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Effect of 60, 90 and 120 days old BG-II cotton leaves on weights of different instars of *Spodoptera litura* (F)

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Abstract
The effect of age of leaves on the larval weights of different instar larvae of *Spodoptera litura* showed that there was significant reduction in the larval weights when fed with dual toxin *Bt* cotton leaves compared non-*Bt* leaves. A perusal of the data at 60 days revealed that the larval weights of first instar after five days ranged from 1.17 mg to 1.40 mg across different BG-II hybrids which were on par and significantly different from non-*Bt* Orugallu Krishna (33.00 mg). Similarly after 10 days of feeding also, it ranged from 2.77 to 3.60 mg in BG-II hybrids which were on par. Significantly higher larval weight (266.00 mg) was registered in Orugallu Krishna. The larval weight of first instar *Spodoptera* fed on 90 days old cotton leaves ranged from 1.13 to 1.60 mg and 1.77 to 3.73 mg when fed for five and 10 days, respectively, in all the BG-II hybrids. Significantly higher larval weights (32 and 254 mg) were gained after five and 10 days, respectively in Orugallu Krishna. The perusal of data on larval weights of first instar *Spodoptera* when fed on 120 day old cotton leaves indicated that all *Bt* cotton hybrids were on par with larval weights ranging from 1.10 to 1.67 mg while significantly higher larval weight (28.00 mg) was registered in Orugallu Krishna after five days. Similarly after 10 days, it ranged from 3.23 to 4.03 mg among *Bt* cotton hybrids and 273.67 mg in Orugallu Krishna.

Keywords: *Spodoptera litura*, bollgard-II, larval weights

Introduction
Cotton (*Gossypium hirsutum*) is an important fiber crop that is grown extensively in many parts of the world. Apart from providing very valuable fiber, cotton seed is used as a source of edible oil and seed cake, which is used as animal feed. In 2014, ~37 million hectares of land was sown with cotton worldwide, mostly under rain fed and dry land conditions. In India alone, the crop was grown in ~12.25 million hectares of land. Cotton is highly susceptible to a large number of lepidopteran pests, and a major one on the Indian subcontinent is *Helicoverpa armigera Spodoptera litura*. A milestone in cotton breeding was the development of transgenic pure-line varieties and hybrids containing the *cry* genes of *Bacillus thuringiensis* (*Bt*) encoding insecticidal proteins that provide protection from lepidopteran pests. Both Bollgard I and Bollgard II utilize a *cry1Ac-like* gene construct that was originally present in the event Mon 531. Since the introduction of *Bt* cotton in India, the cotton production area has increased from 7.7 million hectares to 12.25 million hectares, fiber production has increased from 13.6 million bales to 39.1 million bales, and pesticides usage to control lepidopteran pests has decreased from 5,748 metric tons to 222 metric tons [1]. A number of studies have shown that *Bt* cotton has increased farmer's incomes, including those of smallholder farmers, reduced pesticide usage and even improved natural biocontrol. Based on laboratory bioassays reported that mortality of both *Spodoptera frugiperda* and *S. exigua* was significantly greater in Bollgard-II than on Bollgard or conventional cotton, demonstrating that dual toxin Bollgard-II genotype is highly effective against lepidopteran pests that are not adequately controlled by single toxin Bollgard varieties [2]. Similarly, reported the excellent efficacy of Bollgard II against lepidopteran pests [3]. There were very low larval numbers in the Bollgard II compared to conventional and INGARD cotton and the only lepidopteran pest surviving in the Bollgard II was low numbers of *S. litura* [3]. Bollgard II gave higher yields and provided more effective control against bollworms, beet armyworm and soybean loopers than Bollgard. Bollgard II varieties left untreated for lepidopteran pests averaged 103 kg/ha lint more than Bollgard varieties [4]. A major challenge for transgenic cotton is the management of resistance development in insect pests feeding on the crop, also called insect resistance management, which has garnered considerable attention.

A major weakness of the products (Bollgard I and II) currently used in the field is a drop in the Cry1Ac protein's expression level as the plant matures and sets bolls. Further, there is a high expression level in the roots that provides no resistance against *H. armigera* and other lepidopteran pests, as they do not feed on roots. Another weakness is that a secondary lepidopteran pest on cotton, *Pectinophora gossypiella*, can survive the low Cry1Ac protein dose present in the developing bolls of event Mon 531 and leave progeny.

