Agric Res J **58** (5) : 808-813, *October* 2021
DOI No. 10.5958/2395-146X.2021.00115.0

OVIPOSITIONAL PREFERENCE OF TOBACCO CATERPILLAR, *Spodoptera litura* (FABRICIUS), TO DIFFERENT TRANSGENIC *Bt* COTTON CULTIVARS

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ABSTRACT

The ovipositional preference of *S. litura* on two Bollgard (Bt) cotton hybrids (Yuvraj and JKCH 1050), three Bollgard II hybirids (NCS 855, Ankur 3028 and MRC 7017) and one non Bt cotton cultivar (LH 2076) was studied on 105, 120 and 135 day old plants. Significantly higher number of *S. litura* eggs were recorded on Ankur 3028 BG II (140.77 eggs/plant) and MRC 7017 BG II (139.08 eggs/plant) as compared to NCS 855 BG II, LH 2076, Yuvraj and JKCH 1050. More number of eggs per plant was recorded at 135 day old plants (135.71 eggs/plant) as compared to 105 and 120 day old plants. The study on morphological parameters revealed significantly higher no. of trichomes on Ankur 3028 (36.26/ mm^2) and MRC 7017 (34.11 / mm^2) whereas significantly higher trichome density was observed in 135 day old plants (27.75 / mm^2). Trichomes from leaves of JKCH 1050 (730.96 µm) and Yuvraj (720.44µm) were significantly lengthier as compared to other cultivars.

Keywords: *Bt* cotton, Leaf area, Oviposition, *Spodoptera litura*, Trichomes

Spodoptera litura is a polyphagous pest damaging numerous crops in India (Shivayogeshwara *et al.,* 1991) and is a major pest in many countries in tropical regions of Asia, Africa and Australia (Srivastava, 1987). It attacks over 112 cultivated plant species, belonging to 44 families out of which about 60 species are known from India (Moussa *et al.,* 1960). Among main crop species attacked by *S. litura* are cotton, maize, rice, soybean, tea, jute, lucerne, *colocasia*, castor, cabbage, cauliflower, *Vigna sp.,* etc. Cotton occupied an area of 125.84 lakh hectares and produced approximately 360 lakh bales during 2019-20 (AICCIP, 2020). Whereas, it occupied an area of 2.63 lakh hectares and produced 12.06 lakh bales in Punjab with average yield of 779 kg/ha during 2019-20 (Anonymous, 2020). *Spodoptera* had become a major pest in India in early 80's causing yield losses up to 71 per cent (Amin, 1983) and it started appearing regularly on cotton crop in Punjab in late 90's inflicting economic damage in certain pockets (Singh *et al.,* 1999). In the absence of any effective genetic resistance against these pests, farmers relied heavily on insecticides for their effective management in cotton crop and ended up consuming insecticides worth Rs.16 billion on cotton alone (Manjunath, 2011). Bt cotton cultivars generally referred as Bollgard (Bt) contain only one gene (Cry1Ac) which offers protection against *H. armigera, E. vittella* and *P. gossypiella* in India. Bt cotton hybrids were recommended for cultivation by Government of India during 2002 (Jayaraman, 2004). However, introduction of Bt cotton (Cry1Ac) has led to increase in the population of *S. litura* by 119 per cent that caused considerable losses in cotton by defoliating it (Jeykumar *et al.,* 2007). So, new Bt cotton cultivars have been developed that express multiple insecticidal proteins such as Bollgard II® (Cry1Ac + Cry2Ab). These cultivars express multiple proteins expressed within the same plant and demonstrate higher efficacy against bollworm and many secondary lepidopteran pests as compared to the single protein expressed in Bollgard® cultivars, (Tindall *et al.,* 2009). Though, there are evidences of direct mortality of *S. litura* on BG I and BG II cotton cultivars (Govindan *et al.,* 2010) but the information on the effects of BG II cotton hybrids on ovipositional preference of *S. litura* is scanty. Moreover, during late season when sanitation of cotton fields gets neglected its population builds up on weeds and alternate hosts. Therefore, the experiment was conducted to study the ovipositional preference of tobacco caterpillar *S. litura* (Fabricius) on different Bt cotton hybrids.

