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Evaluation and comparison of different refugia-in-bag (RIB) patterns against boll worm complex with special reference to pink boll worm in Bt cotton

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ABSTRACT

Field study on evaluation and comparison of different refugia-in-bag patterns (RIB) against bollworm complex with special reference to pink bollworm was conducted at RARS, Nandyal during 2021 and 2022. Among different RIB patterns evaluated pooled results of two years indicated no significant difference in green boll and locule damage due to pink bollworm among different refugia patterns. Highest yield of 1002 kg/ha was recorded in 20% structured refugia which is statistically at par with all other refugia patterns except in 100% non Bt which recorded lowest yield of 762 kg/ha. Based on foregoing results it clearly states that refugia is longer effective against pink bollworm population which had already developed resistance therefore further development of resistance can be managed in pink bollworm and can be effectively utilized for other bollworm in slowing down the building up of resistance in Bt cotton.

***Key words**: Resistance, Pink bollworm, Refugia, Refugia-in-bag.*

Introduction

In the production of genetically engineered Bt cotton (Bt for *Bacillus thuringiensis*), the planting of refuge crops (refugia) is the primary insect resistance management (IRM) strategy adopted worldwide to delay the evolution of lepidopteran insects to becoming resistant to the toxin produced by the Bt crop thus, this 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 (Mohan, 2019). While the pest had recently been declared eradicated in the USA (Tabashnik and Carrière, 2019) and had been successfully repressed in China (Tabashnik and Carrière, 2019; Wang *et al.,* 2019; Tabashnik *et al.,* 2021), 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 (Mohan, 2017; Naik *et al.,* 2018 and Fand *et al.,* 2019). 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 specifications for the crop (Mohan, 2018, 2020;

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Shukla *et al*., 2018). Equipped with genes of the Bt bacterium, Bt cotton produces endotoxins that are lethal to lepidopteran insects and thus creates inbuilt pest resistance for the Bt crop (Kathage and Qaim, 2012; Kaviraju *et al*., 2018; Naik *et al.,* 2005).

The predominant strategy for delaying evolution of pest resistance to Bt crops boosts survival of susceptible insects with "refuges" of host plants that do not produce Bt toxins, Ideally, most of the rare resistant insects emerging from Bt crops will mate with the relatively abundant susceptible insects from nearby refuges. If the dose of Bt toxin ingested by larvae is high enough to kill all or nearly all of the hybrid progeny produced by matings between susceptible and resistant insects, refuges are expected to be especially effective for delaying resistance. Retrospective evaluations of global resistance monitoring data suggest that refuges have delayed pest resistance to Bt crops, especially when the plants have met the "high dose" criterion and refuges have been abundant, Pink bollworm resistance to Bt cotton has been reported in the field in India, where farmer compliance with the refuge strategy has been low.

While the USA and China were able to prevent the Bt resistance of pink bollworm populations or even eradicate the pest altogether, in India, pink bollworms are now considered resistant to both authorized Bt cotton generations (Bollgard I and II) (Wan *et al*., 2017; Tabashnik *et al*., 2012, 2021; Tabashnik and Carrière, 2019; Mohan, 2018, 2020). In response, the Indian refuge policy has recently undergone another transformation. In 2016, the implementation of the "refuge-in-bag" (RIB) policy was endorsed and executed in 2020 (Mohan and Sadananda, 2019; Kumar *et al.,* 2021). In contrast to the "structured refuge" policy with RIB, the mandated 5 % of non-Bt cotton seeds are integrated in and blended with the Bt seed package (475 g) (Fig. 5) (Kumar *et al*., 2021). Hence, by withholding farmers from the choice of (refraining from) planting a refuge, this method is referred to as "compliance-assured" (Mohan, 2020; Kranthi *et al*., 2017). Therefore the present study was aimed at evaluation and comparison of different proportions of RIB along with structured refugia against bollworm complex in Bt cotton.

Materials and Method

An experiment was conducted to evaluate and compare different proportions of refugia-in-bag (RIB) along with structured refugia against bollworm complex in Bt cotton with particular reference to pink bollworm at RARS, Nandyal for two years of kharif 2021 and 2022.The experiment was laid down in RBD design with six treatments and four replications. Sowing was completed in first fortnight of July in both the seasons with the popular Bt hybrid jadoo and its isogenic non Bt version for refugia purpose. All the agronomic practices were adopted as prescribed by university. Green boll, open boll, locule damage along with pink bollworm larvae per twenty green bolls in each of the treatment were recorded at 90, 110 and harvest respectively, all the data was suitably converted to square root and arcsine transformation for conducting statistical analysis with LSD.

