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Comparison and Evaluation of Bagged and Structured Refugia against Cotton Bollworms

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Authors' contributions

This work was carried out in collaboration among all authors. Author SGH conceptualized, planned, designed, supervised the entire research work and checked the final draft. Author Shudeer executed the experiment, data collected, statistically analysed, interpreted the results, draft the original manuscript. Author JMN writing Review. Author AM managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluation the Refugia in bag and structured refugia against bollworm complex in cotton.
Study of Design: *Bt* cotton hybrid (KCH-14K59 BG II) and its non *Bt* counterpart was planted, following recommended agronomic practices. Six treatments were designed for the studies are

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detailed below: T_1-100 % *Bt*, T_2-100 % Non *Bt*, T_3-20 % structured refugia, T_4- RIB-Random (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).

Place and Duration of Study: Department of Entomology, College of Agriculture and Main Agricultural Research Station, Raichur, during 2021-22.

Methodology: Observations regarding infestation of cotton bolls by different bollworm complex at 60, 80, 100, 120 and 140 days after sowing (DAS) were recorded along with observation on yield parameters of cotton. For pink bollworm, cotton bolls were randomly collected from the respective treatments, as described in cotton pest scouting method and put them in laboratory for further counting of PBW larvae.

Results: The results in commercial RIB, fixed 5% RIB and Fixed 10% RIB were observed at par with respect to good boll opening (GBO), Bad boll opening (BBO) and locule damage among the all treatments while highest seed cotton yield was observed in T_1 (21.64 q/ha), followed by T_4 (17.04 q/ha) and T_5 (16.74 q/ha), with the least yield recorded in T_2 (4.23 q/ha).

Conclusion: There was no infes of *Helicoverpa* on *Bt* cotton in the different treatments of refugia in bag and structured refugia, However, the pink bollworm incidence and green boll damage caused by PBW was recorded in all the treatments of both *Bt* and non *Bt* cotton.

Keywords: Bt cotton; refugia- in- bag; bollworms; resistance.

1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.), a member of the Malvaceae family, is a vital crop in 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 per cent of the world's total fibre production and contributes to 10 per cent of global edible oil production [1]. India leads in cotton cultivation globally, covering over 120.69 lakh hectares and producing 340.62 lakh bales annually, with an average productivity of 469 kg/ha [2]. In Karnataka alone, cotton is grown on 8.97 lakh hectares, yielding 21.48 lakh bales with a productivity of 407 kg/ha [3].

Since the commercialization of genetically engineered (GE) crops in the United States in 1996, their adoption has surged worldwide. Among these, *Bt* crops-those expressing insecticidal genes from the soil bacterium *Bacillus thuringiensis*-stand out. These crops produce *Cry*1, *Cry*2, *Cry*3, or *Vip*3A proteins to combat Lepidoptera and Coleoptera pests [4]. 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 required to plant 20 per cent 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. This neglect resulted in short-term gains but caused the pink bollworm (PBW) to develop resistance to single-gene *Bt* cotton by 2010 and to Bollgard II by 2015 [5].

Bt crops have enabled farmers to manage agricultural pests more effectively and safely, reducing the need for insecticide applications and benefiting environmental and human health. However, the long-term cultivation of *Bt* crops can lead to *Bt* resistance in target pests, posing a significant threat to the sustainability of *Bt* technology [6,7,8]. To address this, the Ministry of Agriculture and Farmers Welfare, Government of India, issued guidelines in 2016 for the implementation of a Refugia in Bag (RIB) strategy starting in 2017. *Bt* cotton seed packs must now contain 90-95 per cent *Bt* seeds and 5-10 per cent non-*Bt* refuge seeds [9]. The refuge seeds are non-*Bt* hybrids that are isogenic versions of the *Bt* hybrids or have similar flowering and fibre traits. This compliance-assured RIB approach ensures that refuge seeds are indistinguishably blended with *Bt* seeds, as opposed to being provided in separate packets (Mohan and Sadananda, 2019).

