

***Original Research Article***  
**EFFECTIVENESS OF BAGGED AND STRUCTURED REFUGIA  
AGAINST COTTON BOLLWORMS**

Comment [ARF1]: Comparison and evaluation

## ABSTRACT

**Aims:** To evaluate 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 detailed below: T<sub>1</sub>-100 % *Bt*, T<sub>2</sub>-100 % Non *Bt*, T<sub>3</sub>-20 % structured refugia, T<sub>4</sub>. RIB-Random (5-10 % Minimal non *Bt* seeds), T<sub>5</sub>. RIB-Fixed pattern (5 % Minimal non *Bt* seeds), T<sub>6</sub>- 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) 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.

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**Results:** There was no much difference observed 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 and the while highest seed cotton yield was observed in T<sub>1</sub> (21.64 q/ha), followed closely by T<sub>4</sub> (17.04 q/ha) and T<sub>5</sub> (16.74 q/ha), with the least yield recorded in T<sub>2</sub> (4.23 q/ha).

Comment [ARF3]: as described in cotton pest scouting method and put them in laboratory for further counting of PBW larvae

Comment [ARF4]: The results

Comment [ARF5]: T<sub>1</sub> (as 1 may be subscribe formate)

**Conclusion:** There was no incidence 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.

Comment [ARF6]: Infestation

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**Key words:** *Bt* cotton, refugia- in- bag, pink bollworms, cotton yield, locule damage-resistance

## Abbreviations

(Bad opened bolls, Bad opened bolls, Locule damage, Days after sowing)

Comment [ARF8]: No need already explained in text

## 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 (Priyadarshini *et al.*, 2022). 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 (Anon., 2022a). In Karnataka alone, cotton is grown on 8.97 lakh hectares, yielding 21.48 lakh bales with a productivity of 407 kg/ha (Anon., 2022b).

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 *Cry1*, *Cry2*, *Cry3*, or *Vip3A* proteins to combat Lepidoptera and Coleoptera pests (Li *et al.*, 2016). 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 (Mohan and Sadananda, 2019).

*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. 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 (Kranthi *et al.*, 2019). 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.

Comment [ARF9]: Reference????

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. 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 provide insights into the best practices for managing *Bt* resistance against cotton bollworms and that may sustaining the benefits of *Bt* protein in cotton crop technology for more efficient against targeted pests.

Comment [ARF10]: Reference????

## 2. MATERIAL 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<sub>1</sub>.100 % *Bt*, T<sub>2</sub>-100 % Non *Bt*, T<sub>3</sub>.20 % structured refugia, T<sub>4</sub>. RIB-Random (5-10 % Minimal non *Bt* seeds), T<sub>5</sub>. RIB-Fixed pattern (5 % Minimal non *Bt* seeds), T<sub>6</sub>- 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.

### 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<sub>2</sub> on non-*Bt* crops, followed by T<sub>3</sub> (1.17), and the least in T<sub>4</sub> (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<sub>2</sub> and T<sub>3</sub>, and the lowest (0.10) in T<sub>4</sub>, T<sub>5</sub>, and T<sub>6</sub> (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<sub>2</sub> (11.57), followed by T<sub>3</sub> (11.27), and the lowest in T<sub>4</sub> (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<sub>2</sub> exhibited the highest number of larvae (1.87), followed by T<sub>3</sub> (1.67), with the lowest counts in T<sub>5</sub> and T<sub>6</sub> (0.67 each). Similarly, no *Earias* larvae were detected in any *Bt* crop treatments, whereas in non-*Bt* crops, T<sub>2</sub> had the highest larval count (0.80), followed by T<sub>3</sub> (0.70), and the lowest in T<sub>4</sub> and T<sub>5</sub> (0.40 each) (Table 2).

An increased infestation of pink bollworm was noted after 80 DAS. In *Bt* cotton, T<sub>6</sub> recorded the highest number of larvae (0.27), with the lowest observed in T<sub>3</sub> (0.07). Conversely, in non-*Bt* cotton, T<sub>2</sub> and T<sub>3</sub> both had the highest larval count (0.37). Treatments T<sub>4</sub>, T<sub>5</sub>, and T<sub>6</sub> exhibited no square damage from pink bollworm in *Bt* cotton. In non-*Bt* crops, T<sub>2</sub> showed the highest square damage (15.57), followed by T<sub>3</sub> (14.96), and the lowest in T<sub>4</sub> (13.75).

