicoverpa* larvae/ 5 plant		% FD PBW (Rossette flowers/50 flowers)		Green boll damage (%) (ABW/SBW/TC)		Boll damage (%) from 50 green bolls due to PBW		PBW larval recovery from 10 green bolls	
		Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T_1	Pure Bt 100 %	2.79 (9.60)	-	0.00 (0.71)	-	5.18 (13.15)	-	0.00 (0.71)	-	11.58 (12.92)	-	4.95 (2.33)	-
T_2	Pure NBt 100 %	-	16.78 (24.17)	-	4.34 (2.20)	-	14.19 (22.12)	-	23.10 (28.71)	-	32.76 (34.90)	-	15.38 (3.98)
T_3	Structured 20 % NBt	3.84 (11.29)	13.15 (21.25)	0.00 (0.71)	3.73 (2.06)	8.07 (16.50)	10.84 (19.21)	0.00 (0.71)	19.26 (26.02)	20.85 (24.72)	26.03 (30.66)	6.63 (2.67)	13.38 (3.72)
T_4	RIB commercial	3.60 (10.93)	10.25 (18.66)	0.00 (0.71)	2.68 (1.78)	6.80 (15.11)	8.72 (17.16)	0.00 (0.71)	13.98 (21.94)	14.46 (20.69)	19.91 (26.49)	5.50 (2.45)	9.38 (3.14)
T_5	5 % RIB	3.55 (10.85)	9.85 (18.28)	0.00 (0.71)	2.59 (1.76)	6.67 (14.96)	8.89 (17.34)	0.00 (0.71)	13.59 (21.62)	14.02 (18.18)	19.09 (25.89)	5.75 (2.50)	9.00 (3.08)
T_6	10 % RIB	3.58 (10.90)	10.24 (18.65)	0.00 (0.71)	2.78 (1.81)	6.95 (15.28)	8.85 (17.30)	0.00 (0.71)	15.27 (22.99)	15.75 (22.63)	22.73 (28.46)	6.13 2.57)	10.00 (3.24)
	SEm	0.04	0.86	-	0.04	0.80	3.11	-	1.43	1.03	1.71	0.11	0.13
	CD (%)	NS	2.83	-	0.12	NS	9.82	-	4.50	3.18	5.24	0.32	0.51
	CV	11.02	10.15	-	9.11	11.30	7.31	-	12.02	10.41	13.20	10.92	12.12

ABW- American bollworm; **SBW**- Spotted bollworm; **TC**- Tobacco caterpillar; **PBW**-Pink bollworm; **NBt**-Non-Bt; **FD**- Flower damage.

Table 2. Observation on cotton bollworms and their damage in different treatments at 100 days after sowing (2021-2023)

Tr. No.	Treatments	Square damage ABW/SBW/TC		*Helicoverpa* larvae/5 plant		% FD PBW (Rossette flowers/50 flowers)		Green boll damage (%) (ABW/SBW/TC)		Boll damage (%) from 50 green bolls due to PBW		PBW larval recovery from 10 green bolls	
		Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T_1	Pure Bt 100 %	3.89 (11.36)	-	0.00 (0.71)	-	3.75 (11.15)	-	0.00 (0.71)	-	26.61 (31.04)	-	8.18 (2.95)	-
T_2	Pure NBt 100 %	-	22.08 (28.01)	-	5.55 (2.46)	-	12.72 (20.89)	-	31.17 (33.92)	-	36.09 (36.91)	-	33.22 (5.81)
T_3	Structured 20 % NBt	6.05 (14.24)	15.39 (23.09)	0.00 (0.71)	4.78 (2.30)	9.72 (18.16)	10.01 (18.43)	0.00 (0.71)	22.94 (28.60)	26.11 (30.72)	27.78 (31.79)	8.61 (3.02)	24.50 (5.00)
T_4	RIB commercial	4.85 (12.72)	12.22 (20.46)	0.00 (0.71)	3.65 (2.04)	6.79 (15.09)	8.82 (17.27)	0.00 (0.71)	16.25 (23.76)	19.38 (26.10)	23.47 (28.97)	7.08 (2.75)	21.35 (4.67)
T_5	5 % RIB	4.31 (11.98)	11.17 (19.52)	0.00 (0.71)	4.06 (2.14)	6.50 (14.77)	8.54 (16.99)	0.00 (0.71)	16.33 (23.82)	18.52 (25.48)	22.88 (28.56)	6.95 (2.73)	19.96 (4.52)
T_6	10 % RIB	4.83 (12.69)	12.78 (20.94)	0.00 (0.71)	4.31 (2.19)	7.01 (15.35)	8.56 (17.00)	0.00 (0.71)	19.85 (26.44)	20.88 (27.18)	26.24 (30.80)	7.81 (2.88)	24.28 (4.98)
	SEm	0.42	1.02	-	0.07	0.92	3.74	-	2.12	1.23	1.45	0.12	0.82
	CD (%)	NS	3.12	-	0.20	2.83	11.15	-	6.59	3.88	4.52	0.33	2.61
	CV	9.24	10.52	-	11.40	12.41	8.51	-	14.51	10.08	10.48	9.92	7.93

