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Influence of *Cry* proteins against pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera; Gelechiidae)

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ABSTRACT : To evaluate the efficacy of *Cry 1 Ac* and *Cry 1 Ac + Cry 2 Ab,* laboratory experiment was carried out at Insectary, Tamil Nadu Agricultural University, Coimbatore. Per cent mortality was recorded by conducting plant bioassay (squares, flowers, tiny bolls and matured bolls) at different crop growth stages (70, 85, 100, 115 and 130 DAS). Results showed that above 90 per cent mortality at 70 DAS of the crop in both *Bt* and stacked *Bt* cotton. At 130 DAS mortality of one day old larvae reduced drastically in Bollgard and Bollgard II. Non *Bt* cotton (Bunny non *Bt* and RCH 2 non *Bt*) recorded less than 15 per cent mortality in all the plant parts at different crop growth stages. Single and stacked gene caused higher mortality during early stage of the crop, compared to later stage. Apart from *Cry* toxin protein, various other factors also influence the survival of pink bollworm.

Key words : Bioassay; PBW; per cent mortality; single and double gene

Cotton *Gossypium hirsutum* L., is one of the principal commercial crops playing a key role in economic, social and political affairs of the country. Cotton is also called as "White Gold" and it is an economically vital crop for developing countries like India. The insect pests spectrum of cotton is quite complex and as many as 1326 species of insect pests have been reported on this crop throughout the world. Among the bollworms, the pink bollworm assumed major pest status in recent past. World over, pink bollworm *Pectinophora gossypiella* (Saunders) has become economically the most destructive pest of cotton and has known to cause 2.8 to 61.9 per cent loss in seed cotton yield, 2.1 to 47.10 per cent loss in oil content and 10.7 to 59.2 per cent loss in normal opening of bolls.

In India, *Bt* cotton has gained considerable importance in recent years. In Tamil Nadu, cotton production (lakh bales) during 2012-2013 was 5 lakh bales and cotton productivity (kg/ha) during 2012-2013 was 831.33 kg/ha. *Bt* cotton (Bollgard®) offers high level of resistance against cotton bollworm complex ie., *Helicoverpa armigera* (Hubner), *Earias vittella* (Fabricius) and *Pectinophora gossypiella* (Saunders) under field conditions. To evaluate the efficacy of *Cry* 1 *Ac* and *Cry* 1 *Ac* + *Cry* 2 *Ab* against pink bollworm under laboratory, the present investigation was undertaken.

The dual toxin *Bt* cotton (*CryIAc+2Ab*), single toxin *Bt* cotton (*Cry I Ac*) cultivars of RCH 2BG II and Bunny *Bt*, respectively with along their corresponding non *Bt* versions were chosen for bioassay studies in the laboratory against *P. gossypiella* at Insectary, Tamil Nadu Agricultural University, Coimbatore during *kharif*, 2012-2013. One day old larvae of pink bollworm were collected from the damaged fruiting bodies and were reared on natural fresh fruit parts in the laboratory. Bioassays were conducted using detached plant parts *viz.*, fully opened flower, squares, tiny boll and mature bolls from two to three nodes below the terminal end of 70, 85, 100, 110 and 130 days old Bollgard and Bollgard II *Bt* cottons were taken for this study. The detached flowers and squares

and bolls were placed on moist filter paper in petriplates and polypots, respectively. The fresh plant materials were provided for the surviving larvae after 24 hr. From the culture, one day old larvae were separated out and released on fresh plant materials *Bt*, stacked *Bt* and non *Bt*. In case of bolls it was wrapped with gum tape to avoid the larval escape and was kept in petridishes at room temperature in laboratory. The experiment was conducted in a factorial completely randomized block design (FCRD) with four treatments and four replications. Larval survival was recorded after 72 hr of infestation and larval mortalities were worked out as per cent of total larvae used in the experiments. The larvae were marked dead when they did not move when probed. All the experiments were carried out in a room with photoperiod of 14: 10 (L: D) and average temperature of 27°C with 60 per cent relative humidity (Soujanya *et al.*, 2010).

The data collected were analyzed by completely randomized design to compare the age of the crop, varieties/hybrids used for studying survival of pink bollworm on *Bt* and stacked *Bt* cottons. The per cent mortality was transformed using arcsine transformation.