Comment [ARF 11]: Tucky 's honestly Test more accurate than DMRT

Comment [ARF 12]: Mention which programme used for analysis of data with reference

Regarding green boll damage, *Bt* cotton treatment T<sub>3</sub> recorded the highest damage (22.34), followed by T<sub>1</sub> (19.56), with T<sub>6</sub> showing the least damage (18.65). In non-*Bt* cotton, T<sub>2</sub> exhibited the highest green boll damage (42.52), followed by T<sub>3</sub> (40.12), and the lowest in T<sub>4</sub> (39.56) (Table 2).

UNDER PEER REVIEW

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

Treatments	At 60 days after sowing									
	<i>Helicoverpa</i> (No. of larvae/10 plants) *		<i>Erias</i> (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		Square damage (%) **		Green boll damage (%) **	
	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	0.00 (0.71)	--	0.00 (0.71)	--	0.00 (0.71)	--	0.00 (0.00)	--	0.00 (0.00)	--
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	1.37 (1.37)	--	0.20 (0.84)	--	0.00 (0.71)	--	11.57 (19.88)	--	0.00 (0.00)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>	<b>NS</b>	<b>0.08</b>	<b>NS</b>	<b>0.07</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>0.28</b>	<b>NS</b>	<b>NS</b>
<b>CD (%)</b>		<b>0.24</b>		<b>0.21</b>				<b>0.84</b>		

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF13]: Where is alphabets letters after stat.analysis???

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

Treatments	At 80 days after sowing									
	<i>Helicoverpa</i> (No. of larvae/10 plants) *		<i>Erias</i> (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		Square damage (%) **		Green boll damage (%) **	
	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	0.00 (0.71)	--	0.00 0.71	--	0.17 (0.82)	--	0.00 (0.00)	--	19.56 (26.25)	--
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	1.87 (1.54)	--	0.80 (1.14)	--	0.37 (0.93)	--	15.57 (23.24)	--	42.52 (40.70)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>	NS	<b>0.11</b>	NS	<b>0.03</b>	<b>0.06</b>	<b>0.04</b>	NS	<b>0.21</b>	<b>0.96</b>	<b>0.71</b>
<b>CD (%)</b>		<b>0.32</b>		<b>0.09</b>	<b>0.18</b>	<b>0.12</b>		<b>0.63</b>	<b>2.89</b>	<b>2.14</b>

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF14]: Where is alphabets letters after stat.analysis???

### 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<sub>3</sub> (2.17), followed by T<sub>2</sub> (2.07), with the lowest in T<sub>4</sub> (0.67). *Erias* larval infestation was highest in T<sub>3</sub> (1.20), followed by T<sub>2</sub> (1.10), and was lowest in T<sub>4</sub> and T<sub>5</sub> (0.70) (Table 3).

Pink bollworm infestation in *Bt* cotton was highest in T<sub>4</sub> and T<sub>6</sub> (0.27) and lowest in T<sub>3</sub> and T<sub>5</sub> (0.17). In non-*Bt* cotton, the highest pink bollworm infestation was recorded in T<sub>3</sub>, T<sub>5</sub>, and T<sub>6</sub> (0.57), with the lowest in T<sub>2</sub> and T<sub>4</sub> (0.47). No square damage due to pink bollworm was noticed in *Bt* cotton. However, in non-*Bt* cotton, T<sub>2</sub> (19.57) had the highest square damage, followed by T<sub>3</sub> (19.07), with the lowest in T<sub>5</sub> (15.07) (Table 3).

Regarding green boll damage due to pink bollworm, *Bt* cotton recorded the highest damage in T<sub>4</sub> (25.68), followed by T<sub>1</sub> (25.13), with the lowest in T<sub>6</sub> (24.24). In non-*Bt* cotton, T<sub>3</sub> (86.48) had the highest green boll damage, followed by T<sub>6</sub> (86.46), with the lowest in T<sub>2</sub> (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<sub>3</sub>(0.87) recorded the highest larval infestation of *Helicoverpa* larvae, followed by T<sub>2</sub>(0.77) and the lowest infestation was observed in T<sub>5</sub>(0.17). The highest *Erias* larval infestation was observed in T<sub>3</sub>(1.60), while the lowest was in T<sub>4</sub>(0.20) (Table 4).

In *Bt* cotton, the highest pink bollworm infestation was observed in T<sub>6</sub>(0.37), followed by T<sub>1</sub>(0.33), and the lowest infestation was recorded in T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub>, each with 0.27 larvae. In non-*Bt* cotton, the highest pink bollworm infestation was observed in T<sub>3</sub> and T<sub>4</sub>, each with 0.87 larvae, while the lowest was in T<sub>5</sub>(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<sub>2</sub> (20.47) followed by T<sub>3</sub>(19.67) and the lowest in T<sub>5</sub>(16.67). Green boll damage due to pink bollworm in *Bt* cotton was highest in T<sub>6</sub>(36.82) and lowest in T<sub>4</sub>(34.16). In non-*Bt* cotton, T<sub>2</sub> recorded the highest green boll damage (83.14), followed by T<sub>3</sub>(82.64), and the lowest was in T<sub>4</sub>(80.67) (Table 4).