The success of sustaining *Bt* cotton technology under the RIB directive relies on the quality stewardship of all stakeholders. High standards in refuge seed production are essential to meet key requirements, such as matching bloom and boll-setting periods with *Bt* hybrids (Mohan and Sadananda, 2019). To evaluate the effectiveness of refugia in bag and structured refugia against the bollworm complex in cotton, an experiment was conducted at the Department of Entomology, College of Agriculture and Main

Agricultural Research Station, Raichur. This study aims to evaluate the best practices for managing *Bt* resistance against cotton bollworms that may sustain *Bt* protein in cotton crop for more efficient against targeted pests.

2. MATERIALS AND METHODS

A field experiment was conducted at the Department of Entomology, College of Agriculture and Main Agricultural Research Station, Raichur, during 2021-22 to evaluate refugia in bag and structured refugia against bollworm complex in cotton. *Bt* cotton hybrid (KCH-14K59 BG II) and its non *Bt* counterpart was planted, following recommended agronomic practices. Six treatments were designed for the studies are detailed below: T_1-100 % *Bt*, T_2-100 % Non *Bt*, T_3-20 % structured refugia, T_4- RIB-Random (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).

Sowing was performed as per 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 ETL level. Observations regarding infestation of cotton bolls by different bollworm complex at 60, 80, 100, 120 and 140 DAS was recorded along with observation on yield parameters and yield of cotton. For pink bollworm, cotton bolls were randomly collected from the respective treatments, and destructive sampling was done in the laboratory to count the number of larvae. The results were subjected to DMRT for statistical analysis with SPSS version 16.0 software.

3. RESULTS AND DISCUSSION

3.1 Cotton Bollworms and Its Damage in Different Treatments at 60 Days after Sowing

Helicoverpa larvae were not observed in any of the six treatments on the *Bt* cotton. In contrast, the highest number of larvae (1.37) was observed in T_2 on non-*Bt* crops, followed by T_3 (1.17), and the least in T_4 (0.27). Similarly, no *Earias* larvae were observed in any *Bt* crop treatment. The highest number of larvae (0.20) was observed in the non-*Bt* crop in T_2 and T_3, and the lowest (0.10) in T_4, T_5, and T_6 (Table 1).

Pink bollworm larvae were not observed on *Bt* or non-*Bt* cotton because cotton typically starts producing squares after 50-55 DAS. No square damage was observed in *Bt* cotton due to pink bollworm, whereas the highest square damage was observed in T_2 (11.57), followed by T_3 (11.27), and the lowest in T_4 (9.87) for non-*Bt* cotton. Furthermore, no green boll damage caused by pink bollworm was observed in either *Bt* or non-*Bt* crops because the cotton plants had not produced bolls yet, as flowering begins 20-25 days after the squaring stage (Table 1).

3.2 Cotton Bollworms and Its Damage in Different Treatments at 80 Days after Sowing

No *Helicoverpa* larvae were observed on *Bt* cotton across all treatments. In contrast, the non-*Bt* cotton treatment T_2 exhibited the highest number of larvae (1.87), followed by T_3 (1.67), with the lowest counts in T_5 and T_6 (0.67 each). Similarly, no *Erias* larvae were detected in any *Bt* crop treatments, whereas in non-*Bt* crops, T_2 had the highest larval count (0.80), followed by T_3 (0.70), and the lowest in T_4 and T_5 (0.40 each) (Table 2).

An increased infestation of pink bollworm was noted after 80 DAS. In *Bt* cotton, T_6 recorded the highest number of larvae (0.27), with the lowest observed in T_3 (0.07). Conversely, in non-*Bt* cotton, T_2 and T_3 both had the highest larval count (0.37). Treatments T_4, T_5, and T_6 exhibited no square damage from pink bollworm in *Bt* cotton. In non-*Bt* crops, T_2 showed the highest square damage (15.57), followed by T_3 (14.96), and the lowest in T_4 (13.75).

Regarding green boll damage, *Bt* cotton treatment T_3 recorded the highest damage (22.34), followed by T_1 (19.56), with T_6 showing the least damage (18.65). In non-*Bt* cotton, T_2 exhibited the highest green boll damage (42.52), followed by T_3 (40.12), and the lowest in T_4 (39.56) (Table 2).

