Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 2170-2177

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 2170-2177**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.610.257

Effect of BG-II Cotton Hybrids and Non Bt Cotton on Weight of Different Instars of *Spodoptera litura* (FAB.)

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Keywords

Dual toxin Bt cotton, *S. litura*, weight.

Article Info

Accepted:
21 September 2017
Available Online:
10 October 2017

A B S T R A C T

Laboratory assays showed that dual toxin *Bacillus thuringiensis* (Bt) cotton cultivars expressing both Cry 1 Ac and Cry 2 Ab endotoxins (BG-II) were more toxic to *Spodoptera litura* (Fab.) larvae compared to non Bt cotton. The weight of first, second, third and fourth instar larvae were recorded, which were fed with leaves, squares and bolls of eleven dual toxin Bt cotton cultivars at 60, 75, 90 and 125 days after sowing of the crop. The final weight of each instar was reduced at seven days after release when compared to non Bt cotton cultivars. Reduction in the weight was more in case of first, second and third instars than the fourth instar larvae of *S. litura*. Hence the BG-II cotton was more toxic to first, second, third instar larvae than fourth instar.

Introduction

Transgenic cotton, *Gossypium hirsutum* (L.), expressing insecticidal *δ*-endotoxin protein of *Bacillus thuringiensis* Berliner, hereafter referred to as Bt cotton[1]. The introduction of Bt transgenic cotton hybrids in the year 2002-03 in all the cotton growing tracts of Andhra Pradesh revolutionized boll worm management of cotton resulting in a total shift in pesticide use pattern in the crop[2]. The single (Cry 1 Ac) gene Bt transgenic hybrids are highly resistant to boll worms, especially American boll worm, *Helicoverpa armigera* affording very good protection from this pest. However, the incidence of tobacco caterpillar, *S. litura* has been noticed on Bt cotton hybrids during vegetative phage to levels necessitating use of pesticide sprays to manage the pest. So, the BG-I cotton is slowly replaced by BG-II expressing dual genes (Cry 1Ac+Cry2Ab). Bollgard-II cotton expressing both Cry 1 Ac and Cry 2 Ab proteins have provided increased efficacy against the budworm-bollworm complex and enhanced spectrum of activity against beet armyworm, *Spodoptera exigua* (Hub.) fall armyworm, *Spodoptera frugiperda* (Smith)

and common cutworm or tobacco caterpillar *Spodoptera litura* (Fab.) which have been predicted to be major pests in emerging scenario[3,4,5]. The present study aims to understand the effect of double gene Bt transgenic cotton hybrids and non Bt cotton on the weight of different larval instars of *S. litura* through laboratory studies.

Materials and Methods

Laboratory experiments were carried out during 2013-14 to study the effect of eleven BG-II cotton hybrids *viz.*, Ajeeth-155, Ankur-3034, Chetak, ATM, Bhakti, Brahma, Rasi-665, Rasi-668, Denim, Sudarshan, Yuva and non Bt cotton plant parts (leaves, squares and bolls) as control against first, second, third and fourth instar larvae of *S. litura* at 60, 75, 90 and 125 DAS.

Eleven BG-II cotton hybrids and non Bt cotton plots were maintained at College Farm, College of Agriculture, Rajendranagar, Hyderabad during Kharif-2013. Field plots were maintained by standard agronomic practices, including herbicide and fertilizer applications.

Rearing of *S. litura* culture was carried out at BT lab, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad. Initially the culture was collected from the field on castor plants. After *S. litura* larvae were reared on castor leaves in BT lab. Immediately after first generation the experiments was conducted with the first, second, third and fourth instar larvae.

Leaves, squares and bolls were plucked with 0.5 cm of petiole remaining to prevent desiccation from the crop of 60, 75, 90 and 125 days old dual toxin Bt cotton hybrids and non Bt cotton. Later these were cleaned with distilled water and wiped off with blotting paper to remove excess moisture and air dried. The cleaned leaves, squares and bolls were placed after suitably trimming (to fit into the cups) individually into rearing cups. Within 1-2 h of harvesting, a single leaf/ 3-4 squares/ one boll were placed into individual rearing cups and larvae were placed into each cup. *S. litura* larval instars cultured on natural diet were used, three replications of 15 larvae for each treatment kept in rearing cups at ambient temperature in laboratory.