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Date of receipt: 07.10.2020, Date of acceptance: 23.07.2021

MATERIALS AND METHODS

Six cotton hybrids including two Bt hybrids (Yuvraj and JKCH1050), three BG II hybrids (NCS 855, Ankur 3028 and MRC 7017) and one non Bt cultivar (LH 2076) were raised in earthen pots placed in iron framed cages covered with muslin at Entomological Research Farm, Department of Entomology, Punjab Agricultural University, Ludhiana as per PAU recommendations and were kept unsprayed. The field collected egg masses of *S. litura* were used to initiate the mass culture under laboratory conditions. The egg masses were kept under laboratory conditions on the fresh leaves of unsprayed non Bt cotton. After hatching, first instar larvae were transferred to the cotton leaves. The leaves were changed daily and the faecal pellets were also removed from the container after every 24 hours. The grown up larvae were allowed to pupate in glass jars filled with soil. After emergence of moths, they were collected with the help of plastic tube and released into battery jar, which was closed at the top with a muslin cloth for ventilation and a small cotton wick soaked in 10 per cent honey solution was placed in the small petriplates for adult feeding. The newly emerged adults were used for studying the ovipositional preference. The plants of different Bt cotton cultivars of different age intervals were raised in earthen pots. The pots were arranged so as to provide multiple choice to the females of *S. litura* to lay eggs. Ten pairs of newly emerged adults of *S. litura* after pairing were released in the centre of pots placed in screen cages covered with muslin to study the ovipositional preference on different cultivars. The ovipositional preference was studied on 105, 120 and 135 day old plants. Plants were observed for egg laying two days after release of adults. Morphological parameters of different cultivars were studied under Scanning Electron Microscope. Fresh leaves belonging to each cultivar were collected and immediately immersed in individual vials containing 2.5% glutaraldehyde solution for primary fixation and kept overnight at a temperature of 4°C. The leaf specimens were then washed thrice with distilled water. For secondary fixation, the specimens were immersed in 4% osmium tetraoxide solution for a period of 2-4 hours at 4°C. After post-fixation, the specimens were again washed thrice (each washing of 5 to 10 minute duration) using distilled water. Dehydration of the specimen discs was performed using different grades of ethanol (25, 50, 70, 95 and 100%) each for a period of 20 minutes whereas the final dehydration (with 100% ethanol) was performed for 30 minutes. The specimens were dried in CO_2 at 5°C and mounted on aluminium stub using double-sided carbon tape. Each specimen leaf disc was mounted with its lower surface up allowing the lower epidermal surfaces of each leaf to be examined. The mounted leaf specimens were sputter-coated with a thin layer of gold using an automated sputter coater. Finally, the specimens were examined and imaged using Hitachi S-3400N Scanning Electron Microscope (Bassola and Russel, 1999). Numbers of eggs per plant were counted by touch count under stereo microscope, leaf area was determined using CI-203 Laser Leaf Area Meter (CID, Inc, USA). Trichome density and trichome length were measured using Scanning Electron Microscope (SEM) at the Electron Microscopy and Nano science (EMN) Laboratory, PAU, Ludhiana as per standard protocol. The data was subjected to Factorial CRD (Completely Randomized Design) for test of significance after applying appropriate transformations wherever necessary using CPCSI software (Cheema and Singh, 1990). Correlation between number of eggs laid by *S. litura* and morphological parameters was also worked out.

RESULTS AND DISCUSSION

Ovipositional preference of *S. litura* (Fabricius) on different Bt cotton cultivars

The data on the ovipositional preference of *S.litura* on different Bt, BG II and non Bt cultivars revealed that female moths laid eggs in cluster/masses only on the abaxial side of the leaves of different cultivars and also recorded significant differences among them. The BG II cultivars (Ankur 3028 and MRC 7017) were more preferred by female moths of *S. litura* for oviposition to other cultivars (Table 1). Significantly higher number of eggs were laid on Ankur 3028 (140.77 eggs/plant) which were on par with MRC 7017 (139.08 eggs/ plant) but significantly higher as compared to NCS 855 BG II (95.88 eggs/plant) followed by non Bt LH 2076 (94.50 eggs/plant), BG Yuvraj (87.91 eggs/female) and JKCH 1050 (87.00 eggs/plant) that were on par with each other. Significant differences in the ovipositional