3.3 Cotton Bollworms and Its Damage in Different Treatments at 100 Days after Sowing

In *Bt* cotton, no *Helicoverpa* or *Erias* larvae were observed in any of the treatments. In contrast, in non-*Bt* cotton, the highest *Helicoverpa* larval infestation was recorded in T_3 (2.17), followed by T_2 (2.07), with the lowest in T_4 (0.67). *Erias* larval infestation was highest in T_3 (1.20), followed by T_2 (1.10), and was lowest in T_4 and T_5 (0.70) (Table 3).

Table 1. Observation on cotton bollworms and its damage in different treatments at 60 days after sowing

Treatments	At 60 days after sowing									
	***Helicoverpa* (No. of larvae/10 plants) ***		***Erias* (No. of larvae/10 plants) ***		**Pink bollworm (No. of larvae/10 bolls) ***		**Square damage (%) ****		**Green boll damage (%) ****	
	Bt	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***
T_1: Pure *Bt*	0.00 (0.71)	--	0.00 (0.71)	--	0.00 (0.71)	--	0.00 (0.00)	--	0.00 (0.00)	--
T_2: Pure N *Bt*	--	1.37 (1.37)	--	0.20 (0.84)	--	0.00 (0.71)	--	11.57 (19.88)	--	0.00 (0.00)
T_3: Structured N *Bt*	0.00 (0.71)	1.17 (1.29)	0.00 (0.71)	0.20 (0.84)	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)	11.27 (19.61)	0.00 (0.00)	0.00 (0.00)
T_4: RIB Commercial	0.00 (0.71)	0.27 (0.88)	0.00 (0.71)	0.10 (0.77)	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)	9.87 (18.31)	0.00 (0.00)	0.00 (0.00)
T_5: 5 % RIB	0.00 (0.71)	0.37 (0.93)	0.00 (0.71)	0.10 (0.77)	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)	9.97 (18.40)	0.00 (0.00)	0.00 (0.00)
T_6: 10 % RIB	0.00 (0.71)	0.47 (0.98)	0.00 (0.71)	0.10 (0.77)	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)	10.17 (18.59)	0.00 (0.00)	0.00 (0.00)
S. Em (±)	**NS**	**0.08**	**NS**	**0.07**	**NS**	**NS**	**NS**	**0.28**	**NS**	**NS**
CD (%)		**0.24**		**0.21**				**0.84**		

Table 2. Observation on cotton bollworms and its damage in different treatments at 80 days after sowing

Treatments	At 80 days after sowing									
	***Helicoverpa* (No. of larvae/10 plants) ***		***Erias* (No. of larvae/10 plants) ***		**Pink bollworm (No. of larvae/10 bolls) ***		**Square damage (%) ****		**Green boll damage (%) ****	
	Bt	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***
T_1: Pure *Bt*	0.00 (0.71)	--	0.00 0.71	--	0.17 (0.82)	--	0.00 (0.00)	--	19.56 (26.25)	--
T_2: Pure N *Bt*	--	1.87 (1.54)	--	0.80 (1.14)	--	0.37 (0.93)	--	15.57 (23.24)	--	42.52 (40.70)
T_3: Structured N *Bt*	0.00 (0.71)	1.67 (1.47)	0.00 (0.71)	0.70 (1.10)	0.07 (0.75)	0.37 (0.93)	0.00 (0.00)	14.96 (22.76)	22.34 (28.20)	40.12 (39.30)
T_4: RIB Commercial	0.00 (0.71)	0.77 (1.13)	0.00 (0.71)	0.40 (0.95)	0.17 (0.82)	0.27 (0.88)	0.00 (0.00)	13.75 (21.76)	18.94 (25.80)	39.56 (38.97)
T_5: 5 % RIB	0.00 (0.71)	0.67 (1.08)	0.00 (0.71)	0.40 (0.95)	0.17 (0.82)	0.27 (0.88)	0.00 (0.00)	13.87 (21.86)	19.46 (26.18)	39.84 (39.14)
T_6: 10 % RIB	0.00 (0.71)	0.67 (1.08)	0.00 (0.71)	0.50 (1.00)	0.27 (0.88)	0.27 (0.88)	0.00 (0.00)	14.12 (22.07)	18.65 (25.59)	39.68 (39.04)
S. Em (±)	**NS**	**0.11**	**NS**	**0.03**	**0.06**	**0.04**	**NS**	**0.21**	**0.96**	**0.71**
CD (%)		**0.32**		**0.09**	**0.18**	**0.12**		**0.63**	**2.89**	**2.14**