Treatments

Instars/treatmnets	First Instar	Second Instar	Third Instar	Fourth Instar
Treatments (Eleven BG-II hybrids)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)
	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)
		Bolls (90 and 125 DAS)	Bolls (90 and 125 DAS)	Bolls (90 and 125 DAS)
Control (Non Bt cotton)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)	Leaves (60, 75, 90 and 125 DAS)
	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)	Squares (60, 75, 90 and 125 DAS)
		Bolls (90 and 125 DAS)	Bolls (90 and 125 DAS)	Bolls (90 and 125 DAS)

Fresh leaves, squares and bolls collected as before, were provided to larvae up to seven days after release. Seven days after release the survived larval weight was recorded.

The data recorded on weight of *S. litura* larvae on Bollgard-II cotton were subjected to completely randomized design (CRD)[6]. The CRD analysis was done by using the statistical programme opstat.

Results and Discussion

First instar larvae of *S. litura* were more susceptible to leaves and squares of dual toxin hybrids than non Bt cotton. The weight of the first instar larvae was not recorded at seven days after release due to the 100 per cent mortality of first instar larvae on leaves of BG-II cotton.

The weight of second, third and fourth instar larvae of *S. litura* fed with leaves of eleven BG-II cotton hybrids was lowest when compared to non-Bt leaves (Table 1).

Minimum weight of second, third and fourth instar larvae fed with leaves of BG-II cotton hybrids recorded as 0.021, 0.055 and 0.173 g per larvae, respectively at seven days after release.

Similar results were also obtained by Naik[7] where in 0.016, 0.035 and 0.059 g per larval weight was observed in *S. litura* larvae fed with BG-II cotton hybrid leaves at 72 h after release.

The lowest weight of first, second, third and fourth instar larvae of *S. litura* fed with leaves were recorded on Rasi-665 BG-II hybrid. These observations were similar with the results of Soujanya[8] the weight of the larvae fed with dual toxin Bt cultivars was significantly reduced compared to the larvae fed with Bt and non-Bt cultivars and the reduction in the larval weight progressively increased from 24 to 72 h of feeding up to fifth instar and there was no significant difference in larval weights that were fed with Bt and non-Bt hybrids.

Squares

Minimum weight of first, second, third and fourth instar larvae fed with squares of BG-II cotton hybrids was recorded as 0.009, 0.020, 0.055 and 0.175 g per larvae at seven days after release in between 60 and 125 DAS (Tables 2 and 3).

Similar results were also obtained by Naik[7], where the weight of second, third and fourth instar larvae was 0.020, 0.039 and 0.066 g per larvae when fed with BGII cotton hybrids squares at 72 h after release.

Among eleven BG-II cotton hybrids Rasi-665 recorded lowest weight on squares at 60, 75, 90 and 125 DAS. On non-Bt the weight was more when compared to eleven BG-II cotton hybrids. The weight was less on squares, compared to leaves. These findings are in line with the results of Naik[7].

Bolls

Minimum weight of second, third and fourth instar larvae fed with bolls of BG-II cotton hybrid was recorded as 0.017, 0.053 and 0.191 g per larvae at seven days after release in between 60 and 125 DAS (Table 4).

Among all eleven BG-II cotton hybrids minimum weight of second, third and fourth instars at 90 and 125 DAS was recorded on bolls of Rasi-665.

On leaves, squares and bolls the weight of first, second, third and fourth instar larvae on BG-II cotton hybrids was significantly different from non-Bt cotton.

Table.1 Effect of test hybrid leaves on larval weight of *S. litura*.

