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Larval Movement of *Helicoverpa armigera* (Hübner) under Mosaic or Mixed Refuge Patterns on *Bt* Cotton

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Abstract As an alternative to structured refuge concept, seed mix refuge or Refuge in Bag (RIB) may become effective strategy which is still under experimentation. Under such mosaic conditions concerns exist regarding bollworm infestation, larval movement between *Bt* and non-*Bt* plants and yield parameters in a seed mix field. Therefore an experiment was conducted at the Department of Agricultural Entomology and Main Agricultural Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka. Significantly more number of larvae were recorded on top of the canopy by registering 2.20 and 2.60 larvae in treatments T_2 (1 non *Bt* plant surrounded by 24 *Bt* plants) and T_4 (All non *Bt*), respectively. At both 24 and 48 hours after release where larvae preferred to stay in the top portion of canopy indicating least downward movement on non *Bt* plants compared to larvae released on *Bt* plants. Frequent movement, potentially within and between plants, implies that larvae may be able to detect and avoid the *Bt* toxin. These behaviors, coupled with temporal and spatial variability of *Bt* toxin expression in *Bt* cotton, can result in a proportion of the population becoming established. Non overlapping of cotton foliage at 60 DAS can also be reason for lower larval movement.

Keywords Larval movement, *Bt* cotton, *Helicoverpa*, Mixed/Mosaic refuge pattern.

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Introduction

Cotton (*Gossypium hirsutum* L.) a white gold is considered as an important fiber and commercial crop extensively grown in India and contributes 85% of raw material to textile industry and earns about 33% of total foreign exchange. Globally cotton is cultivated in an area of 29.8 million hectares with a production of 25 million tons [1] among which India ranks first in area having 11.61 million hectares with a production of 6.2 million tons. India ranks first in area and second in production after China with an average productivity of 494 kg lint ha^{-1} which is low as compared to world average of 725 kg lint ha^{-1} [2].

When *Bt* cotton was commercially released first time during 1996 in USA and other countries, it has found favorable to farmers and responsible for tremendous reduction in the overall use of insecticides against bollworm [3]. However, it is important to address the issue of probability of resistance development in target pest to *Bt* cotton, if the technology has to sustain itself for a long time.

The larvae of *Helicoverpa armigera* (Hübner) generally has a tendency to feed on same or few

adjacent plants. Plot to plot enmass larval movement generally does not occur. Only the adult moths are highly migrant and known to travel long distances of 100 m to two km. Studies on relative abundance and host preference of *H. armigera* revealed that pigeonpea and chickpea are preferred over cotton by this pest and can also act as alternative refuge. However until it is reviewed and officially altered, the prevailing recommendation for structured refuge should be followed. In fact, in China, in view of the availability of alternate host crops, growing refuge is not mandatory. However, since Pink bollworm does not have alternate hosts, it has to be considered carefully, especially in areas where this pest is endemic [4].

Due to this, a seed mix refuge interspersed with a pyramided *Bt* product is an alternative strategy that addresses the risk of growers not planting a refuge. However, concerns exist regarding how larval movement between *Bt* and non *Bt* plants might influence resistance evolution in a seed mix field. Some simulation models showed that, across a range of conditions, seed mix refugia provide an effective alternative IRM tactic for delaying resistance evolution. Under some conditions use of seed mix refugia may be a superior IRM tactic leading to longer delays to resistance, and greater durability, compared to structured refugia [5].

Materials and Methods

An experiment was carried out at the Department of Agricultural Entomology, College of Agriculture and Main Agricultural Research Station, Raichur during 2012-13 and 2013-14 to determine the movement of *H. armigera* larvae under mixed or mosaic refuge pattern of *Bt* cotton (MRC 7351 BG II) and its non *Bt* counterpart. Untreated *Bt* and non *Bt* cotton seeds were procured from M/s Mahyco-Monsanto company. Four treatments were designed for the studies are detailed below and were replicated five times using randomized block design: T_1-One *Bt* cotton plant surrounded by 24 non *Bt* cotton plants, T_2-One Non *Bt* cotton plant surrounded by 24 *Bt* cotton plants, T_3-All *Bt* cotton plants, T_4-All non *Bt* cotton plants.

Sowing was performed as per the treatments in a plot size of 24 m^2 with a spacing of 90 cm between rows and 60 cm between plants. *Helicoverpa* culture was reared in the laboratory till they attained third instar and later used for this experiment.