Table 3. Observation on cotton bollworms and its damage in different treatments at 100 days after sowing

Treatments	At 100 days after sowing									
	***Helicoverpa* (No. of larvae/10 plants) ***		***Erias* (No. of larvae/10 plants) ***		**Pink bollworm (No. of larvae/10 bolls) ***		**square damage (%) ****		**Green boll damage (%) ****	
	Bt	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***
T_1: Pure *Bt*	0.00 (0.71)	--	0.00 (0.71)	--	0.23 (0.85)	--	0.00 (0.00)	--	25.13 (30.09)	--
T_2: Pure N *Bt*	--	2.07 (1.60)	--	1.10 (1.26)	--	0.47 (0.98)	--	19.57 (6.02)	--	84.36 (66.70)
T_3: Structured N *Bt*	0.00 (0.71)	2.17 (1.63)	0.00 (0.71)	1.20 (1.30)	0.17 (0.82)	0.57 (1.03)	0.00 (0.00)	19.07 (6.29)	24.96 (29.97)	86.48 (68.43)
T_4: RIB Commercial	0.00 (0.71)	0.67 (1.08)	0.00 (0.71)	0.70 (1.10)	0.27 (0.88)	0.47 (0.98)	0.00 (0.00)	15.17 (4.80)	25.68 (30.45)	85.56 (67.67)
T_5: 5 % RIB	0.00 (0.71)	0.77 (1.13)	0.00 (0.71)	0.70 (1.10)	0.17 (0.82)	0.57 (1.03)	0.00 (0.00)	15.07 (4.80)	24.85 (29.90)	86.12 (68.13)
T_6: 10 % RIB	0.00 (0.71)	0.87 (1.17)	0.00 (0.71)	0.80 (1.14)	0.27 (0.88)	0.57 (1.03)	0.00 (0.00)	16.57 (5.13)	24.24 (29.49)	86.46 (68.41)
S. Em (±)	**NS**	**0.12**	**NS**	**0.03**	**0.09**	**0.07**	**NS**	**0.23**	**0.48**	**0.62**
CD (%)		**0.36**		**0.09**	**0.27**	**0.23**		**0.68**	**1.45**	**1.86**

Table 4. Observation on cotton bollworms and its damage in different treatments at 120 days after sowing

Treatments	At 120 days after sowing									
	***Helicoverpa* (No. of larvae/10 plants) ***		***Erias* (No. of larvae/10 plants) ***		**Pink bollworm (No. of larvae/10 bolls) ***		**square damage (%) ****		**Green boll damage (%) ****	
	Bt	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***
T_1: Pure *Bt*	0.00 (0.71)	--	0.00 (0.71)	--	0.33 (0.91)	--	0.00 (0.00)	--	36.45 (37.14)	--
T_2: Pure N *Bt*	--	0.77 (1.13)	--	0.40 (0.95)	--	0.77 (1.13)	--	20.47 (26.90)	--	83.14 (65.76)
T_3: Structured N *Bt*	0.00 (0.71)	0.87 (1.17)	0.00 (0.71)	1.60 (1.45)	0.27 (0.88)	0.87 (1.17)	0.00 (0.00)	19.67 (26.33)	35.48 (36.56)	82.64 (65.38)
T_4: RIB Commercial	0.00 (0.71)	0.47 (0.98)	0.00 (0.71)	0.20 (0.84)	0.27 (0.88)	0.77 (1.13)	0.00 (0.00)	17.17 (24.48)	34.16 (35.77)	80.67 (63.92)
T_5: 5 % RIB	0.00 (0.71)	0.17 (0.82)	0.00 (0.71)	0.30 (0.89)	0.27 (0.88)	0.67 (1.08)	0.00 (0.00)	16.67 (24.09)	34.85 (36.18)	81.64 (64.63)
T_6: 10 % RIB	0.00 (0.71)	0.47 (0.98)	0.00 (0.71)	0.40 (0.95)	0.37 (0.93)	0.87 (1.17)	0.00 (0.00)	17.77 (24.93)	36.82 (37.36)	82.46 (65.24)
S. Em (±)	**NS**	**0.04**	**NS**	**0.06**	**0.07**	**0.08**	**NS**	**0.37**	**0.71**	**0.78**
CD (%)		**0.12**		**0.18**	**0.21**	**0.24**		**1.12**	**2.14**	**2.35**