Treatments	Weight of Second Instar larvae (g)				Weight of third Instar larvae (g)				Weight of fourth Instar larvae (g)			
	60 DAS	75 DAS	90 DAS	125 DAS	60 DAS	75 DAS	90 DAS	125 DAS	60 DAS	75 DAS	90 DAS	125 DAS
Ankur-3034	0.037 (1.02)^{a}	0.045 (1.02)^{a}	0.050 (1.03)^{a}	0.034 (1.02)^{a}	0.082 (1.04)^{a}	0.070 (1.04)^{a}	0.120 (1.06)^{a}	0.101 (1.05)^{a}	0.192 (1.09)^{a}	0.218 (1.10)^{a}	0.248 (1.12)^{a}	0.224 (1.11)^{a}
Ajeet-155	0.046 (1.02)^{a}	0.042 (1.02)^{a}	0.032 (1.02)^{a}	0.036 (1.02)^{a}	0.109 (1.05)^{a}	0.086 (1.04)^{a}	0.089 (1.04)^{a}	0.063 (1.03)^{a}	0.265 (1.13)^{a}	0.230 (1.11)^{a}	0.251 (1.12)^{a}	0.237 (1.11)^{a}
Chetak	0.046 (1.02)^{a}	0.034 (1.02)^{a}	0.038 (1.02)^{a}	0.032 (1.02)^{a}	0.097 (1.05)^{a}	0.086 (1.04)^{a}	0.087 (1.04)^{a}	0.069 (1.03)^{a}	0.281 (1.13)^{a}	0.225 (1.11)^{a}	0.248 (1.12)^{a}	0.233 (1.11)^{a}
ATM	0.031 (1.02)^{a}	0.038 (1.02)^{a}	0.042 (1.02)^{a}	0.036 (1.02)^{a}	0.091 (1.05)^{a}	0.069 (1.03)^{a}	0.074 (1.04)^{a}	0.062 (1.03)^{a}	0.210 (1.10)^{a}	0.201 (1.10)^{a}	0.235 (1.11)^{a}	0.224 (1.11)^{a}
Bhakti	0.023 (1.01)^{a}	0.038 (1.02)^{a}	0.037 (1.02)^{a}	0.025 (1.01)^{a}	0.084 (1.04)^{a}	0.086 (1.04)^{a}	0.072 (1.04)^{a}	0.072 (1.04)^{a}	0.254 (1.12)^{a}	0.221 (1.11)^{a}	0.209 (1.10)^{a}	0.231 (1.10)^{a}
Brahma	0.028 (1.01)^{a}	0.039 (1.02)^{a}	0.040 (1.020)^{a}	0.027 (1.01)^{a}	0.093 (1.05)^{a}	0.078 (1.04)^{a}	0.129 (1.06)^{a}	0.061 (1.03)^{a}	0.210 (1.10)^{a}	0.195 (1.09)^{a}	0.215 (1.10)^{a}	0.212 (1.10)^{a}
Denim	0.028 (1.01)^{a}	0.022 (1.01)^{a}	0.035 (1.02)^{a}	0.032 (1.02)^{a}	0.087 (1.04)^{a}	0.072 (1.04)^{a}	0.112 (1.05)^{a}	0.058 (1.03)^{a}	0.284 (1.13)^{a}	0.187 (1.09)^{a}	0.223 (1.11)^{a}	0.217 (1.10)^{a}
Rasi-665	0.021 (1.01)^{a}	0.021 (1.01)^{a}	0.025 (1.01)^{a}	0.025 (1.01)^{a}	0.093 (1.05)^{a}	0.069 (1.03)^{a}	0.071 (1.04)^{a}	0.055 (1.03)^{a}	0.191 (1.09)^{a}	0.175 (1.08)^{a}	0.173 (1.08)^{a}	0.175 (1.10)^{a}
Rasi-668	0.026 (1.01)^{a}	0.027 (1.01)^{a}	0.028 (1.01)^{a}	0.027 (1.01)^{a}	0.071 (1.04)^{a}	0.082 (1.04)^{a}	0.084 (1.04)^{a}	0.061 (1.03)^{a}	0.198 (1.10)^{a}	0.198 (1.10)^{a}	0.201 (1.10)^{a}	0.217 (1.10)^{a}
Sudarshan	0.025 (1.01)^{a}	0.036 (1.02)^{a}	0.034 (1.02)^{a}	0.040 (1.02)^{a}	0.101 (1.05)^{a}	0.078 (1.04)^{a}	0.083 (1.04)^{a}	0.083 (1.04)^{a}	0.252 (1.12)^{a}	0.189 (1.10)^{a}	0.208 (1.10)^{a}	0.177 (1.09)^{a}
Yuva	0.026 (1.01)^{a}	0.043 (1.02)^{a}	0.033 (1.02)^{a}	0.053 (1.03)^{a}	0.087 (1.04)^{a}	0.081 (1.04)^{a}	0.089 (1.04)^{a}	0.091 (1.05)^{a}	0.226 (1.11)^{a}	0.242 (1.11)^{a}	0.185 (1.09)^{a}	0.233 (1.11)^{a}
Control	0.032 (1.02)^{a}	0.048 (1.02)^{a}	0.033 (1.02)^{a}	0.038 (1.02)^{a}	0.09 (1.04)^{a}	0.087 (1.04)^{a}	0.096 (1.05)^{a}	0.084 (1.04)^{a}	0.231 (1.11)^{a}	0.287 (1.13)^{a}	0.239 (1.11)^{a}	0.256 (1.12)^{a}
CD	0.004	0.005	0.004	0.003	0.004	0.005	0.009	0.003	0.026	0.01	0.005	0.008
SE (m)	0.002	0.002	0.001	0.001	0.001	0.002	0.003	0.001	0.01	0.004	0.002	0.003