Rearing of *H. armigera* larvae

Helicoverpa population found on *Bt* or non *Bt* cotton and or other hosts were collected. Such collected population was reared in laboratory using artificial diet till F_1 generation under controlled atmospheric conditions having temperature and relative humidity of 26 ± 2°C and 70±5%, respectively. As the larvae attained pupal stage they were sexed separately and three pairs were kept in glass jars, which were in turn, placed in moth emergence cages having sterilized moist sand. Upon emergence, moths were provided with 10% honey solution containing yeast in cotton swabs and allowed almost for three days for copulation. Once moths started laying eggs, the one day old eggs laid on black muslin cloth were kept on previously raised bengalgram seedlings in pots and covered properly so as to avoid escape of neonate larvae. The larvae were reared on bengalgram seedlings until 2nd instar, later they were transferred to multicavity trays containing semi-synthetic diet [6] until 3rd instar. Uniform sized larvae were used for larval movement studies.

In each treatment five larvae were released at apical portion of plant located in the center of the plot surrounded by 24 plants at two intervals of 60 and 90 days after sowing. Prior to release of larvae set of treatments were enclosed with nylon mesh cage of size 5.5x3.5 m. All the plants were numbered, observations were recorded on larval movement and their distribution on the plant at different position (top, middle and bottom) and untraceable larvae were also recorded at 24, 48 and 72 h after release. Effect of larval movement on yield and boll opening was also recorded.

Bt toxin estimation

Quantification of Cry 1 Ac as well as Cry2Ab in BG II leaf, squares, bolls and seed samples was carried out using commercial ELISA kits "Quan-T plates" sup-

Table 1. Larval movement of *H. armigera* under mixed refuge conditions at 60 DAS during 2012-13 and 2013-14. *Figures in parentheses are square root transformed ($\sqrt{x + 1}$) values.

	H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 2012-13 (n = 5)											
	24 h				48 h				72 h			
Treatments	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt*+24 non *Bt*	1.40 (1.54)*	1.80 (1.66)	0.60 (1.25)	1.20 (1.46)	1.60 (1.60)	1.40 (1.54)	0.20 (1.08)	1.80 (1.67)	1.60 (1.60)	1.40 (1.54)	0.20 (1.08)	1.80 (1.67)
T_2 : 1 non *Bt*+24 *Bt*	2.20 (1.78)	1.20 (1.48)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.20 (1.48)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.20 (1.48)	0.40 (1.17)	1.20 (1.48)
T_3 : All *Bt*	2.00 (1.73)	1.20 (1.48)	0.60 (1.25)	1.20 (1.48)	2.20 (1.79)	1.20 (1.46)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.20 (1.46)	0.60 (1.23)	1.20 (1.48)
T_4 : All non *Bt*	2.60 (1.89)	1.20 (1.48)	0.40 (1.17)	1.00 (1.41)	2.60 (1.89)	1.00 (1.41)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.40 (1.54)	0.40 (1.17)	1.00 (1.41)
SEm±	0.07	0.08	0.09	0.09	0.05	0.08	0.10	0.06	0.06	0.09	0.12	0.05
CD at 0.05	0.22	0.24	0.29	0.28	0.17	0.24	0.31	0.17	0.19	0.28	0.36	0.16
CV (%)	9.01	11.53	17.14	13.78	6.88	11.84	19.81	8.29	8.01	13.50	22.20	7.92

	H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 2013-14 (n = 5)											
	24 h				48 h				72 h			
Treatments	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt*+24 non *Bt*	1.80 (1.67)*	1.80 (1.67)	0.60 (1.25)	1.80 (1.33)	2.20 (1.79)	1.60 (1.60)	0.40 (1.17)	1.40 (1.54)	2.00 (1.72)	1.80 (1.67)	0.40 (1.17)	1.20 (1.48)
T_2 : 1 non *Bt*+24 *Bt*	2.20 (1.78)	1.40 (1.54)	0.80 (1.33)	0.60 (1.25)	1.60 (1.60)	1.20 (1.48)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.20 (1.48)	0.40 (1.17)	0.60 (1.25)
T_3 : All *Bt*	1.00 (1.41)	1.60 (1.60)	1.60 (1.60)	0.80 (1.33)	2.20 (1.79)	1.20 (1.46)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.00 (1.38)	0.60 (1.25)	1.20 (1.48)
T_4 : All non *Bt*	2.20 (1.78)	1.60 (1.59)	0.40 (1.17)	0.60 (1.25)	2.60 (1.89)	1.40 (1.54)	0.40 (1.17)	0.60 (1.25)	2.40 (1.84)	1.40 (1.54)	0.40 (1.17)	0.80 (1.33)
SEm±	0.08	0.10	0.09	0.10	0.05	0.10	0.11	0.09	0.06	0.10	0.09	0.07
CD at 0.05	0.25	0.30	0.28	0.32	0.17	0.30	0.35	0.27	0.18	0.30	0.29	0.23
CV (%)	11.16	13.70	14.96	18.07	6.88	14.49	21.76	13.61	7.29	14.22	17.75	11.87