ABW- American bollworm; **SBW**- Spotted bollworm; **TC**- Tobacco caterpillar; **PBW**-Pink bollworm; **NBt**-Non-Bt; **FD**- Flower damage**.**

As the crop stage advanced to 120 days after sowing. No *Helicoverpa* or *Erias* larvae were observed on any of the Bt plants. But on non-Bt plants, T_2 (2.63 /5 plants) and T_3(2.10 /5 plants) recorded the highest larval infestation of *Helicoverpa*, and the least was observed in T_4 (1.735/plants) and T_5(1.72/5 plants) (Table 3). The highest PBW infestation per 10 green bolls was observed on Bt plants of T_3(17.65), followed by T_6(15.22), and the lowest was observed in T_1 with 12.19 larval recovery from 10 green bolls. But on non-Bt plants, the highest PBW larval recovery was observed in T_2 (37.63) and T_3 (27.56) and T_6 (27.49) each with 0.87 larvae, while the lowest was in T_5(23.13). There was no square damage noticed on Bt plant due to PBW. On non-Bt plants, the highest square damage was perceived in T_2 (20.58), followed by T_3(16.41) and the lowest in T_5 (12.97). Green boll damage due to PBW in Bt plants was maximum in T_1(35.69) and lowest in T_4(24.14). But in the non-Bt cotton block, T_2 showed the highest green boll damage (40.72), followed by T_3(32.37) and the lowest was in T_5(26.06) (Table 3).

At 140 days after sowing in Bt cotton, no other bollworms were observed except the PBW. In non-Bt cotton, T_2 (1.18) and T_3 (0.27) recorded the highest infestation by *Helicoverpa* larvae per 5 plants, while the least was in T_5 (0.93) (Table 4). From Bt plants, T_3 (25.14) recorded the highest PBW larval recovery from 10 green bolls, followed by T_5(17.19), T_6 (17.09) and T_1(17.03), with the least in T_4 (16.91). In non-Bt cotton, T_2 (26.65) recorded the highest PBW larval infestation per 10 bolls, while the least was in T_5 (17.72). The highest % boll damage registered in Bt plants of T_3(36.53 %) due to PBW out of 50 bolls, with the least noticed in T_5 (32.89 %) While on non-Bt cotton block, T_2 (39.27 %) registered the highest % boll damage due to PBW, and the lowest in T_5(31.60 %) (Table 4).

About the yield parameters, in Bt cotton, % open boll damage was highest in T_3 (25.21 %), followed by T_6 (22.67 %) and least in T_5(20.52 %). In non-Bt cotton, the highest % open boll damage was observed in T_2 (38.44 %), followed by T_3 (33.07 %), with the least observed in T_5 (25.45 %). The highest % locule damage in Bt cotton plants was observed in T_3 (31.13 %), and the least in T_1 (19.32 %). In non-Bt cotton, T_2 (44.49 %) recorded the highest % locule damage, while the least was observed in T_5 (28.98 %) (Table 5). The highest seed cotton yield of 13.45 q/ha was registered in T_1,which is outstandingly superior over all other treatments (Table 5).