Figures in parentheses are square root transformed values; numbers followed by same superscript are not statistically different.
*First instar recorded 100 per cent mortality.

Table.2 Effect of test hybrid squares on weight of first and second instar larvae of *S. litura*.

Treatments	Weight of first Instar larvae (g)				Weight of second Instar larvae (g)			
	60 DAS	75 DAS	90 DAS	125 DAS	60 DAS	75 DAS	90 DAS	125 DAS
Ankur-3034	0.010 (1.01)[a]	0.010 (1.01)[a]	0.011 (1.01)[a]	0.009 (1.01)[a]	0.028 (1.01)[a]	0.024 (1.01)[a]	0.038 (1.02)[a]	0.033 (1.02)[a]
Ajeet-155	0.015 (1.01)[a]	0.013 (1.01)[a]	0.012 (1.01)[a]	0.012 (1.01)[a]	0.052 (1.03)[a]	0.039 (1.02)[a]	0.041 (1.02)[a]	0.036 (1.02)[a]
Chetak	0.011 (1.01)[a]	0.009 (1.00)[a]	0.010 (1.01)[a]	0.011 (1.01)[a]	0.038 (1.02)[a]	0.033 (1.02)[a]	0.032 (1.02)[a]	0.031 (1.02)[a]
ATM	0.009 (1.01)[a]	0.008 (1.00)[a]	0.008 (1.00)[a]	0.010 (1.01)[a]	0.022 (1.01)[a]	0.021 (1.01)[a]	0.035 (1.02)[a]	0.029 (1.01)[a]
Bhakti	0.010 (1.01)[a]	0.009 (1.00)[a]	0.008 (1.00)[a]	0.010 (1.01)[a]	0.028 (1.01)[a]	0.035 (1.02)[a]	0.033 (1.02)[a]	0.029 (1.01)[a]
Brahma	0.010 (1.01)[a]	0.008 (1.00)[a]	0.012 (1.01)[a]	0.011 (1.01)[a]	0.027 (1.01)[a]	0.030 (1.02)[a]	0.041 (1.02)[a]	0.028 (1.01)[a]
Denim	0.010 (1.01)[a]	0.008 (1.00)[a]	0.009 (1.00)[a]	0.007 (1.00)[a]	0.033 (1.02)[a]	0.030 (1.02)[a]	0.041 (1.02)[a]	0.027 (1.01)[a]
Rasi-665	0.009 (1.01)[a]	0.005 (1.00)[a]	0.007 (1.00[a]	0.007 (1.00)[a]	0.020 (1.01)[a]	0.021 (1.01)[a]	0.026 (1.01)[a]	0.024 (1.01)[a]
Rasi-668	0.009 (1.00)[a]	0.011 (1.01)[a]	0.011 (1.01)[a]	0.009 (1.01)[a]	0.034 (1.02)[a]	0.025 (1.01)[a]	0.029 (1.01)[a]	0.027 (1.01)[a]
Sudarshan	0.009 (1.01)[a]	0.008 (1.00)[a]	0.009 (1.00)[a]	0.009 (1.00)[a]	0.035 (1.02)[a]	0.033 (1.02)[a]	0.038 (1.02)[a]	0.029 (1.01)[a]
Yuva	0.012 (1.01)[a]	0.012 (1.01)[a]	0.012 (1.01)[a]	0.012 (1.01)[a]	0.043 (1.02)[a]	0.029 (1.02)[a]	0.035 (1.02)[a]	0.031 (1.02)[a]
Control	0.010 (1.01)[a]	0.015 (1.01)[a]	0.016 (1.01)[a]	0.015 (1.01)[a]	0.046 (1.02)[a]	0.038 (1.02)[a]	0.033 (1.02)[a]	0.031 (1.02)[a]
CD	0.001	0.001	0.001	0.001	0.004	0.003	0.002	0.002
SE (m)	0	0	0	0	0.001	0.001	0.001	0.001