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

Treatments	At 100 days after sowing									
	<i>Helicoverpa</i> (No. of larvae/10 plants) *		<i>Erias</i> (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		square damage (%) **		Green boll damage (%) **	
	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>	<i>Bt</i>	<i>Non-Bt</i>
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	0.00 (0.71)	--	0.00 (0.71)	--	0.23 (0.85)	--	0.00 (0.00)	--	25.13 (30.09)	--
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	2.07 (1.60)	--	1.10 (1.26)	--	0.47 (0.98)	--	19.57 (6.02)	--	84.36 (66.70)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>	<b>NS</b>	<b>0.12</b>	<b>NS</b>	<b>0.03</b>	<b>0.09</b>	<b>0.07</b>	<b>NS</b>	<b>0.23</b>	<b>0.48</b>	<b>0.62</b>
<b>CD (%)</b>		<b>0.36</b>		<b>0.09</b>	<b>0.27</b>	<b>0.23</b>		<b>0.68</b>	<b>1.45</b>	<b>1.86</b>

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF15]: Where is alphabets letters after stat.analysis???

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

Treatments	At 120 days after sowing									
	<i>Helicoverpa</i> (No. of larvae/10 plants) *		<i>Erias</i> (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		square damage (%) **		Green boll damage (%) **	
	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	0.00 (0.71)	--	0.00 (0.71)	--	0.33 (0.91)	--	0.00 (0.00)	--	36.45 (37.14)	--
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	0.77 (1.13)	--	0.40 (0.95)	--	0.77 (1.13)	--	20.47 (26.90)	--	83.14 (65.76)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>	<b>NS</b>	<b>0.04</b>	<b>NS</b>	<b>0.06</b>	<b>0.07</b>	<b>0.08</b>	<b>NS</b>	<b>0.37</b>	<b>0.71</b>	<b>0.78</b>
<b>CD (%)</b>		<b>0.12</b>		<b>0.18</b>	<b>0.21</b>	<b>0.24</b>		<b>1.12</b>	<b>2.14</b>	<b>2.35</b>

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF16]: Where is alphabets letters after stat.analysis???

### 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<sub>3</sub>(0.27) recorded the highest infestation by *Helicoverpa* followed by T<sub>2</sub>, T<sub>4</sub> and T<sub>6</sub> with 0.17 each, while the least was observed in T<sub>5</sub> (0.07). With respect to *Erias*, T<sub>2</sub> recorded the highest larval infestation, and the least was observed in T<sub>4</sub>, T<sub>5</sub>, and T<sub>6</sub> (0.10) (Table 5).

In *Bt* cotton, T<sub>6</sub> (0.57) recorded the highest pink bollworm larval infestation, followed by T<sub>1</sub> and T<sub>3</sub> (0.47), with the least observed in T<sub>4</sub> and T<sub>5</sub> (0.37). In non-*Bt* cotton, T<sub>2</sub> (1.17) recorded the highest larval infestation, while the least was observed in T<sub>4</sub> (0.77). No square damage due to pink bollworm was noticed in *Bt* cotton. In non-*Bt* cotton, T<sub>2</sub> (7.85) recorded the highest square damage due to pink bollworm followed by T<sub>3</sub>(7.77), while the least was observed in T<sub>5</sub> (6.87). T<sub>4</sub> (45.85) recorded the highest green boll damage due to pink bollworm, with the least noticed in T<sub>3</sub> (44.36). In non-*Bt* cotton, T<sub>6</sub> (95.48) recorded the highest green boll damage due to pink bollworm, and the lowest was observed in T<sub>4</sub> (93.85) (Table 5).

