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RESEARCH ARTICLE

SEASON LONG BIO-EFFICACY OF FIRST AND SECOND GENERATION BT COTTON GENOTYPES AGAINST *HELICOVERPA ARMIGERA* (HUBNER), *EARIAS VITTELLA* (FABRICIUS), *SPODOPTERA LITURA* (FABRICIUS) AND *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) UNDER RAINFED CONDITIONS

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ABSTRACT

Season long bioefficacy of *Bacillus thuringiensis* (Bt) insecticidal protein against *Helicoverpa armigera* (Hubner), *Earias vittella* (Fabricius), *Spodoptera litura* (Fabricius) and *Pectinophora gossypiella* (Saunders) in Bt transgenic cotton RCH-2 BG-II (*cry*1Ac+*cry*2Ab) and RCH-2 Bt (*cry*1Ac) were investigated in Dharwad region of Karnataka, India, in 2007-08. The results showed that, the toxin content (larval mortality) in Bt cotton changed/ gradually declined as the advancement of the cropping season. Generally, insecticidal protein (larval mortality) levels were high during the early stage of the crop growth, gradually declined over time. Compared with RCH-2Bt, the expression (mortality) of *cry*1Ac+*cry*2Ab protein in RCH-2BG-II was significantly high during the whole cropping period.

INTRODUCTION

Helicoverpa armigera (Hubner), *Earias vittella* (Fabricius), *Spodoptera litura* (Fabricius) and *Pectinophora gossypiella* (Saunders) are the most important pests of cotton and developing resistance against insecticides in India as well as throughout the world. Bollgard-I (BG-I) contains only one gene (*cry*1Ac) which offers protection against lepidopteran pests and known to be effective against *H. armigera*, *E. vittella* and *P. gossypiella* in India. The use of refugia to mitigate the expected resistance development found to be inconvenient and later two genes *viz.*, *cry*1Ac + *cry*2Ab (referred as Bollgard-II) concept came in to existence. Bollgard-II (BG-II) provides season long control of key pests of cotton including *Spodoptera litura* (Fabricius) and *H. armigera* pests.

Investigations on Bt cotton in the Dharwad region in 2007-08 shown that, the expression of toxin (cause mortality) at a high level in early stage of the cropping period but gradually declined as the advancement of the crop growth has resulted in reduced bio-efficacy late in the season (Sun *et al.*(2002), Olsen *et al.* (2003), Olsen *et al.*(2005), Kranthi *et al.*(2005), Udikeri (2006), Shelkar and Regupathi (2004), Wan *et al.*(2005)). However, BG-II genotype significantly superior with respect to larval mortality over BG-I genotype.

MATERIAL AND METHODS

Cotton lines: Three cotton genotypes were evaluated. Two transgenic cotton genotypes, RCH-2 BG-II, containing a *cry*1Ac+*cry*2Ab fused genes, RCH-2 Bt, expressing a Cry 1Ac gene and one non Bt cotton, RCH-2.

Experiments were conducted during 2007-08 in Dharwad region of Karnataka, India. The field experimental design was RBD, replicated four times and seeds were dibbled at a spacing of 90X60 cm and the space between treatments was 1.00m and replications were placed 1.50 m apart. All plots were nonirrigated and maintained using the standard package of practice except plant protection measures.

Bio-assay of Bt cotton genotypes

Season long bio-efficacy of two cotton genotypes (RCH-2BG-II, RCH-2 Bt) was assessed through bio-assay in 2007-08. Top canopy leaves, squares and bolls were collected randomly from

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different cotton plants of respective treatments excluding border rows at 20 interval and collected material from individual plants were pooled and assayed. The bioassay was carried out in laboratory at 60, 80, 100, and 120 DAS by leaf feeding method for *H. armigera* and *S. litura*. Squares were used for *E. vitella* and tiny bolls for *P. gossypiella*. In each treatment, there were 10 larvae replicated 4 times and in all the cases, two days old neonates were used for bio-assay. The larvae were released @ 1/well on leaf disc of 2.0 cm diameter and closed tightly with serene wrap and lid. The discs were changed every day. The leaf discs were placed on semi-wet filter paper disc of similar size to avoid drying of test material. Rearing trays of 25 wells were used for bioassay. Small plastic cups and pet jars were used for squares and bolls. The squares and bolls were placed in the jars/cup having 0.5 per cent solidified agar solution at the bottom for maintenance of moisture. The lid of the jar/cup was closed tightly after releasing the larvae at the rate of one per square or boll (Udikeri, 2006). The mortality of the larvae at 24 hours interval till seven days was recorded and converted as per cent mortality and later corrected using mortality in the control treatment (RCH-2 non Bt) and only corrected mortality in each treatment were considered for analysis.