Figures in parentheses are square root transformed values; numbers followed by same superscript are not statistically different.

Table.3 Effect of test hybrid squares on weight of third and fourth instar larvae of *S. litura*.

Treatments	Weight of third Instar larvae (g)				Weight of fourth Instar larvae (g)			
	60 DAS	75 DAS	90 DAS	125 DAS	60 DAS	75 DAS	90 DAS	125 DAS
Ankur-3034	0.076 (1.04)[a]	0.075 (1.04)[a]	0.096 (1.05)[a]	0.080 (1.04)[a]	0.198 (1.10)[a]	0.192 (1.09)[a]	0.196 (1.09)[a]	0.181 (1.09)[a]
Ajeet-155	0.099 (1.05)[a]	0.094 (1.05)[a]	0.107 (1.05)[a]	0.099 (1.05)[a]	0.222 (1.11)[a]	0.226 (1.11)[a]	0.234 (1.11)[a]	0.221 (1.11)[a]
Chetak	0.088 (1.04)[a]	0.081 (1.04)[a]	0.082 (1.04)[a]	0.070 (1.04)[a]	0.216 (1.10)[a]	0.187 (1.09)[a]	0.215 (1.10)[a]	0.188 (1.09)[a]
ATM	0.090 (1.04)[a]	0.069 (1.03)[a]	0.079 (1.04)[a]	0.065 (1.03)[a]	0.196 (1.10)[a]	0.199 (1.10)[a]	0.208 (1.10)[a]	0.193 (1.09)[a]
Bhakti	0.091 (1.05)[a]	0.089 (1.04)[a]	0.064 (1.03)[a]	0.083 (1.04)[a]	0.235 (1.11)[a]	0.189 (1.09)[a]	0.214 (1.10)[a]	0.201 (1.10)[a]
Brahma	0.086 (1.04)[a]	0.083 (1.04)[a]	0.083 (1.04)[a]	0.060 (1.03)[a]	0.224 (1.11)[a]	0.218 (1.10)[a]	0.207 (1.10)[a]	0.234 (1.11)[a]
Denim	0.092 (1.05)[a]	0.085 (1.04)[a]	0.080 (1.04)[a]	0.055 (1.03)[a]	0.219 (1.10)[a]	0.215 (1.10)[a]	0.232 (1.11)[a]	0.227 (1.10)[a]
Rasi-665	0.074 (1.04)[a]	0.066 (1.03)[a]	0.077 (1.04)[a]	0.058 (1.03)[a]	0.199 (1.10)[a]	0.177 (1.09)[a]	0.191 (1.09)[a]	0.175 (1.08)[a]
Rasi-668	0.076 (1.04)[a]	0.074 (1.04)[a]	0.084 (1.04)[a]	0.055 (1.03)[a]	0.193 (1.09)[a]	0.198 (1.02)[a]	0.207 (1.10)[a]	0.179 (1.08)[a]
Sudarshan	0.091 (1.05)[a]	0.088 (1.04)[a]	0.090 (1.04)[a]	0.082 (1.04)[a]	0.209 (1.10)[a]	0.197 (1.09)[a]	0.213 (1.10)[a]	0.177 (1.09)[a]
Yuva	0.077 (1.04)[a]	0.076 (1.04)[a]	0.079 (1.04)[a]	0.084 (1.04)[a]	0.234 (1.11)[a]	0.194 (1.09)[a]	0.240 (1.11)[a]	0.223 (1.11)[a]
control	0.095 (1.05)[a]	0.089 (1.04)[a]	0.084 (1.04)[a]	0.083 (1.04)[a]	0.225 (1.11)[a]	0.281 (1.13)[a]	0.255 (1.12)[a]	0.224 (1.11)[a]
CD	0.005	0.004	0.003	0.005	N.S.	0.017	0.01	0.014
SE (m)	0.002	0.002	0.001	0.002	0.006	0.006	0.003	0.005

Figures in parentheses are square root transformed values; numbers followed by same superscript are not statistically different.