### 3.6 Yield parameters

In *Bt* cotton, the number of GOB was highest in T<sub>1</sub> (41.50/plant), followed by T<sub>5</sub> (39.80/plant) and T<sub>6</sub> (39.20/plant) and least was observed in T<sub>3</sub>(37.50). In non-*Bt* cotton, the highest number of GOB was observed in T<sub>4</sub> (4.20/plant), followed by T<sub>5</sub> (4.00/plant) and T<sub>6</sub> (3.80/plant), with the least observed in T<sub>2</sub> (2.40/plant). In *Bt* cotton, the highest number of BOB was noticed in T<sub>6</sub> (2.40/plant), followed by T<sub>3</sub> and T<sub>4</sub> with similar counts (2.30/plant), and the least observed in T<sub>1</sub> and T<sub>5</sub> (2.10/plant). In non-*Bt* cotton, the highest number of BOB was observed in T<sub>2</sub> (31.80/plant), followed closely by T<sub>4</sub> (26.10/plant), T<sub>3</sub> (26.20/plant), and T<sub>6</sub> (26.30/plant), with the least observed in T<sub>5</sub> (25.80/plant). The highest number of LD in *Bt* cotton was observed in T<sub>1</sub> (6.34), and the least in T<sub>5</sub> (5.01). In non-*Bt* cotton, T<sub>2</sub> (94.24) recorded the highest number of LD, while the least was observed in T<sub>4</sub> (86.13) and T<sub>5</sub> (86.17), with no significant difference between them (Table 6).

### 3.7 Seed cotton yield

The highest seed cotton yield was observed in T<sub>1</sub> (21.64 q/ha), followed closely by T<sub>4</sub> (17.04 q/ha) and T<sub>5</sub> (16.74 q/ha), with the least yield recorded in T<sub>2</sub> (4.23 q/ha) (Table 7).

There was no *Helicoverpa* as well as *Erias* infestation observed in any of the treatment on *Bt* cotton including T<sub>3</sub> and T<sub>4</sub> (Table 1, 2, 3, 4 and 5) it might be due to the presence of cry toxin in the *Bt* cotton. Whereas 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<sub>2</sub> and T<sub>3</sub> (Table 1 and 2) and T<sub>3</sub> and T<sub>2</sub> (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<sub>4</sub> (Table 1 and 3) and T<sub>5</sub> (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.* (2006) 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  $T_6$  (Table 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 (2022) 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.

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

Treatments	At 140 days after sowing									
	<i>Helicoverpa</i> (No. of larvae/10 plants) *		<i>Erias</i> (No. of larvae/10 plants) *		Pink bollworm (No. of larvae/10 bolls) *		square damage (%) **		Green boll damage (%) **	
	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	0.00 (0.71)	--	0.00 (0.71)	--	0.47 (0.98)	--	0.00 (0.00)	--	45.67 (42.52)	--
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	0.17 (0.82)	--	0.40 (0.95)	--	1.17 (1.29)	--	7.85 (16.27)	--	95.26 (77.43)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>		<b>0.06</b>		<b>0.03</b>	<b>0.07</b>	<b>0.09</b>		<b>0.19</b>	<b>0.82</b>	<b>0.89</b>
<b>CD (%)</b>		<b>0.19</b>		<b>0.09</b>	<b>0.22</b>	<b>0.28</b>		<b>0.57</b>	<b>2.45</b>	<b>2.68</b>

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF17]: Where is alphabets letters after stat.analysis???

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

Treatments	GOB* (Numbers/plant)		BOB* (Numbers/plant)		LD ** (%)		Yield* (q/ha)
	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	<i>Bt</i>	Non- <i>Bt</i>	
<b>T<sub>1</sub>: Pure <i>Bt</i></b>	41.50 (6.48)	--	2.10 (1.61)	--	6.34 (14.58)	--	21.64 (4.71)
<b>T<sub>2</sub>: Pure N <i>Bt</i></b>	--	2.40 (1.70)	--	31.80 (5.68)	--	94.24 (76.11)	4.23 (2.17)
<b>T<sub>3</sub>: Structured N <i>Bt</i></b>	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)
<b>T<sub>4</sub>: RIB Commercial</b>	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)
<b>T<sub>5</sub>: 5 % RIB</b>	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)
<b>T<sub>6</sub>: 10 % RIB</b>	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)
<b>S. Em (±)</b>	<b>0.13</b>	<b>0.09</b>	<b>0.11</b>	<b>0.16</b>	<b>0.81</b>	<b>0.89</b>	<b>0.15</b>
<b>CD (%)</b>	<b>0.39</b>	<b>0.27</b>	<b>0.34</b>	<b>0.48</b>	<b>2.43</b>	<b>2.68</b>	<b>0.45</b>

\* - Values in parenthesis are  $\sqrt{x+0.5}$  transformed, \*\* - Values in parenthesis are arcsine transformed

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

Means followed by same alphabet in columns did not differ significantly ( $P = .05$ ) by DMRT

Comment [ARF18]: Where is alphabets letters after stat.analysis???

#### 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 incidence and green boll damage were recorded in all treatments for both *Bt* and non-*Bt* cotton.

Comment [ARF19]: Infestation

#### CONSENT FOR PUBLICATION

Manuscript has not been published, or submitted for publication elsewhere.

#### ETHICAL APPROVAL

Not applicable

Comment [ARF20]: No need

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Comment [ARF21]: Write web link

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