RESULTS

Mortality of *H. armigera*

When the neonates of *H. armigera* subjected for bioassay with top canopy leaves of RCH-2 BG-II and RCH-2 Bt, the toxin caused significantly highest mortality of 94.38 per cent in RCH-2 BG-II compared to RCH-2 Bt (79.85%). In general, there was gradual decrease in per cent mortality from 60 to 120 DAS. The interaction effect on larval mortality indicated significantly highest mortality in RCH-2 BG-II at 60 (100%), 80 (100%), and 100DAS (95.00%) and RCH-2 Bt at 60 (100%), 80DAS (92.00%) which was statistically superior to RCH-2 BG-II at 120DAS (82.55%), RCH-2 Bt at 100 DAS (79.15%) and 120 DAS (47.75%). The per cent mortality in RCH-2 BG-II at 120 DAS (82.55%) and RCH-2 Bt at 100DAS (79.15%) recorded same as they were statistically at par with each other. Significantly lowest per cent mortality of 47.75 was recorded in RCH-2 Bt at 120DAS compared to others (Table 1, Fig 1).

Mortality of *E. vittella*

Neonates of *E. vittella* subjected for bioassay with squares of RCH-2 BG-II and RCH-2 Bt revealed that RCH-2 BG-II (96.96%) recorded significantly highest mortality compared to RCH-2 Bt (86.77%). In general, there was gradual decline in per cent larval mortality from 60 to 120 DAS. The interaction effect revealed that the per cent mortality in RCH-2 BG-II at all the periods of observation and RCH-2 Bt at 60 DAS (97.21%), 80 DAS (91.66%) remained same as they were statistically at par with each other. However, significantly lowest per cent mortality of 70.05 was recorded in RCH-2 Bt at 120 DAS (Table 2, Fig 2).

Mortality of *S. litura*

When the neonates of *S. litura* subjected for bioassay with top canopy leaves of RCH-2 BG-II and RCH-2 Bt indicated that RCH-2 BG-II (77.76%) recorded significantly highest mortality of *S. litura* larvae compared to RCH-2 Bt (8.76%). Further the mortality of *S. litura* larvae remained same at all the four periods of observations. The interaction effect revealed that the per cent mortality was significantly higher in RCH-2 BG-II compared to RCH-2 Bt from 60 to 120DAS. Thus it is very clear from the results that the mortality in RCH-2 BG-II was highest (71.86 to 84.72%) compared to RCH-2 Bt where the mortality was negligible which ranged from 6.25 to 11.80 per cent (Table 3, Fig 3).

Mortality of *P. gossypiella*

When the neonates of *P. gossypiella* subjected for bioassay with tiny bolls of RCH-2 BG-II and RCH-2 Bt revealed that RCH-2 BG-II recorded significantly highest mortality of 88.92 per cent compared to RCH-2 Bt (75.21%). The per cent mortality of *P. gossypiella* declined gradually from 89.18 to 75.17 per cent with advancement of cropping season. The interaction effect revealed significantly highest larval mortality in RCH-2 BG-II at 80 (94.75%) and 100DAS (88.47%). Significantly lowest per cent larval mortality of 66.80 was recorded in RCH-2 Bt at 120 DAS being at par with RCH-2 Bt at 100DAS (75.45%), RCH-2 BG-II AT 120 DAS (83.55%) and RCH-2 Bt at 80 DAS (83.61%) (Table 4, Fig 4).