Table.4 Effect of test hybrid bolls on larval weight of *S. litura*.

Treatments	Weight of second Instar larvae (g)		Weight of third Instar larvae (g)		Weight of fourth Instar larvae (g)	
	90 DAS	125 DAS	90 DAS	125 DAS	90 DAS	125 DAS
Ankur-3034	0.035 (1.02)[a]	0.034 (1.02)[a]	0.090 (1.04)[a]	0.087 (1.04)[a]	0.234 (1.11)[a]	0.231 (1.11)[a]
Ajeet-155	0.045 (1.02)[a]	0.037 (1.02)[a]	0.081 (1.04)[a]	0.092 (1.05)[a]	0.267 (1.13)[a]	0.335 (1.16)[a]
Chetak	0.029 (1.01)[a]	0.028 (1.01)[a]	0.087 (1.04)[a]	0.083 (1.04)[a]	0.211 (1.10)[a]	0.228 (1.11)[a]
ATM	0.033 (1.02)[a]	0.029 (1.01)[a]	0.084 (1.04)[a]	0.089 (1.04)[a]	0.204 (1.10)[a]	0.267 (1.13)[a]
Bhakti	0.028 (1.01)[a]	0.028 (1.01)[a]	0.086 (1.04)[a]	0.086 (1.04)[a]	0.255 (1.12)[a]	0.271 (1.13)[a]
Brahma	0.030 (1.02)[a]	0.022 (1.01)[a]	0.077 (1.04)[a]	0.062 (1.03)[a]	0.243 (1.12)[a]	0.218 (1.10)[a]
Denim	0.029 (1.01)[a]	0.031 (1.02)[a]	0.084 (1.04)[a]	0.063 (1.03)[a]	0.234 (1.11)[a]	0.253 (1.12)[a]
Rasi-665	0.024 (1.01)[a]	0.017 (1.01)[a]	0.073 (1.04)[a]	0.053 (1.03)[a]	0.191 (1.09)[a]	0.249 (1.12)[a]
Rasi-668	0.028 (1.01)[a]	0.021 (1.01)[a]	0.086 (1.04)[a]	0.062 (1.03)[a]	0.217 (1.10)[a]	0.224 (1.11)[a]
Sudarshan	0.033 (1.02)[a]	0.043 (1.02)[a]	0.083 (1.04)[a]	0.082 (1.04)[a]	0.191 (1.09)[a]	0.313 (1.15)[a]
Yuva	0.039 (1.02)[a]	0.042 (1.02)[a]	0.076 (1.04)[a]	0.086 (1.04)[a]	0.219 (1.104)[a]	0.221 (1.11)[a]
Control	0.030 (1.02)[a]	0.031 (1.02)[a]	0.090 (1.04)[a]	0.087 (1.04)[a]	0.252 (1.12)[a]	0.371 (1.17)[a]
CD	0.004	0.003	0.005	0.004	0.006	0.008
SE (m)	0.001	0.001	0.002	0.001	0.002	0.003

Figures in parentheses are square root transformed values; numbers followed by same superscript are not statistically different.

However, statistically all eleven BG-II cotton hybrids were on par with each other. Rasi-665 BG-II cotton recorded minimum weight of first, second, third and fourth instar in between 60 and 125 DAS. The present results suggest that dual toxin Bt cottons will provide substantially better control of *S. litura.*

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How to cite this article:

Ramanjali, T., T.V.K. Singh and Narendra Reddy, C. 2017. Effect of BG-II Cotton Hybrids and Non Bt Cotton on Weight of Different Instars of *Spodoptera litura* (FAB.). *Int.J.Curr.Microbiol.App.Sci.* 6(10): 2170-2177. doi: https://doi.org/10.20546/ijcmas.2017.610.257