plied by M/S Desi-Gen company, Jalna, (Maharashtra). The kits for Cry 1 Ac and Cry2Ab2 were purchased separately [7].

The samples viz., leaf, squares, bolls and seed were collected in gel cool (Ice) box, carried to laboratory and stored at -20°C. The samples were lyophilized in a lyophilizer to remove moisture content completely, powdered and stored at –20°C. Exactly 15 mg of each sample was utilized for further analysis. Weighed samples were taken in eppendorf tubes by adding standard extraction buffer 500 µl and then macerated for 10 minutes. Macerated samples were subjected for centrifugation at 3000 rpm for 30 seconds, after 10 minutes, the sample was again subjected to second round of centrifugation at 8,000 rpm for 30 seconds. Then the supernatant was separated and stored at 4°C. Before loading the sample extract to plate the positive and negative standards were diluted with standard buffer (both provided in kit). Before loading, ELISA plate was washed 2-3 times with standard buffer with multi channel pipette. The standards were loaded to the plate (three each). Later test samples

Table 2. Larval movement of *H. armigera* under mixed refuge conditions at 90 DAS during 2012-13 and 2013-14. *Figures in parentheses are square root transformed ($\sqrt{x + 1}$) values.

H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 2012-13 (n = 5)

Treatments	24 h				48 h				72 h			
	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt*+24 non *Bt*	0.80 (1.33)*	2.20 (1.79)	0.80 (1.33)	1.20 (1.48)	0.40 (1.17)	2.00 (1.72)	1.40 (1.54)	1.20 (1.48)	0.20 (1.08)	1.60 (1.60)	2.20 (1.79)	1.00 (1.41)
T_2 : 1 non *Bt*+24 *Bt*	2.40 (1.84)	1.40 (1.54)	0.40 (1.17)	1.20 (1.48)	2.20 (1.79)	1.20 (1.46)	0.60 (1.25)	1.00 (1.41)	2.00 (1.73)	1.20 (1.48)	0.60 (1.25)	1.20 (1.48)
T_3 : All *Bt*	1.60 (1.60)	1.20 (1.48)	0.80 (1.33)	1.40 (1.54)	0.60 (1.25)	2.20 (1.79)	1.40 (1.54)	0.80 (1.33)	0.40 (1.17)	1.80 (1.67)	2.20 (1.79)	0.60 (1.25)
T_4 : All non *Bt*	2.40 (1.83)	1.00 (1.41)	0.60 (1.25)	1.00 (1.41)	2.00 (1.72)	1.20 (1.48)	0.80 (1.33)	1.00 (1.41)	2.00 (1.72)	1.60 (1.59)	0.60 (1.25)	0.80 (1.33)
SEm±	0.09	0.06	0.09	0.06	0.10	0.09	0.08	0.06	0.09	0.07	0.08	0.07
CD at 0.05	0.29	0.18	0.27	0.18	0.30	0.29	0.24	0.17	0.28	0.20	0.24	0.22
CV (%)	12.64	8.26	15.48	9.00	14.49	12.84	12.42	8.29	14.04	9.32	11.28	11.80

H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 2013-14 (n = 5)