Table 1. Ovipositional preference of *Spodoptera litura* (Fabricius) to different Bt cotton cultivars

Cultivar	*Number of eggs per plant			
	105 DAS	120 DAS	135 DAS	Mean (Cultivars)
Yuvraj (Bt)	70.50	81.00	112.25	87.91
JKCH 1050 (Bt)	72.00	79.25	109.75	87.00
NCS 855 (BG II)	78.75	94.40	114.50	95.88
Ankur 3028 (BG II)	92.00	149.50	180.75	140.77
MRC 7017 (BG II)	91.75	147.75	177.50	139.08
LH 2076 (Non-Bt)	79.50	84.75	119.25	94.50
Mean (DAS)	80.75	106.12	135.71	-

*Mean of 3 replications; DAS-days after sowing

CD (p=0.05)	Cultivars	DAS	Interaction
	21.46	30.34	NS

Table 2. Morphological characters of Bt cotton cultivars at different stages of plant growth

Cultivar	Leaf area (cm^2)*				Number of trichome* (per mm^2)				Trichome length *(μm)			
	105 DAS	120 DAS	135 DAS	Mean	105 DAS	120 DAS	135 DAS	Mean	105 DAS	120 DAS	135 DAS	Mean
Yuvraj (Bt)	65.75	69.41	73.75	69.64	19.32	21.00	21.00	20.44	634.32	757.67	769.32	720.44
JKCH 1050 (Bt)	67.12	68.28	69.08	68.16	16.82	18.00	19.32	18.05	675.00	739.00	778.9	730.96
NCS 855 (BG II)	79.58	82.61	86.05	82.75	19.50	22.67	23.67	21.95	563.32	629.72	643.02	612.02
Ankur 3028 (BG II)	81.68	88.31	93.35	87.78	30.32	38.67	38.80	36.26	570.32	623.67	649.00	614.33
MRC 7017 (BG II)	73.82	74.68	75.41	74.64	32.00	34.00	36.32	34.11	590.67	608.32	658.67	619.22
LH 2076 (Non-Bt)	66.08	69.08	73.58	69.58	24.67	25.50	26.32	25.50	551.67	570.32	572.32	564.77
Mean (DAS)	72.34	75.40	78.54	-	23.78	26.63	27.75	-	597.55	654.78	678.54	-

*Mean of 3 replications; DAS-days after sowing

CD (p=0.05)	Cultivars	DAS	Interaction
Leaf Area	5.46	3.10	NS
Trichome length	42.25	29.87	NS
Trichome density	2.76	1.35	NS

preference were also recorded among different plant stages by *S. litura*. Maximum number of eggs per plant were recorded on 135 day old plants (135.71 eggs/ plant) which were at par with 120 day old plants (106.12 eggs /plant) but significantly higher as compared to 105 days old plants (80.75 eggs /plant) (Table 1).

Saini *et al.* (2013) reported significantly more number of eggs laid by female moth of *S. litura* on the leaves of BG II genotype, RCH 134 whereas minimum eggs were laid on non Bt Ankur Jai at 90 day old plants. Whereas, Kumar and Prasad (2016) reported that oviposition behavior in moths did not show any preference, *viz.,* Mallika BG, Mallika BGII or non-*Bt* control for egg laying. A similar non-significant trend was towards the number of egg masses laid and number of fertile egg masses was also recorded in their studies. Parker and Luttrell (1998) also reported no difference in egg densities on plant terminals (only the upper three nodes) between Bt and non-Bt cotton fields. Similarly, non significant differences in oviposition preference by the adult of *S. litura*, *H. armigera* and *E. vittella* among different Bt and non Bt cultivars were reported by Anooshka *et al.* (2013), Basavaraja *et al.* (2012) and Saini *et al*. (2012), respectively. Macintosh *et al.* (1990) studied and reported that ovipositional preference of bollworm between Bt and non-Bt cotton did not yield any significant differences because the Cry1Ac protein in Bt cotton does not affect bollworm adults.