The data on per cent mortality in bunny *Bt* against PBW were recorded 96.67 and 98.33per cent when fed on squares of 70 days old crop of both stacked *Bt* and *Bt* hybrids, compared to 3.33 and 15.30 per cent only, on Bunny non *Bt* and RCH non *Bt* hybrids (Table 1). At 115 and 130 days, the mortality of one day old larvae larva in Bunny *Bt* (*Cry 1Ac*) it was 43.33 and 27.50 percent respectively. Similarly trend was noticed in RCH BG II. It was in accordance with the finding of Kranthi *et al.*, (2005) that the mortality was high during early stages due to decline in *CryIAc* content as the age of the crop advances (Olsen and Daly, 2000). Per cent mortality of one day old larvae was 95.83 and 15.00 at 70 and 130 DAS respectively in flowers of Bunny *Bt* (BG), whereas respective non *Bt* (Bunny non *Bt*) showed 9.17 (70 DAS) and 17.50 (130 DAS) per cent mortality. Per cent mortality of one day old larvae larva of pink bollworm was 97.50 and 19.17 at 70 and 130 DAS respectively in RCH 2 BG II whereas per cent mortality of one day old larvae in RCH non *Bt* was 3.33 and 7.50 at 70 and 130 DAS respectively (Table 1). Early stage crop gives effective mortality as compared to late stage crop as sited in present findings has been reported in studies conducted by various scientists (Adamczyk *et al.*, 2004; Sun *et al.*, 2002; Gujar *et al.*, 2004; Gujar *et al.*, 2007; Gujar *et al.*, 2000).

In case of tiny bolls, per cent mortality of one day old larvae was 93.33 and 94.17 when fed on 70 days old crop of both stacked *Bt* and *Bt* hybrids, compared to 3.33 and 10.83 per cent only, on Bunny non *Bt* and RCH non *Bt* hybrids, which differed significantly from non *Bt* hybrids with 10.83 and 17.50 per cent mortality in bunny non *Bt* and RCH 2 non *Bt* hybrids, respectively (Table 2). Per cent mortality of one day old larvae was 93.33 and 97.5 when fed on tiny bolls of 70 days old crop of both stacked *Bt* and *Bt* hybrids, compared to 6.67 and 97.50 per cent, on Bunny non *Bt* and RCH non *Bt* hybrids whereas the mortality of one day old larvae fed with bolls of 130 days old crop was 5.83 and 13.33 in bunny non *Bt* and RCH 2 non *Bt* cotton hybrids, respectively (Table 2). The present investigation revealed that in *Bt* cotton (single and stacked gene) mortality of pink bollworm at 75 DAS was higher when compared to 130 DAS. The present findings also corroborate the reports of Jech and Henneberry (2005), Henneberry *et al.*, (2006) and Rahaman and Goud (2007), who reported higher mortality of pink bollworm larvae when fed on *Bt* cotton. Studies conducted by various scientists (Soujanya *et al.*, 2010; Santhosh, 2008) also revealed that the toxin content in *Bt* cotton changed significantly overtime, structure, growth stage and variety. From the present study it can be concluded that at 130 DAS mortality of pink bollworm reduced drastically in Bollgard and Bollgard II as compared to 70 DAS. Non *Bt* cotton (Bunny non *Bt* and RCH 2 non *Bt*) recorded

Table 1. Per cent mortality of *Pectinophora gossypiella* larvae on squares and flowers of single and stacked gene *Bt* hybrids