Table 3. Observation on cotton bollworms and their damage in different treatments at 120 days after sowing (2021-2023)

Tr. No.	Treatments	Square damage ABW/SBW/TC		*Helicoverpa* Larvae/5plant		% FD PBW (Rossette flowers/50 flowers)		Green boll damage (%) (ABW/SBW/TC)		Boll damage (%) from 50 green bolls due to PBW		PBW larval recovery from 10 green bolls	
		Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T_1	Pure Bt 100 %	2.39 (8.88)	-	0.00 (0.71)	-	0.00 (0.71)	-	0.00 (0.71)	0.00 (0.71)	35.69 (36.55)	-	12.19 (3.56)	-
T_2	Pure NBt 100 %	-	20.58 (26.97)	-	2.63 (1.77)	-	0.00 (0.71)	-	-	-	40.72 (39.64)	-	37.63 (6.17)
T_3	Structured 20 % NBt	3.60 (10.93)	16.41 (23.89)	0.00 (0.71)	2.10 (1.61)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	33.41 (31.69)	32.37 (34.66)	17.65 (4.26)	27.56 (5.30)
T_4	RIB Commercial	2.78 (9.59)	13.76 (21.77)	0.00 (0.71)	1.73 (1.49)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	25.31 (27.50)	28.55 (32.28)	13.94 (3.80)	23.80 (4.93)
T_5	5 % RIB	2.65 (9.37)	12.97 (21.10)	0.00 (0.71)	1.72 (1.49)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	24.14 (26.24)	26.06 (30.68)	13.33 (3.72)	23.13 (4.86)
T_6	10 % RIB	2.72 (9.49)	13.58 (21.61)	0.00 (0.71)	1.84 (1.53)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	25.81 (28.48)	30.64 (33.60)	15.22 (3.96)	27.49 (5.29)
	SEm	0.41	1.03	-	0.04	-	-	-	-	1.57	6.86	0.16	0.34
	CD (%)	NS	3.16	-	0.13	-	-	-	-	4.84	21.31	0.49	1.05
	CV	10.60	10.07	-	8.32	-	-	-	-	10.44	8.21	8.59	13.49

ABW- American bollworm; **SBW**- Spotted bollworm; **TC**- Tobacco caterpillar; **PBW**-Pink bollworm; **NBt**-Non-Bt; **FD**- Flower damage.

Table 4. Observation on cotton bollworms and their damage in different treatments at 140 days after sowing (2021-2023)

Tr. No.	Treatments	Square damage ABW/SBW/TC		*Helicoverpa* larvae/ 5 plant		% FD PBW (Rossette flowers/50 flowers)		Green boll damage (%) (ABW/SBW/TC)		Boll damage (%) from 50 green bolls due to PBW		PBW larval recovery from 10 green bolls	
		Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T_1	Pure Bt 100 %	0.00 (0.71)	-	0.00 (0.71)	-	0.00 (0.71)	-	0.00 (0.71)	0.00 (0.71)	33.82 (35.54)	-	17.03 (4.19)	-
T_2	Pure NBt 100 %	-	0.00 (0.71)	-	1.18 (1.30)	-	0.00 (0.71)	-	-	-	39.27 (38.79)	-	26.65 (5.21)
T_3	Structured 20 % NBt	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.08 (1.25)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	36.53 (37.17)	37.72 (37.88)	25.14 (5.06)	22.69 (4.82)
T_4	RIB commercial	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.97 (1.21)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	33.19 (35.16)	32.97 (35.03)	16.91 (4.17)	18.34 (4.34)
T_5	5 % RIB	0.00 (0.71)	0.00(0.71)	0.00 (0.71)	0.93 (1.19)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	32.89 (34.98)	31.60 (34.19)	17.19 (4.21)	17.72 (4.27)
T_6	10 % RIB	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.01 (1.23)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	35.72 (36.69)	34.15 (35.74)	17.09 (4.19)	18.80 (4.39)
	SEm	-	-	-	0.05	-	-	-	-	2.41	1.94	0.14	0.19
	CD (%)	-	-	-	NS	-	-	-	-	7.44	6.02	0.52	0.68
	CV	-	-	-	10.76	-	-	-	-	13.82	11.03	8.13	10.03

ABW- American bollworm; **SBW**- Spotted bollworm; **TC**- Tobacco caterpillar; **PBW**-Pink bollworm; **NBt**-Non-Bt; **FD**- Flower damage.