Morphological characters of Bt cotton cultivars at different stages of plant growth

The data on the morphological characters of different Bt (Yuvraj and JKCH 1050), BG II (Ankur 3028, NCS 855 and MRC 7017) and non Bt LH 2076 cultivars showed significant differences among different cultivars and different plant stages (105, 120 and 135 days old plants) (Table 2). Larger leaf area was recorded in case of the BG II cultivars, Ankur 3028 (87.78 cm^2) and NCS 855 (82.75 cm^2) which was on par with each other but significantly large as compared to MRC 7017 BG II (74.64 cm^2) followed by Yuvraj Bt (69.64 cm^2), LH 2076 non Bt (69.58cm^2) and JKCH BG I (68.16 cm^2) which were again on par with each other (Table 2). Among different plant stages, large leaf area was recorded from 135 days old plants (78.54 cm^2) which was on par with 120 days (75.40 cm^2) but significantly large than 105 days old plants (72.34 cm^2) however, the latter two recorded non significant differences with each other.

However, the data on the trichome density among the different cultivars revealed significantly higher number of trichomes on leaves of BG II cultivars, Ankur 3028 (36.26 trichomes / mm^2) (Fig. 1) and MRC 7017 (34.11 trichomes / mm^2) as compared to non Bt LH 2076 (25.50 trichomes / mm^2) followed by Bt cultivars

Table 3. Correlation between morphological leaf characteristics of Bt cotton hybrids and oviposition by *S. litura*

Character	Eggs/plant	
	Correlation coefficient (r)	Coefficient of determination (R^2)
Leaf area (cm^2)	0.69	0.47
Trichome density (mm^2)	0.95*	0.90
Trichome length (μm)	-0.35	0.12

* Significant at 1% level of significance

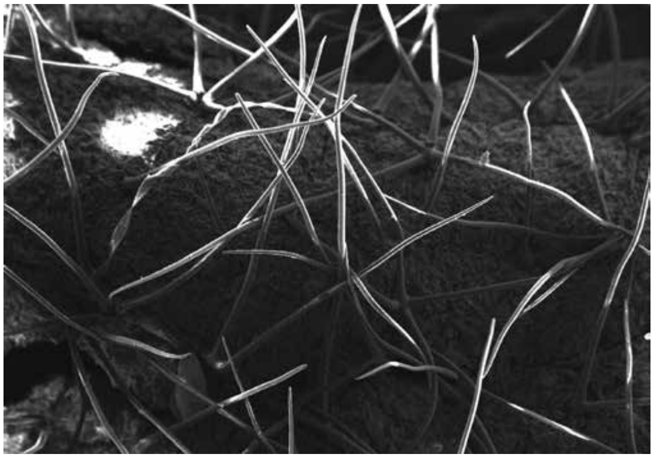

Fig. 1. Ankur 3028 (BG II) with maximum number of trichomes per mm^2

Fig. 2. JKCH 1050 (BG I) with least number of trichome per mm^2.

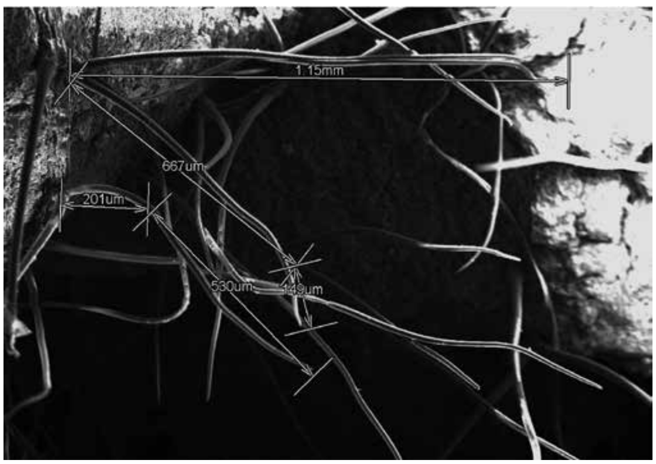

Fig. 3. JKCH 1050 (BG I) with maximum trichome length (um)

Fig. 4. LH 2076 (non Bt) with minimum trichome length (um)

Yuvraj (20.44 trichomes / mm^2) and JKCH 1050 (18.05 trichomes / mm^2) (Fig. 2); whereas, among the different plant stages, trichome density was higher in 135 day old plants (27.75 trichomes / mm^2) followed by 120 days old plants (26.63 trichomes / mm^2) which were at par but significantly different from leaves of 105 days old plants (23.78 trichomes / mm^2) (Table 2).