DAS	Per cent mortality of PBW against squares of *Bt* hybrids after 72 hr of infestation					DAS	Per cent mortality of PBW against flowers of *Bt* hybrids after 72 hr of infestation				
	Bunny *Bt*	Bunny non *Bt*	RCH 2 BG	RCH 2 non *Bt*	**Mean**		Bunny *Bt*	Bunny non *Bt*	RCH 2 BG	RCH 2 non *Bt*	**Mean**
70	96.67(80.87)a	3.33(11.62)	98.33(84.48)a	15.00(22.76)	**53.33**	70	95.83 (81.39)a	9.17 (17.45)	97.50 (82.00)a	3.33 (10.51)	**51.46**
85	83.33(66.00)b	15.00(22.76)	86.67(68.91)b	10.83(19.00)	**48.96**	85	80.00 (64.08)b	13.33 (21.34)	82.50 (65.34)b	15.00 (22.76)	**47.71**
100	60.00(50.00)c	10.83(19.00)	64.17(53.24)c	6.67(14.72)	**35.42**	100	59.17 (50.30)c	17.50 (24.71)	64.17 (53.23)c	10.83 (19.00)	**37.92**
115	43.33(41.16)d	6.67(14.72)	52.50(46.45)d	7.50(15.50)	**27.50**	115	26.67 (30.98)d	11.67 (19.73)	30.00 (33.20)d	6.67 (14.72)	**18.75**
130	27.50(31.60)e	9.17(17.45)	35.00(36.20)e	14.17(22.01)	**21.46**	130	15.00 (22.63)e	17.50 (24.66)	19.17 (25.91)e	7.50 (15.83)	**14.79**
Mean	**62.17**	**9.00**	**67.33**	**10.83**	**37.33**	**55.33**	**13.83**	**58.67**	**8.67**		**34.13**
Interaction	**SEd**	**CD (p = 0.05)**	**Interaction**	**SEd**	**CD (p = 0.05)**						
Days	1.32	2.65	Days	1.39	2.78						
Variety	1.18	2.37	Variety	1.24	2.48						
Days x Variety	2.65	5.31	Days x Variety	2.78	5.60						

Mean of four replications; 30 observations per replication; DAS - Days after sowing;
PBW-Pink bollworm; Figures in parentheses are arcsine transformed values.

Table 2. Per cent mortality of *Pectinophora gossypiella* larvae on tiny boll and bolls of single and stacked gene *Bt* hybrids

DAS	Per cent mortality of PBW against tiny boll of *Bt* hybrids after 72 hr of infestation					DAS	Per cent mortality of PBW against boll of *Bt* hybrids after 72 hr of infestation				
	Bunny *Bt*	Bunny non *Bt*	RCH 2 BG	RCH 2 non *Bt*	**Mean**		Bunny *Bt*	Bunny non **Bt**	RCH 2 BG	RCH 2 non *Bt*	**Mean**
70	93.33 (75.53)a	3.33 (10.51)	94.17 (78.02)a	10.83 (19.00)	**50.42**	70	93.33 (75.53)a	6.67 (14.72)	97.50 (82.00)a	7.50 (15.83)	**51.25**
85	73.33 (55.02)b	15.00 (22.76)	86.67 (68.99)b	11.67 (19.73)	**46.67**	85	83.33 (69.30)b	3.33 (10.51)	89.17 (73.26)b	10.00 (18.11)	**46.46**
100	43.33 (41.16)c	10.83 (19.00)	61.67 (51.82)c	17.50 (24.62)	**33.33**	100	55.00 (47.87)c	15.00 (22.62)	63.33 (52.90)c	14.17 (22.00)	**36.88**
115	33.33 (35. 23)d	6.67 (14.72)	40.00 (39.21)d	10.00 (18.12)	**22.50**	115	35.83 (36.74)d	10.83 (19.00)	33.33 (35.23)d	9.17 (17.45)	**22.29**
130	20.00 (26.37)e	9.17 (17.45)	27.50 (31.60)e	6.67 (14.72)	**15.84**	130	20.83 (27.04)e	5.83 (13.60)	25.00 (29.94)e	13.33 (21.21)	**16.46**
Mean	**52.66**	**9.00**	**62.00**	**11.33**	**33.75**	**57.67**	**8.50**	**61.67**	**10.83**		**34.67**
Interaction	**SEd**	**CD (p = 0.05)**	**Interaction**	**SEd**	**CD (p = 0.05)**						
Days	1.40	2.81	Days	2.00	4.00						
Variety	1.25	2.51	Variety	1.75	3.51						
Days x Variety	2.81	5.63	Days x Variety	4.00	7.85						

Mean of four replications; 30 observations per replication; DAS - Days after sowing;
PBW-Pink bollworm; Figures in parentheses are arcsine transformed values.

meager per cent mortality in all the plant parts at different crop growth stages. Single and stacked gene inflicted higher mortality during early phase of the crop compared to later phase of the crop growth.

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