Treatments	24 h				48 h				72 h			
	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt* +24 non *Bt*	1.20 (1.48)*	2.00 (1.72)	1.40 (1.54)	0.60 (1.25)	1.00 (1.39)	1.80 (1.66)	1.40 (1.54)	0.60 (1.25)	0.80 (1.33)	1.80 (1.66)	1.80 (1.67)	0.80 (1.33)
T_2 : 1 non *Bt*+24 *Bt*	2.20 (1.78)	1.40 (1.54)	0.80 (1.33)	0.40 (1.17)	2.20 (1.79)	1.60 (1.60)	0.60 (1.25)	1.20 (1.48)	2.00 (1.72)	1.60 (1.60)	0.60 (1.25)	0.60 (1.25)
T_3 : All *Bt*	1.00 (1.41)	2.00 (1.72)	1.60 (1.60)	0.60 (1.25)	0.80 (1.33)	2.20 (1.78)	1.60 (1.60)	0.40 (1.17)	0.80 (1.33)	2.40 (1.84)	1.20 (1.48)	0.60 (1.25)
T_4 : All non *Bt*	2.40 (1.84)	1.40 (1.54)	0.60 (1.25)	0.40 (1.17)	2.00 (1.72)	1.60 (1.60)	0.80 (1.33)	0.40 (1.17)	1.80 (1.67)	1.60 (1.60)	0.00 (1.41)	0.60 (1.25)
SEm±	0.08	0.08	0.10	0.12	0.09	0.11	0.10	0.09	0.09	0.10	0.06	0.09
CD at 0.05	0.23	0.23	0.29	0.36	0.29	0.33	0.29	0.28	0.27	0.30	0.18	0.29
CV (%)	10.41	10.32	14.95	21.70	13.43	14.53	14.95	16.17	13.18	12.77	8.88	16.59

(stored at 4°C) were diluted at 1:1000 with standard buffer were loaded to plate (250 µl each sample). Then the sample in the plate was washed with conjugate buffer and the plate was incubated at room temperature for 30 minutes for color development. The plate was subjected to ELISA reader and based on absorption values the quantification of Cry protein was assessed with help of regression analysis. Based on the toxin concentration in seeds, different concentrations of toxins were prepared by diluting the *Bt* cotton seed powder.

Results and Discussion

Larval movement of *H. armigera* on the *Bt* cotton canopy under mixed refuge conditions at 60 DAS

The movement and distribution of *H. armigera* was recorded for three consecutive days after releasing larvae during 2012-13 and 2013-14 year of experimentation and results of pooled analysis of two seasons data indicated significantly more number of larvae on

Table 3. Larval movement of *H. armigera* under mixed refuge conditions at 60 and 90 DAS (Pooled 2012–2013 and 2013–2014 seasons). (n=5). *Figures in parentheses are square root transformed ($\sqrt{x}$ + 1) values.

	H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 60 DAS (Pooled 2013 and 2014 season) (n = 5)											
		24 h					48 h			72 h		
Treatments	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt*+24 non *Bt*	1.60 (1.61)*	1.80 (1.67)	0.60 (1.26)	1.00 (1.41)	1.90 (1.70)	1.50 (1.58)	0.30 (1.14)	1.60 (1.61)	1.80 (1.67)	1.60 (1.61)	0.30 (1.14)	1.50 (1.58)
T_2 : 1 non *Bt*+24 *Bt*	2.20 (1.79)	1.30 (1.52)	0.60 (1.26)	0.90 (1.38)	1.90 (1.70)	1.20 (1.48)	0.40 (1.18)	1.20 (1.48)	2.20 (1.79)	1.20 (1.48)	0.40 (1.18)	0.90 (1.38)
T_3 : All *Bt*	1.50 (1.58)	1.40 (1.55)	0.10 (1.45)	1.00 (1.41)	2.20 (1.79)	1.20 (1.48)	0.40 (1.18)	1.20 (1.48)	2.20 (1.79)	1.10 (1.45)	0.60 (1.26)	1.20 (1.48)
T_4 : All non *Bt*	2.40 (1.84)	1.40 (1.55)	0.40 (1.18)	0.80 (1.34)	2.60 (1.90)	1.20 (1.48)	0.40 (1.18)	0.90 (1.38)	2.30 (1.82)	1.40 (1.55)	0.40 (1.18)	0.90 (1.38)
SEm±	0.05	0.08	0.09	0.06	0.04	0.09	0.10	0.06	0.06	0.09	0.10	0.05
CD at 0.05	0.16	0.24	0.27	0.20	0.14	0.27	0.31	0.18	0.17	0.27	0.30	0.15
CV (%)	6.82	11.19	15.06	10.48	5.68	12.91	19.14	8.81	7.17	12.95	18.81	7.64