Materials and Methods

Experimental site

The field experiment was conducted in the research farm of RARS, Warangal, which is geographically situated at an altitude of 265 m above mean sea level on $18^{0}01'$ 04.8 "N" latitude and $79^{o}3611.3$ "E" longitude .The experimental field had black cotton soils suitable for cotton cultivation

Seed material

The experiment consisted of seven second generation *Bt* transgenic hybrids with *Cry* 1 Ac and *Cry* 2 Ab genes, and one conventional non-*Bt* hybrid. The test genotypes used for the investigations were intraspecific hybrids the seeds were procured as commercial sale packets in open market, while the non-*Bt* seed was supplied by cotton breeder, RARS, Warangal. All the seven hybrids had minimum germination of 75%, pure seeds of 98%, genetic purity of 90% and protein toxin expression of 90%.

Laboratory studies

To know the season long changes on the mortality of larvae to cry proteins, each of the 8 treatments were sown separately in 4.8 x 4.8 m^2 plots without any plant protection measures except seed treatment with imidacloprid 70 WS @ 10 g/kg seed. The plant leaves, were collected at 60, 90 and 120 days and brought to laboratory for bioassay studies using *S. litura*

Maintenance of insect culture

Tobacco caterpillar, *S. litura*

To establish initial culture of *S. litura*, the larvae were collected from weeds and groundnut fields. The larvae were reared on castor leaves instead of artificial diet. The handling of the larvae was in mass for early instars and individually in late instars. The initial instars were reared on castor leaves in plastic basins and after a week transferred to pet jars containing castor leaves till pupation. The oviposition, egg brushing and other procedures remained same. The eggs were deposited in mass and each mass was placed in large size box having netted lid. Each day fresh castor leaf was placed over the leaf containing egg mass. Immediately after hatching the larvae crowded on fresh leaf and were shifted for further rearing in small jars / buckets. The larvae of F1 and F2 generations were used for bio assay. The experiment was laid out in Randomized Block Design (RBD) with three replications. The data were subjected to stastical analysis after suitable transformation and the means were separated by DMRT (P=0.05%).

Results and Discussion

Effect of age of leaves on larval weights of different instars of *S. litura* 60 days old leaves

A perusal of the data (Table 1) at 60 days revealed that the larval weights of first instar after five days ranged from 1.17 mg to 1.40 mg across different BG-II hybrids which were on par and significantly different from non-*Bt* Orugallu Krishna (33.00 mg). Similarly after 10 days of feeding also, it ranged from 2.77 to 3.60 mg in BG-II hybrids which were on par. Significantly higher larval weight (266.00 mg) was registered in Orugallu Krishna.

The larval weights of second instar larvae fed with 60 days old cotton leaves for 5 days registered lower larval weight ranging from 25.97 to 29.90 mg in BG-II hybrids and were on par but significantly different from Orugallu Krishna (non-*Bt*) with larval weight of 265.67mg. After feeding for 10 days the larval weights in BG-II hybrids ranged from 109.13 to 118.57 mg which were on par and significantly different from Orugallu Krishna (non *Bt*) with larval weight of 868.67 mg.

The larval weights of 3rd instar larvae after five days of feeding on BG-II hybrids ranged from 126.83 to 133.60 mg which were significantly different from non-*Bt* Orugallu Krishna (795.00 mg). All the BG-II hybrids registered lower larval weights than Orugallu Krishna (1254.33 mg).

90 days old leaves

The larval weight of first instar *Spodoptera* fed on 90 days old cotton leaves ranged from 1.13 to 1.60 mg and 1.77 to 3.73 mg (Table 2) when fed for five and 10 days, respectively, in all the BG-II hybrids. Significantly higher larval weights (32 and 254 mg) were gained after five and 10 days, respectively in Orugallu Krishna.

In second instar, larval weights after five days ranged from 37.67 to 40.00 mg across BG-II hybrids which were on par and significantly different from Orugallu Krishna, which registered larval weight of 273.00 mg. Among the BG-II hybrids significantly highest larval weight was noticed in RCH-2. But, Orugallu Krishna (non-*Bt*) registered significantly higher larval weight (764.67 mg) than all the *Bt* cotton hybrids.

The third instar larvae showed significant differences in larval weights among BG-II hybrids. After five days, significantly lowest larval weights were found in Neeraja (131.77 mg) and Mallika (132.20 mg) followed by Bunny (134.47 mg), Brahma (142.70 mg) and RCH-530 (140.37 mg) which were on par with each other. Tulasi-9 and RCH-2 recorded significantly higher larval weights (149.33 and 150.33 mg, respectively) than Mallika and Neeraja. However, highest larval weight of 791.97 mg was noticed in Orugallu Krishna.