The data on the trichome length (µm) among the different cultivars showed that trichome length was significantly more on leaves of Bt cultivars, JKCH 1050 (730.96 µm) (Fig. 3) and Yuvraj (720.44µm) as compared to BG II cultivars, MRC 7017 (619.22 µm), Ankur 3028 (614.33 µm) and NCS 855 (612.02 µm) and non Bt LH 2076 (564.77µm) (Fig. 4). Among different stages of plant growth, trichome length was more on leaves of 135 day old plants (678.54 µm) followed by 120 days old plants (654.78 µm) which were on par with each other but significantly different from leaves of 105 days old plants (597.55µm) (Table 2).

Correlation studies revealed that among morphological characteristics, trichome density has significantly positive and leaf area showed positive but non significant correlation (r=0.95) with oviposition of *S. litura.* Whereas, the trichome length showed negative but non significant correlation to oviposition. Among the morphological traits, trichome density was more important in determining the extent of oviposition by *S. litura* (Fig. 5). Similar results were reported by Ramnath *et al.* (1993) that higher trichome density was strongly favoured for oviposition by *H. armigera* and they further submitted that leaf pubescence provided a better foothold for females for oviposition.

Although there is difference in the egg laying preference of *S .litura* female among different cultivars/ genotypes but there is no relation of Bt cotton with egg laying of *S.litura* because *Bacillus thuringiensis* makes toxins that target insect larvae when eaten and toxins are activated in their gut. The activated toxin breaks down

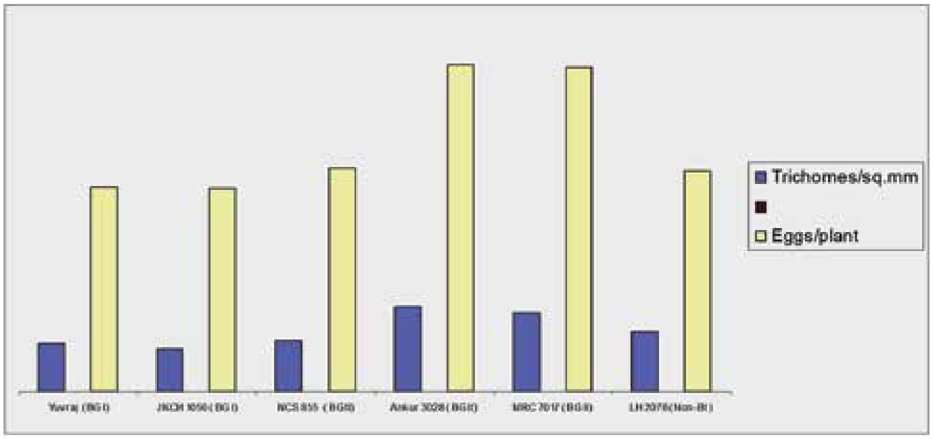

Fig. 5. Relationship between trichome density and eggs of *S. litura* in different cotton cultivars

their gut, and the insects die of infection and starvation. The difference in the oviposition preference in different cultivars could be due to genotype characters such as leaf area, trichome length, trichome density, leaf laminar thickness or leaf colour which varies among different cultivars.

So, it can be concluded from the studies that there was significantly positive correlation between oviposition by *S. litura* females and trichome density. Therefore, cultivars with low trichome density may be preferred in areas susceptible to *S.litura* infestation.

Acknowledgements

The authors are grateful to Dr. Anu Kalia, Assistant Professor (Nanoscience) for help in conducting research and for providing other facilities at EMN laboratory, P.A.U. Ludhiana.

Authors' contribution

Conceptualization of research work and designing of experiments (HSB, RSG); Execution of field/lab experiments and data collection (HSB); Analysis of data and interpretation (HSB); Preparation of manuscript (HSB, RSG)

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