	H. armigera larval distribution on cotton plant at different periods of observation (larvae / plant) during 90 DAS (Pooled 2013 and 2014 season) (n = 5)											
		24 h					48 h			72 h		
Treatments	Top portion	Middle portion	Bottom portion	Untraceable	Top-portion	Middle portion	Bottom portion	Untraceable	Top portion	Middle portion	Bottom portion	Untraceable
T_1 : 1 *Bt*+24 non *Bt*	1.00 (1.41)*	2.10 (1.76)	1.10 (1.45)	0.90 (1.38)	0.70 (1.30)	1.90 (1.70)	1.40 (1.55)	0.90 (1.38)	0.50 (1.22)	1.70 (1.64)	2.00 (1.73)	0.90 (1.38)
T_2 : 1 non *Bt*+24 *Bt*	2.30 (1.82)	1.40 (1.55)	0.60 (1.26)	0.80 (1.34)	2.20 (1.79)	1.40 (1.55)	0.60 (1.26)	1.10 (1.45)	2.00 (1.73)	1.40 (1.55)	0.60 (1.26)	0.90 (1.38)
T_3 : All *Bt*	1.30 (1.52)	1.60 (1.61)	1.20 (1.48)	1.00 (1.41)	0.70 (1.30)	2.20 (1.79)	1.50 (1.58)	0.60 (1.26)	0.60 (1.26)	2.10 (1.76)	1.70 (1.64)	0.60 (1.26)
T_4 : All non *Bt*	2.40 (1.84)	1.20 (1.48)	0.60 (1.26)	0.70 (1.30)	2.00 (1.73)	1.40 (1.55)	0.80 (1.34)	0.70 (1.30)	1.90 (1.70)	1.60 (1.61)	0.80 (1.34)	0.70 (1.30)
SEm±	0.06	0.05	0.08	0.05	0.08	0.08	0.07	0.05	0.04	0.06	0.05	0.06
CD at 0.05	0.20	0.17	0.26	0.16	0.23	0.24	0.21	0.14	0.14	0.19	0.17	0.19
CV (%)	8.70	7.55	13.99	8.35	11.09	10.50	10.86	7.63	6.74	8.34	8.25	10.45

top of the canopy by registering 2.20 and 2.40 larvae in treatments T_2 and T_4 (larvae released on non *Bt* plant in the center), respectively at both 24 and 48 hours after release where larvae preferred to stay in the top portion of canopy indicating least downward movement on non *Bt* plants compared to larvae released directly on *Bt* plants (Table 1–3). Frequent movement, potentially within and between plants, implies that larvae may be able to detect and avoid the *Bt* toxin. These behaviors, coupled with temporal and spatial variability of *Bt* toxin expression in *Bt* cotton, can result in a proportion of the population becoming established. Non overlapping of cotton foliage at 60 DAS can also be reason for lower larval movement (Table 3). In the middle portion of *Bt* cotton more number of larvae (1.80 and 1.50 larvae/plant) were recorded in T_1 (1 *Bt* + 24 non *Bt*) showing higher movement at 24 and 48 hours after release, respectively due to restlessness and crawling in different directions without settling at a point. As the time lapsed number of larvae recorded in bottom portion remained stagnant, however number of untraceable larvae were increase in T_1 i.e. *Bt* plant in the center by registering 1.60 and 1.50 larvae at 48 and 72 h after

Table 4. Effect of larval movement of *H. armigera* on yield parameters and yield of cotton (2012, 2013 and pooled data).

Treatments	2012-13 Yield parameters				2013-14 Yield parameters				Pooled Yield parameters			
	Good opened bolls/ plant	Bad opened bolls/ plant	Total bolls/ plant	Seed cotton yield (q/ ha)	Good opened bolls/ plant	Bad opened bolls/ plant	Total bolls/ plant	Seed cotton yield (q/ ha)	Good opened bolls/ plant	Bad opened bolls/ plant	Total bolls/ plant	Seed cotton yield (q/ ha)
T_1 : 1 *Bt*+24 non *Bt*	4.22	8.92	13.14	5.78	4.40	8.72	13.12	4.30	4.31	8.82	13.13	5.04
T_2 : 1 non *Bt*+24 *Bt*	28.20	3.12	31.32	28.74	30.00	3.42	33.42	29.21	29.10	3.27	32.37	28.97
T_3 : All *Bt*	28.94	3.24	32.18	29.63	31.44	3.44	34.88	30.42	30.19	3.34	33.53	30.02
T_4 : All non *Bt*	3.88	8.32	12.20	4.96	4.65	8.62	12.50	1.70	4.26	8.47	12..35	3.33
SEm±	0.07	0.09	0.09	0.51	0.07	0.09	0.08	0.37	0.07	0.09	0.08	0.44
CD at 0.05	0.22	0.27	0.27	1.56	0.20	0.27	0.25	1.13	0.21	0.27	0.25	1.35
CV (%)	4.16	7.60	4.27	7.28	3.86	7.60	3.94	5.13	3.93	7.60	3.94	6.21

release which may be attributed to movement of larvae away from *Bt* plant at both the intervals in search of non toxic refuge plants.