After 10 days significantly highest larval weight among BG-II hybrids was registered in Bunny. Orugallu Krishna (non-*Bt*) with larval weight of 1295.33 mg was significantly different from all *Bt* cotton hybrids.

120 days old leaves

The perusal of data on larval weights of first instar *Spodoptera* when fed on 120 day old cotton leaves indicated that all *Bt* cotton hybrids were on par with larval weights ranging from 1.10 to 1.67 mg (Table 3), while significantly higher larval weight (28.00 mg) was registered in Orugallu Krishna after five days. Similarly after 10 days, it ranged from 3.23 to 4.03 mg among *Bt* cotton hybrids and 273.67 mg in Orugallu Krishna.

During second instar the larval weight of Mallika, RCH-530, Tulasi-9, Bunny, Brahma and RCH-2 after 5 days ranged from 33.73 to 37.93 mg and all were on par except Neeraja (41.17 mg). Significantly highest larval weight was recorded in Orugallu Krishna (273.67 mg).

After 10 days significantly lowest larval weight was observed in Brahma (126.10 mg) followed by Bunny (129.80 mg),

Mallika (132.73mg), RCH-530 (140.47 mg) which were on par with each other. Among BG-II hybrids significantly higher larval weight was registered in RCH-2 (149.97 mg) and Tulasi-9 (151.40 mg) while, highest larval weight was on Orugallu Krishna (753.67 mg).

During 3rd instar lowest larval weight after 5 days was observed in Bunny (132 mg) followed by Mallika and Neeraja (138.50 and 141.90, respectively). They were significantly different from RCH-530 (148.87 mg) and RCH-2 (159.53 mg). Orugallu Krishna recorded highest larval weight (851.00 mg). After 10 days, Orugallu Krishna (non-*Bt*) recorded highest larval weight (1195.00 mg).

Significant reduction in the larval and pupal weights when fed with dual toxin *Bt* cotton plant parts. Though the fourth and fifth instar larvae were able to survive when exposed to leaves and squares of dual toxin *Bt* cotton cultivars, they were unable to develop normally with significant reduction in the weight of larvae, formation of smaller pupae [5, 6]. Similar vews were expressed in more studies [7, 5]. Bollgard-II was highly effective against *S. frugiperda* by causing mortality, retarding larval weight, pupal duration and adult duration [8]. Exposure of later instars to plant parts of *Bt* event hybrids exhibited adverse effect on growth and development in terms of reduced larval weight, prolonged larval duration, reduced pupation, smaller pupae and reduction in adult emergence [9].

Table 1: Effect of 60 days old BG-II cotton leaves on weights of different instars of *S. litura*

	Treatments	Weight in mg/larva								
		1st instar			2nd instar			3rd instar		
		Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR
T_1	Bunny BG-II	0.06	1.17^{b}	3.13^{b}	21.38	29.53^{b}	117.93^{b}	109.87	130.10^{b}	228.67^{de}
T_2	Mallika BG-II	0.06	1.27^{b}	3.23^{b}	20.70	29.90^{b}	118.57^{b}	106.27	128.10^{b}	216.67^{e}
T_3	Neeraja BG-II	0.06	1.17^{b}	2.77^{b}	20.97	27.47^{b}	122.50^{b}	111.07	130.07^{b}	291.00^{b}
T_4	Brahma BG-II	0.05	1.27^{b}	3.13^{b}	19.93	26.60^{b}	109.13^{b}	105.20	126.83^{b}	255.67^{c}
T_5	Tulasi-9 BG-II	0.06	1.13^{b}	3.27^{b}	20.73	25.97^{b}	111.20^{b}	103.60	131.40^{b}	236.00^{cde}
T_6	RCH-2 BG-II	0.06	1.40^{b}	2.93^{b}	20.57	26.43^{b}	118.57^{b}	111.07	133.60^{b}	243.33^{cd}
T_7	RCH-530 BG-II	0.05	1.17^{b}	3.60^{b}	21.45	26.27^{b}	116.83^{b}	113.40	130.30^{b}	241.00^{cd}
T_8	Orugallu Krishna (Non *Bt*)	0.06	33.00^{a}	266.00^{a}	20.60	265.67^{a}	868.67^{a}	109.80	795.00^{a}	1254.33^{a}
CD at 5%		NS	0.71	3.31	NS	3.86	15.05	NS	8.01	20.62
SE m±		0.002	0.23	1.09	0.43	1.27	4.97	2.36	2.65	6.82
CV %		5.13	7.87	5.26	3.61	3.86	4.09	3.76	2.15	3.18

DAR-Days after release

Table 2: Effect of 90 days old BG-II cotton leaves on weights of different instars of *S. litura*