Table 5. Observation on yield parameters and yield of cotton in different treatments (2021-2023)

Tr. No.	Treatment	Open Boll damage (%)		% Locule damage		Yield (q/ha.)
		Bt	NBt*	Bt	NBt*	
T_1	Pure Bt 100 %	18.88 (25.74)	-	19.32 (26.06)	-	13.45
T_2	Pure NBt 100 %	-	38.44 (38.30)	-	44.49 (41.82)	6.62
T_3	Structured 20 % NBt	25.21 (30.13)	33.07 (35.09)	31.13 (33.90)	36.82 (37.34)	10.27
T_4	RIB commercial	21.18 (27.39)	27.07 (31.34)	24.14 (29.41)	29.95 (33.16)	11.78
T_5	5 % RIB	20.52 (26.92)	25.45 (30.28)	22.75 (28.48)	28.98 (32.56)	12.37
T_6	10 % RIB	22.67 (28.42)	28.98 (32.56)	25.13 (30.07)	31.30 (34.01)	11.69
	SEm	0.66	0.32	0.38	1.60	0.81
	CD (%)	2.04	1.01	1.19	4.95	2.45
	CV	4.77	5.65	2.61	9.01	14.74

*Non- Bt plants.

Discussion

Globally, there are few studies on the effect of different proportions of RIB on PBW incidence. The present findings exhibited that as the cotton crop stage advanced from 80 to 140 days after sowing, the prevalence of PBW also enhanced in both Bt and non-Bt. As documented, PBW has already acquired resistance to Bt toxin since 2018 and further, it can damage and survive both on Bt and non-Bt plants. While the populace of other bollworms was negligible in Bt, their incidence was noticed in non-Bt. Extensively, there are limited studies on the effect of different proportions of RIB on PBW incidence. Our findings align with prior work and support the adoption of new concepts, such as RIB (12). However, this strategy may only slow down the development of resistance in emerging populations, including other bollworms such as *Helicoverpa armigera*, due to the presence of natural refuges and their polyphagous nature. Whereas *Helicoverpa* incidence, square damage, and green boll damage were on non-Bt cotton. However, PBW infestation and green boll damage were seen in all blocks for both Bt and non-Bt plants (13). Earlier findings also state that maintaining 'RIB' for extending the susceptibility of bollworms towards cry protein (17, 18).

To impede the threat of Bt resistance in lepidopteran larvae, it's better to retain a susceptible population by cultivation of a refuge crop strategy. Refuge crops limit the intensity of selection on the target pest and enhance the life span of Bt cotton/ transgenic technology. Suggestions that growing of non-Bt as a refuge crop will be beneficial to minimize the Bt resistance problem in *Pectinophora gossypiella* (19). The field populations of PBW have already developed resistance to both cry genes (20), and it is also put forward that non-Bt cotton as a refuge crop should be cultivated on a large scale and integrated with Bt cotton as a management tactic (21).

Conclusion

In a nutshell, there was zero incidence and damage of *Helicoverpa* on Bt plants across the different RIB and structured refugia treatments. In contrast, *Helicoverpa* incidence, square damage, and green boll damage were observed on non-Bt plants in the same treatments. Pink bollworm infestation and green boll damage occurred in all treatments, emphasizing the need to maintain RIB to prolong bollworm susceptibility to cry proteins. Pink bollworm infestation and green boll damage occurred in all treatments, emphasizing the need to maintain RIB to prolong bollworm susceptibility to cry proteins. Cry proteins remain effective against American and spotted bollworms, despite PBW resistance. Refugia-in-bag not only ensures refuge compliance but also supports sustainable Bt resistance management. Future research should focus on evaluating novel cry traits, monitoring resistance genes in pink bollworm, and integrating natural enemies to strengthen IPM strategies. These approaches will enhance the durability and effectiveness of Bt cotton in controlling major bollworm pests.

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Authors' contributions

This study was conducted by PVM and PMH. PMH and PVM prepared the manuscript. RK provided technical guidance for the smooth conduct of the experiment. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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