Details of treatments

S.No	Treatment	Details of treatment
1	T1	100% Bt
2	T2	100% Non Bt
3	T3	20 % Structured refugia
4	T4	RIB (random 5-10% non Bt seeds)
5	T5	Fixed pattern (5% non Bt seed)
6	T6	Fixed pattern (10% non Bt seed)

Results and Discussion

During 2021

The population of American bollworm and tobacco caterpillar were negligible in all the treatments whereas lowest larval recovery of 16.65 pink bollworm per twenty green bolls were recorded in treatment T3, (20% structured refugia) which is statistically at par with treatments T5 (5% RIB) and T6 (10% (RIB) which recorded 17.67 and 21.67 larvae per twenty green bolls respectively.

Lowest fruiting body damage of 6.65% was recorded in treatment T6, which is statistically at par with all other treatments except T2 which recorded a highest of 15.0% damage. Lowest green boll damage of 28.0% was recorded in treatment T5, which is statistically at par with all other treatments except T2 which recorded a highest of 40%.

Lowest percentage of pink bollworm larval recovery (38.0%) was recorded in treatment T5 which is statistically at par with all other treatments except T2 which recorded a highest of 51.67% .

Lowest open boll damage of 19.56% was recorded in treatment T4, which is statistically at par

with treatments T3,T5 and T6 which recorded a damage of 25.5, 23.0 and 21.64 respectively.

Lowest locule damage of 4.5% was recorded in T5, which is statistically at par with treatment T4, and T6 which recorded a damage of 5.5 and 5.3% respectively.

Highest yield of 1343kg/ha was recorded in treatment T5 which is significantly superior over all other treatments.

During 2022-23

The population of American bollworm and tobacco caterpillar were negligible in all the treatments whereas lowest pink bollworm larval recovery of 23.33% was recorded in treatment T5 fixed 5% non *Bt* refugia which is statistically at par with T4 RIB 5-

Evaluation of different refugee patterns against bollworms in cotton during 2021-22

Tr.no	SBW				ABW				PBW			
	EGG		Larva		EGG		Larva		EGG		Larva	
	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.71)*	0.00	0.00	(5.42)*	(0.71)*
T2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	32.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(1.34)	0.00	0.00	(0.71)	(5.69)
T3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.65	14.67
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.71)	0.00	0.00	(4.14)	(3.89)
T4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.0	25.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.71)	0.00	0.00	(7.99)	(4.98)
T5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.67	32.54
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.71)	0.00	0.00	(4.26)	(5.75)
T6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.67	45.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.71)	0.00	0.00	(4.69)	(6.75)
CD (5%)								0.15			0.76	0.24
SE (m)								0.05			0.24	0.08
SE (d)								0.06			0.34	0.11
CV (%)								10.33			10.06	8.50

*TV are √(x+0.5) transformed values **TV are Arc-sin transformed values

Table 12. Fruiting body damage in cotton in different refugee pattern during 2021-22

Tr.No	Fruiting body damage (%)		Rosette Flower (%)		Green boll damage (%)		PBW Larval Recovery (%)	
	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T1	6.69	0.00	0.00	0.00	35.00	0.00	40.00	0.00
	(14.91)**	(0.00)**	0.00	0.00	(36.15)**	(0.00)**	(39.23)**	(0.00)**
T2	0.00	15.00	0.00	0.00	0.00	40.00	0.00	51.67
	(0.00)	(22.73)	0.00	0.00	(0.00)	(39.23)	(0.00)	(45.99)
T3	7.50	6.67	0.00	0.00	32.0	34.00	43.0	41.67
	(15.90)	(14.77)	0.00	0.00	(34.47)	(35.15)	(41.0)	(40.12)
T4	7.18	10.33	0.00	0.00	35.0	40.00	45.0	36.67
	(15.55)	(18.76)	0.00	0.00	(36.29)	(39.23)	(42.15)	(37.28)
T5	8.52	9.67	0.00	0.00	28.0	43.33	38.0	45.00
	(16.98)	(18.12)	0.00	0.00	(31.96)	(41.15)	(38.08)	(42.11)
T6	6.65	10.00	0.00	0.00	32.0	46.67	41.0	51.67
	(14.95)	(18.43)	0.00	0.00	(34.47)	(43.11)	(39.84)	(45.98)
CD (5%)	2.75	1.80			6.66	2.18	7.62	3.27
SE (m)	0.87	0.57			2.11	0.69	2.42	1.04
SE (d)	1.23	0.81			2.99	0.98	3.42	1.47
CV (%)	10.69	26.08			11.22	18.30	12.28	23.44

*TV are √(x+0.5) transformed values **TV are Arc-sin transformed values

10% refugia which recorded a larval recovery of 30.0%

Lowest green boll damage 21.67% was recorded in treatment T5 fixed 5% refugia which is at par with treatments T6 fixed 10% refugia and T1 100% Bt which recorded a damage of 25.0 and 28.33% respectively.