	Treatments	Weight in mg/larva								
		1st instar			2nd instar			3rd instar		
		Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR
T_1	Bunny BG-II	0.06	1.13^{b}	2.80^{b}	24.03	38.87^{b}	131.67^{de}	114.93	134.47^{bc}	303.77^{b}
T_2	Mallika BG-II	0.06	1.33^{b}	3.13^{b}	24.80	37.87^{b}	148.33^{c}	116.40	132.20^{c}	267.83^{d}
T_3	Neeraja BG-II	0.06	1.30^{b}	3.20^{b}	22.80	39.20^{b}	138.33^{cde}	113.60	131.77^{c}	284.10^{cd}
T_4	Brahma BG-II	0.06	1.53^{b}	3.73^{b}	24.27	39.40^{b}	143.67^{cd}	120.87	142.70^{bc}	239.43^{e}
T_5	Tulasi-9 BG-II	0.06	1.60^{b}	2.63^{b}	22.57	39.07^{b}	125.33^{e}	116.60	149.33^{b}	300.73^{bc}
T_6	RCH-2 BG-II	0.06	1.37^{b}	1.77^{b}	21.87	40.00^{b}	157.67^{b}	116.80	150.33^{b}	277.73^{d}
T_7	RCH-530 BG-II	0.05	1.57^{b}	2.33^{b}	23.97	37.67^{b}	148.00^{cd}	116.73	140.37^{bc}	267.37^{d}
T_8	Orugallu Krishna (Non *Bt*)	0.05	32.00^{a}	254.00^{a}	24.53	273.00^{a}	764.67^{a}	115.77	791.97^{a}	1295.33^{a}
CD at 5%		NS	0.67	4.47	NS	6.27	16.34	NS	14.56	19.62
SE m±		0.002	0.22	1.48	0.95	2.07	5.40	1.51	4.81	6.49
CV %		5.504	7.33	7.49	7.02	5.27	4.26	2.24	3.76	2.77

DAR-Days after release

Table 3: Effect of 120 days old BG-II cotton leaves on weights of different instars of *S. litura*

	Treatments	Weight in mg/larva								
		1st instar			2nd instar			3rd instar		
		Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR	Initial Wt of larvae	After 5 DAR	After 10 DAR
T_1	Bunny BG-II	0.05	1.53^{b}	3.53^{b}	21.30	37.40^{bc}	129.80^{cd}	120.07	132.00^{e}	281.67^{b}
T_2	Mallika BG-II	0.06	1.30^{b}	4.03^{b}	22.13	32.33^{c}	132.73^{bcd}	120.87	138.50^{de}	271.67^{bc}
T_3	Neeraja BG-II	0.06	1.17^{b}	3.63^{b}	21.47	41.17^{b}	147.30^{bc}	118.27	141.90^{cde}	267.00^{bc}
T_4	Brahma BG-II	0.06	1.23^{b}	3.23^{b}	21.33	37.47^{bc}	126.10^{d}	122.10	142.60^{cd}	257.33^{c}
T_5	Tulasi-9 BG-II	0.06	1.53^{b}	4.03^{b}	22.37	36.47^{bc}	151.40^{b}	125.20	146.20^{cd}	289.00^{b}
T_6	RCH-2 BG-II	0.05	1.67^{b}	3.50^{b}	21.93	37.93^{bc}	149.97^{b}	119.87	159.53^{b}	271.00^{bc}
T_7	RCH-530 BG-II	0.06	1.10^{b}	3.63^{b}	20.53	33.73^{c}	140.47^{bcd}	119.07	148.87^{c}	280.33^{b}
T_8	Orugallu Krishna (Non *Bt*)	0.06	28.00^{a}	273.67^{a}	21.87	273.67^{a}	753.67^{a}	117.53	851.00^{a}	1195.00^{a}
CD at 5%		NS	0.67	4.56	NS	5.34	17.78	NS	10.34	20.96
SE m±		0.002	0.22	1.51	0.49	1.76	5.88	1.69	3.42	6.93
CV %		6.789	8.17	6.99	3.95	4.61	4.70	2.43	2.54	3.08

DAR-Days after release

Conclusion

The effect of age of leaves on the larval weights of different instar larvae of *S. litura* showed that there was significant reduction in the larval weights when fed with dual toxin *Bt* cotton leaves compared non-*Bt* leaves. The present studies also indicated that with increase in age of leaves, up to 120 days there were very little decline in the mortality of *S .litura* larvae within the same instars. Also the studies on the effect of different plant parts indicated that within the same larval instars and age of plant parts leaves, squares and bolls were equally effective in controlling the larvae.

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