Pink bollworm infestation in *Bt* cotton was highest in T_4 and T_6 (0.27) and lowest in T_3 and T_5 (0.17). In non-*Bt* cotton, the highest pink bollworm infestation was recorded in T_3, T_5, and T_6 (0.57), with the lowest in T_2 and T_4 (0.47). No square damage due to pink bollworm was noticed in *Bt* cotton. However, in non-*Bt* cotton, T_2 (19.57) had the highest square damage, followed by T_3 (19.07), with the lowest in T_5 (15.07) (Table 3).

Regarding green boll damage due to pink bollworm, *Bt* cotton recorded the highest damage in T_4 (25.68), followed by T_1 (25.13), with the lowest in T_6 (24.24). In non-*Bt* cotton, T_3 (86.48) had the highest green boll damage, followed by T_6 (86.46), with the lowest in T_2 (84.36) (Table 3).

3.4 Cotton Bollworms and Its Damage in Different Treatments at 120 Days after Sowing

There were no *Helicoverpa* and *Erias* larvae observed in any of the treatments in *Bt* cotton. In non-*Bt* cotton, T_3(0.87) recorded the highest larval infestation of *Helicoverpa* larvae, followed by T_2(0.77) and the lowest infestation was observed in T_5(0.17). The highest *Erias* larval infestation was observed in T_3(1.60), while the lowest was in T_4(0.20) (Table 4).

In *Bt* cotton, the highest pink bollworm infestation was observed in T_6(0.37), followed by T_1(0.33), and the lowest infestation was recorded in T_3, T_4, and T_5, each with 0.27 larvae. In non-*Bt* cotton, the highest pink bollworm infestation was observed in T_3 and T_4, each with 0.87 larvae, while the lowest was in T_5(0.67). There was no square damage noticed in *Bt* cotton due to pink bollworm. In non-*Bt* cotton, the highest square damage was observed in T_2 (20.47) followed by T_3(19.67) and the lowest in T_5(16.67). Green boll damage due to pink bollworm in *Bt* cotton was highest in T_6(36.82) and lowest in T_4(34.16). In non-*Bt* cotton, T_2 recorded the highest green boll damage (83.14), followed by T_3(82.64), and the lowest was in T_4(80.67) (Table 4).

3.5 Cotton Bollworms and Its Damage in Different Treatments at 140 Days after Sowing

In *Bt* cotton, no *Helicoverpa* and *Erias* larvae were observed. In non-*Bt* cotton, T_3(0.27) recorded the highest infestation by *Helicoverpa* followed by T_2, T_4 and T_6 with 0.17 each, while the least was observed in T_5 (0.07). With respect to Erias, T_2 recorded the highest larval infestation, and the least was observed in T_4, T_5, and T_6 (0.10) (Table 5).

In *Bt* cotton, T_6 (0.57) recorded the highest pink bollworm larval infestation, followed by T_1 and T_3 (0.47), with the least observed in T_4 and T_5 (0.37). In non-*Bt* cotton, T_2 (1.17) recorded the highest larval infestation, while the least was observed in T_4 (0.77). No square damage due to pink bollworm was noticed in *Bt* cotton. In non-*Bt* cotton, T_2 (7.85) recorded the highest square damage due to pink bollworm followed by T_3(7.77), while the least was observed in T_5 (6.87). T_4 (45.85) recorded the highest green boll damage due to pink bollworm, with the least noticed in T_3 (44.36). In non-*Bt* cotton, T_6 (95.48) recorded the highest green boll damage due to pink bollworm, and the lowest was observed in T_4 (93.85) (Table 5).