Lowest open boll damage of 21.67% was recorded in treatment T1, 100 % *Bt* which is statistically at par with remaining all other treatments

Lowest locule damage of 6.0% was recorded in T5 fixed 5% refugia which is at par with remaining all the treatments

Highest yield of 1147 kg/ha was recorded in treatment T5 which is significantly superior over all other treatments.

Open boll and locule damage in cotton in different refugee pattern during 2021-22

Tr.No	Open boll damage (%)		Locule damage damage (%)		Yield
	Bt	NBt	Bt	NBt	kg/ha
T1	35.0	0.00	6.5	0.00	850
	(36.29)**	(0.00)**	(14.78)**	(0.00)**	
T2	0.00	50.00	0.00	11.00	1063
	(0.00)	(45.02)	(0.00)	(19.37)	
T3	25.5	26.67	6.00	4.67	1070
	(30.35)	(31.33)	(14.19)	(12.47)	
T4	19.56	21.67	5.5	7.00	890
	(26.26)	(27.72)	(13.57)	(15.32)	
T5	23.0	25.00	4.5	7.00	1343
	(28.67)	(29.94)	(12.25)	(15.32)	
T6	21.64	18.33	5.3	7.67	997
	(27.74)	(25.32)	(13.32)	(16.03)	
CD (5%)	4.79	2.13	1.92	0.68	236.54
SE (m)	1.52	0.68	0.61	0.22	106.34
SE (d)	2.15	0.96	0.86	0.31	75.19
CV (%)	11.84	15.62	8.64	11.60	12.58

*TV are √(x+0.5) transformed values **TV are Arc-sin transformed values

Fruiting body damage in cotton in different refugee pattern during 2022-23

Treatment	Fruiting body damage (%)		Rosette Flower (%)		Green boll damage (%)		PBW Larval Recovery (%)	
	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
T1	0.00	0.00	0.00	0.00	28.33	0.00	35.00	0.00
	(0.71)**	(0.71)**	0.00	0.00	(32.03)**	(0.00)**	(36.26)**	(0.00)**
T2	0.67	0.00	0.00	0.00	0.00	40.00	0.00	38.33
	(1.05)	(0.71)	0.00	0.00	(0.00)	(39.14)	(0.00)	(38.21)
T3	0.00	0.00	0.00	0.00	36.67	35.00	35.00	35.00
	(0.71)	(0.71)	0.00	0.00	(37.22)	(36.26)	(36.26)	(36.26)
T4	0.33	0.00	0.00	0.00	41.67	38.33	30.00	33.33
	(0.88)	(0.71)	0.00	0.00	(40.22)	(38.26)	(33.18)	(35.19)
T5	0.00	0.00	0.00	0.00	21.67	26.67	23.33	31.67
	(0.71)	(0.71)	0.00	0.00	(27.53)	(30.96)	(28.68)	(34.20)
T6	0.00	0.00	0.00	0.00	25.00	25.00	40.00	41.67
	(0.71)	(0.71)	0.00	0.00	(29.94)	(29.94)	(39.23)	(40.22)
CD (5%)	0.34				6.85	6.45	5.57	5.96
SE (m)	0.11				2.17	2.05	1.77	1.89
SE (d)	0.15				3.07	2.89	2.50	2.67
CV (%)	23.82				13.53	12.19	10.58	10.67

*TV are √(x+0.5) transformed values **TV are Arc-sin transformed values

Pooled results for the year 2021 and 2022

Two years of pooled results indicated that lowest green boll damage of 31.67% was recorded in T1 100% *Bt* which is statistically at par with remaining all other treatments. Lowest pink bollworm larval recovery of 33.3% was recorded in treatment T4 RIB 5-10% refugia which is at par with all other treatments except T6 which recorded a highest pink bollworm larval recovery of 45.83%.

Lowest open boll damage of 20.83% was recorded in treatment T1 100% Bt which is at par with remaining all other treatments, lowest locule damage of 6.50% was recorded in treatment T1 100% Bt which is at par with remaining all other treatments. Highest yield of 1002kg/ha was recorded in treatment T3 20% Structured refugia which is at par with all other treatments except T2 100% non Bt which recorded a lowest yield of 762kg/ha.