Larval movement of *H. armigera* in the cotton canopy under mixed refuge conditions at 90 DAS

The movement and distribution of *H. armigera* was recorded for three consecutive days after releasing larvae during 2012-13 and 2013-14 year of experimentation and results of pooled analysis of two seasons data indicated that top of the canopy registered 2.30 and 2.40 larvae in treatments T_2 and T_4 (non *Bt* plant in the center) at 24 hours after release and gradually larvae showed downward movement and settled on non *Bt* cotton plants. When the foliage of plants overlapped larvae could crawl from one plant to the other along the row or even across the row which can be correlated to cotton plant architecture. As the time advanced significantly, more number of larvae were settled and showed least downward movement on non *Bt* plants compared to larvae released on treatments with *Bt* plants in the center (Table 3). In the middle portion of plant more number of larvae were recorded on *Bt* plants by registering 2.10 larvae in T_1 (*Bt* plant in the center) at 24 h and 2.20 and 2.10 larvae in T_3 (all *Bt* plants) at 48 and 72 h after release showing higher movement due to wandering in different directions without settling at a point. As the time lapsed number of untraceable larvae were increased wherein, 1.00 larvae were recorded in T_3 (all *Bt* plants) at 24 h after release. Similarly, in the process of their search for non *Bt* cotton plants, showed higher movement away from *Bt* plant even at 48 and 72 h after release (Table 3).

Bommireddy [7] reported that on flowering stage cotton plants, *H. zea* and *H. virescens* were observed on approximately 35 to 40% of the terminals on Coker 312, Vip3A, and VipCot™ cotton plants at 3 h after infestation. Similarly, Li et al. [8] 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.

Yield parameters

Number of GOBs recorded were more (28.94 / plant) in T_3 because it had more number of *Bt* plants (25 in number) which differed significantly with T_2 (24 *Bt* plants) which recorded (28.20/plant) during 2012-13 season. Whereas, the bad opened bolls recorded were more in T_1 (1 *Bt* + 24 non *Bt*) and T_4 (all non *Bt*) treatments where non *Bt* plants were more registering 8.92 and 8.32 bad opened bolls respectively (Table 4). Similar trend was observed during 2013-14 season of experimentation. Pooled data of 2012 and 2013 sea-

son showed that number of GOBs recorded were more (30.19/plant) in T_3 because it had more number of *Bt* plants (25 in number) which differed significantly with T_2 (all 24 *Bt* plants) which recorded (29.10/plant). Whereas, the bad opened bolls recorded were more in T_1 and T_4 treatments where non *Bt* plants were more registering 8.82 and 8.47 bad opened bolls repectively (Table 4).

Seed cotton yield

Significantly highest yield of 29.63 and 30.42 q/ha was harvested from T_3 (all *Bt* plants) which showed no significant difference with T_2 (1 non *Bt* + 24 *Bt*) which registered 28.74 and 29.21 q/ha during 2012-13 and 2013-14, respectively. Conversely, T_1 (1 *Bt* + 24 non *Bt*) has recorded 5.78 and 4.30 q/ha and showed no significant difference with T_4 (all non *Bt*) which registered 4.96 and 1.70 q/ha during 2012-13 and 2013-14, respectively. There was sudden decrease in yield levels with increase in number of non *Bt* plants in both the years of experimentation. Pooled analysis of two years data indicated that significantly highest yield of 30.02 q/ha was harvested from T_3 (all *Bt*) which showed no significant difference with T_2 (1 non *Bt* + 24 *Bt*) by recording 28.97 q/ha. Conversely, T_1 (1 *Bt* + 24 non *Bt*) has recorded 5.04 q/ha and showed significant difference with T_4 (all non *Bt*) which registered 3.33 q/ha (Table 4). The movement of larvae to *Bt* plants from non-*Bt* plants in a mixed planting may result in a significant increase in damage and reduced yields in these plots.

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