3.6 Yield Parameters

In *Bt* cotton, the number of GOB was highest in T_1 (41.50/plant), followed by T_5 (39.80/plant) and T_6 (39.20/plant) and least was observed in T_3(37.50). In non-*Bt* cotton, the highest number of GOB was observed in T_4 (4.20/plant), followed by T_5 (4.00/plant) and T_6 (3.80/plant), with the least observed in T_2 (2.40/plant). In *Bt* cotton, the highest number of BOB was noticed in T_6 (2.40/plant), followed by T_3 and T_4 with similar counts (2.30/plant), and the least observed in T_1 and T_5 (2.10/plant). In non-*Bt* cotton, the highest number of BOB was observed in T_2 (31.80/plant), followed closely by T_4 (26.10/plant), T_3 (26.20/plant), and T_6 (26.30/plant), with the least observed in T_5 (25.80/plant). The highest number of LD in *Bt* cotton was observed in T_1 (6.34), and the least in T_5 (5.01). In non-*Bt* cotton, T_2 (94.24) recorded the highest number of LD, while the least was observed in T_4 (86.13) and T_5 (86.17), with no significant difference between them (Table 6).

3.7 Seed Cotton Yield

The highest seed cotton yield was observed in T_1 (21.64 q/ha), followed closely by T_4 (17.04 q/ha) and T_5 (16.74 q/ha), with the least yield recorded in T_2 (4.23 q/ha) (Table 6).

Table 5. Observation on cotton bollworms and its damage in different treatments at 140 days after sowing

Treatments	At 140 days after sowing									
	Helicoverpa (No. of larvae/10 plants) *		*Erias* (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		square damage (%) **		Green boll damage (%) **	
	Bt	Non-*Bt*	*Bt*	Non-*Bt*	*Bt*	Non-*Bt*	*Bt*	Non-*Bt*	*Bt*	Non-*Bt*
T_1: Pure *Bt*	0.00 (0.71)	--	0.00 (0.71)	--	0.47 (0.98)	--	0.00 (0.00)	--	45.67 (42.52)	--
T_2: Pure N *Bt*	--	0.17 (0.82)	--	0.40 (0.95)	--	1.17 (1.29)	--	7.85 (16.27)	--	95.26 (77.43)
T_3: Structured N *Bt*	0.00 (0.71)	0.27 (0.88)	0.00 (0.71)	0.30 (0.89)	0.47 (0.98)	1.07 (1.25)	0.00 (0.00)	7.77 (16.18)	44.36 (41.76)	94.18 (76.04)
T_4: RIB Commercial	0.00 (0.71)	0.17 (0.82)	0.00 (0.71)	0.10 (0.77)	0.37 (0.93)	0.77 (1.13)	0.00 (0.00)	7.07 (15.42)	45.85 (42.62)	93.85 (75.64)
T_5: 5 % RIB	0.00 (0.71)	0.07 (0.75)	0.00 (0.71)	0.10 (0.77)	0.37 (0.93)	0.87 (1.17)	0.00 (0.00)	6.87 (15.19)	44.48 (41.83)	94.56 (76.51)
T_6: 10 % RIB	0.00 (0.71)	0.17 (0.82)	0.00 (0.71)	0.10 (0.77)	0.57 (1.03)	0.97 (1.21)	0.00 (0.00)	7.17 (15.53)	46.25 (42.85)	95.48 (77.73)
S. Em (±)		**0.06**		**0.03**	**0.07**	**0.09**		**0.19**	**0.82**	**0.89**
CD (%)		**0.19**		**0.09**	**0.22**	**0.28**		**0.57**	**2.45**	**2.68**

Table 6. Observation on yield parameters and yield of cotton in different treatments