Table 9. Open boll ,locule damage and yield in cotton in different refugee pattern during 2022-23

Treatment	Open boll damage (%)		Locule damage damage (%)		Yield kg/ha
	Bt	NBt	Bt	NBt	
T1	21.67 (27.53)**	0.00 (0.00)**	7.00 (15.32)**	0.00 (0.00)**	607
T2	0.00 (0.00)	25.00 (29.94)	0.00 (0.00)	8.67 (17.11)	483
T3	26.67 (31.09)	28.33 (32.11)	7.33 (15.67)	8.00 (16.42)	940
T4	28.33 (32.03)	26.67 (31.09)	7.00 (15.32)	8.00 (16.42)	687
T5	30.00 (33.18)	25.00 (29.94)	6.67 (14.86)	7.00 (15.32)	1147
T6	26.67 (30.96)	28.33 (32.03)	6.00 (14.15)	5.67 (13.69)	980
CD (5%)	6.24	6.08	2.65	2.25	165
SE (m)	1.98	1.93	0.84	0.72	52.43
SE (d)	2.80	2.72	1.18	1.01	74.14
CV (%)	13.30	12.92	11.58	9.41	11.25

*TV are √(x+0.5) transformed values **TV are Arc-sin transformed values

Table 10. Pooled data of different refugia patterns on rosette flower, boll damage and yield in kg/ha for the years 2021 and 2022

Treatment	Rosette Flower (%)		Green boll damage (%)		PBW Larval Recovery (%)		Open boll damage (%)		Open boll Loucle damage (%)		Yield kg/ha
	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt	
T1	0.00 (0.00)**	0.00 0.00	31.67 (34.12)**	0.00 (0.00)**	37.50 (37.76)**	0.00 (0.00)**	20.83 (27.04)**	0.00 (0.00)**	6.50 (14.75)**	0.00 (0.00)**	957
T2	0.33 (2.70)	0.00 0.00	0.00 (0.00)	40.00 (39.22)	0.00 (0.00)	45.00 (42.13)	0.00 (0.00)	37.50 (37.76)	0.00 (0.00)	9.83 (18.27)	762
T3	0.00 (0.00)	0.00 0.00	35.83 (36.72)	17.50 (24.71)	38.33 (38.24)	17.50 (24.71)	21.67 (27.75)	14.17 (22.06)	6.00 (14.16)	4.00 (11.53)	1002
T4	0.17 (1.35)	0.00 0.00	40.83 (39.73)	19.17 (25.97)	33.33 (35.28)	16.67 (24.01)	25.00 (30.00)	13.33 (21.41)	7.00 (15.34)	4.00 (11.53)	788
T5	0.00 (0.00)	0.00 0.00	32.50 (34.68)	13.33 (21.30)	34.17 (35.67)	15.83 (23.40)	27.50 (31.58)	12.50 (20.65)	6.83 (15.10)	3.50 (10.77)	1000
T6	0.00 (0.00)	0.00 0.00	35.83 (36.77)	12.50 (20.65)	45.83 (42.63)	20.83 (27.16)	22.50 (28.24)	14.17 (21.99)	6.83 (15.12)	2.83 (9.63)	985
CD (5%)	2.69		6.17	3.43	4.09	4.00	4.92	4.22	2.01	1.64	231
SE (m)	0.86		1.96	1.09	1.30	1.27	1.56	1.34	0.64	0.52	73.55
SE (d)	1.21		2.76	1.54	1.83	1.79	2.20	1.89	0.90	0.73	104.01
CV (%)	19.22		11.17	8.58	7.12	9.33	11.22	12.23	8.90	8.78	13.91

Discussion

Globally there are scanty studies on effect of different proportions of RIB refugia-in-bag on pink bollworm incidence however Murali Mohan and Mahesh (2020) feels that that the field populations of PBW have already developed resistance to both Cry genes It is either failure in the implementation of resistance management strategies or an issue related to the technology that might have led to the recorded resistance. Any corrective action that is made now (like introducing RIB) holds little water which is in agreement with the present study which clearly indicated no significant difference among different refugia-in-bag proportions on pink bollworm incidence, they also opined that that the requisite quantity of non-Bt seeds was already present in the seed packets planted by the farmers, which raises concerns about the recommendations made in the RIB strategy for deliberate inclusion of non-Bt seeds for resistance management. It appears that the development of resistance might not be delayed by such deliberate inclusion. Moreover, rigorously conducted studies have shown that the RIB strategy could accelerate the rate of resistance development in target insects

Conclusion

Adoption of new concept like refugia-in-bag (RIB) in delaying the development of resistance in already developed population of pink bollworm holds little relevance in countries like India, however it may slow down the development of resistance to the new population with regard to other bollworms also like *Helicoverpa armigera* as it contains other natural refugees and polyphagous nature , non Bt cotton refugia also holds little relevance

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