Treatments	GOB* (Numbers/plant)		BOB* (Numbers/plant)		LD ** (%)		Yield* (q/ha)
	Bt	Non-*Bt*	*Bt*	Non-*Bt*	*Bt*	Non-*Bt*	
T_1: Pure *Bt*	41.50 (6.48)	--	2.10 (1.61)	--	6.34 (14.58)	--	21.64 (4.71)
T_2: Pure N *Bt*	--	2.40 (1.70)	--	31.80 (5.68)	--	94.24 (76.11)	4.23 (2.17)
T_3: Structured N *Bt*	37.50 (6.16)	3.70 (2.05)	2.30 (1.67)	26.20 (5.17)	5.77 (13.90)	87.62 (69.40)	14.61 (3.89)
T_4: RIB Commercial	38.60 (6.25)	4.20 (2.17)	2.30 (1.67)	26.10 (5.16)	5.48 (13.54)	86.13 (68.13)	17.04 (4.19)
T_5: 5 % RIB	39.80 (6.35)	4.00 (2.12)	2.10 (1.61)	25.80 (5.13)	5.01 (12.93)	86.17 (68.17)	16.74 (4.15)
T_6: 10 % RIB	39.20 (6.30)	3.80 (2.07)	2.40 (1.70)	26.30 (5.18)	5.64 (13.74)	87.37 (69.18)	15.80 (4.04)
S. Em (±)	**0.13**	**0.09**	**0.11**	**0.16**	**0.81**	**0.89**	**0.15**
CD (%)	**0.39**	**0.27**	**0.34**	**0.48**	**2.43**	**2.68**	**0.45**

GOB- Good opened bolls, BOB- Bad opened bolls, LD- Locule damage

There was no *Helicoverpa* as well as *Erias* infestation observed in any of the treatment on *Bt* cotton including T_3 and T_4 (Table 1, 2, 3, 4 and 5) it might due to present of cry toxin present in the *Bt* cotton. Where as in non *Bt* cotton the incidence of *Helicoverpa* was observed to be highest where the population of non *Bt*-cotton was high which was observed in T_2 and T_3 (Table 1 and 2) and T_3 and T_2 (Table 3, 4 and 5). And the population of *Helicoverpa* was observed to be lowest where the non *Bt* cotton was lowest as observed in T_4 (Table 1 and 3) and T_5 (Table 2, 4 and 5). It might be due to the absence of *cry* toxin in the non *Bt* cotton or migration of *Helicoverpa* larvae from the *Bt* cotton to the non *Bt* cotton. As in the treatment where non *Bt* cotton was less, there were very a smaller number of plant available for the *Helicoverpa* larvae to infect and feed. Li et al. [10] reported the movement of *T. ni* larvae between *Bt* and non *Bt* leaves is generally unidirectional, i.e. from *Bt* leaves to non *Bt* leaves, and not vice versa.

The pink bollworm incidence was observed to be present in all the treatments on *Bt* and non *Bt* cotton, but the T_3 recorded the lowest incidence which was on par with the T_4, T_5 and T_6 (Tables 1, 2, 3, 4 and 5).

Globally, there are few studies on the effect of different proportions of RIB (refugia-in-bag) on pink bollworm incidence. Murali Mohan and Mahesh [11] suggest that field populations of pink bollworm have already developed resistance to both Cry genes. This resistance might be due to a failure in implementing resistance management strategies or issues related to the technology itself. Any corrective actions, such as introducing RIB, are considered ineffective, aligning with the present study, which found no significant difference in pink bollworm incidence among different refugia-in-bag proportions. They also noted that the necessary quantity of non-*Bt* seeds was already present in the seed packets used by farmers, raising concerns about the RIB strategy's recommendation for the deliberate inclusion of non-*Bt* seeds for resistance management. It seems that such inclusion might not delay the development of resistance and could even accelerate it, as shown by rigorously conducted studies [12].

4. CONCLUSION

In conclusion, there was no incidence of *Helicoverpa* on *Bt* cotton across the different refugia-in-bag and structured refugia treatments. No square damage was recorded in *Bt* cotton. In contrast, *Helicoverpa* incidence, square damage, and green boll damage were observed on non-*Bt* cotton in the same treatments. However, pink bollworm infestation and green boll damage were recorded in all treatments for both *Bt* and non-*Bt* cotton.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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