Table 1 Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *Helicoverpa armigera*

	Neonate mortality (%)				
Bt cotton genotype/ DAS	60	80	100	120	Mean
RCH-2 BG-II	100.00 a (89.96)	100.00 a (89.96)	95.00 a (80.75)	82.55b (65.61)	94.38a (81.57)
RCH-2 Bt	100.00 a (89.96)	92.50 a (78.71)	79.15 b (63.45)	47.75 c (43.62)	79.85 b (68.94)
Mean	100.00 a (89.96)	96.25 a (84.34)	87.07 b (72.10)	65.15 c (54.61)	
	SEm±			CD at 5%	
Genotype	2.03			5.97	
DAS	2.88			8.47	
Interaction	4.07			11.96	

DAS: Days after sowing Figures in parentheses are arcsine values
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table 2 Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *Earias vittella*

	Neonate mortality (%)				
Bt cotton genotype / DAS	60	80	100	120	Mean
RCH-2 BG-II	100.00 a (89.96)	97.25 ab (85.09)	96.85 ab (84.79)	93.75 ab (82.46)	96.96 a (85.58)
RCH-2 Bt	97.21 ab (85.09)	91.66 ab (78.07)	88.19 b (72.58)	70.05 c (56.87)	86.77 b (73.15)
Mean	98.60 a (87.53)	94.55 a (81.58)	92.52 ab (78.68)	81.90 b 969.67)	
	SEm±			CD at 5%	
Genotype	2.41			7.11	
DAS	3.41			10.06	
Interaction	4.83			14.22	

DAS: Days after sowingFigures in parentheses are arcsine values
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table 3 Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *Spodoptera litura*

Bt cotton genotype / DAS	Neonate mortality (%) 60	80	100	120	Mean
RCH-2 BG-II	84.72 a (67.25)	80.83 a (64.27)	73.65 a (59.49)	71.86 a (58.35)	77.76 a (62.34)
RCH-2 Bt	11.80b (20.08)	8.68 b (14.91)	8.33b (14.60)	6.25 b (10.35)	8.76 b (14.98)
Mean	48.26a (43.67)	44.75 a (39.59)	40.99a (37.04)	39.05a (36.35)	
		SEm±		CD at 5%	
Genotype		2.10		6.17	
DAS		2.96		8.71	
Interaction		4.20		12.35	

DAS: Days after sowing
Figures in parentheses are arcsine values
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table 4 Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *Pectinophora gossypiella*

Bt cotton genotype / DAS	Neonate mortality (%) 80	100	120	Mean
RCH-2 BG-II	94.75 a (80.49)	88.47ab (72.99)	83.55 bc (66.26)	88.92 a (73.25)
RCH-2 Bt	83.61bc (66.44)	75.45 bc (60.65)	66.80 c (54.91)	75.28 b (60.66)
Mean	89.18 a (73.46)	81.96 ab (66.82)	75.17 b (60.58)	
	SEm±		CD at 5%	
Genotype	2.36		7.10	
DAS	2.88		8.69	
Interaction	4.08		12.3	

DAS: Days after sowing
Figures in parentheses are arcsine values
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

DISCUSSION

The superiority of RCH2-BG-II over RCH-2 Bt was evident from the results (Table 1 to 4 and Fig. 1 to 4) where the mortality of neonate larvae of all the four lepidopteran pests *viz*., *H. armigera*, *E vittella*, *S. litura* and *P. gossypiella* was significantly higher, which aggress with the bioassay as well as toxin quantification reports of Udikeri (2006) and field performance (Udikeri *et al*, 2011)

The exact impact of Bt transgenic cottons expressing one (Cry 1 Ac) or two (Cry 1 Ac+ 2 Ab) toxin producing genes on *E.vittella* has been better explained by Somashekara *et al.*, (2011) which hold good for other pests also considered in this study.

The difference in larval mortality of *H. armigera*, *E vittella*, and *P. gossypiella* between BG-II and BG-I was very narrow ranging from 10.19 to 14.53 per cent, while in case of *S. litura* there was wide difference of 69 per cent between RCH-2 BG-II and RCH-2 Bt. which is due to gene pyramiding in RCH-2 BG-II (*cry*1Ac + *cry*2Ab) having an additional advantage of mortality of *S. litura* apart from controlling other bollworms.

In general there was gradual decline in the larval mortality of all the four lepidopteran pests with the advancement of cropping season, which may due to decline in the *cry* protein expression. The present findings are in close agreement with Sun *et al.*(2002), Olsen *et al.* (2003), Olsen *et al.*(2005), Kranthi *et al.*(2005), Shelkar and Regupathi (2004), Wan *et al.* (2005).

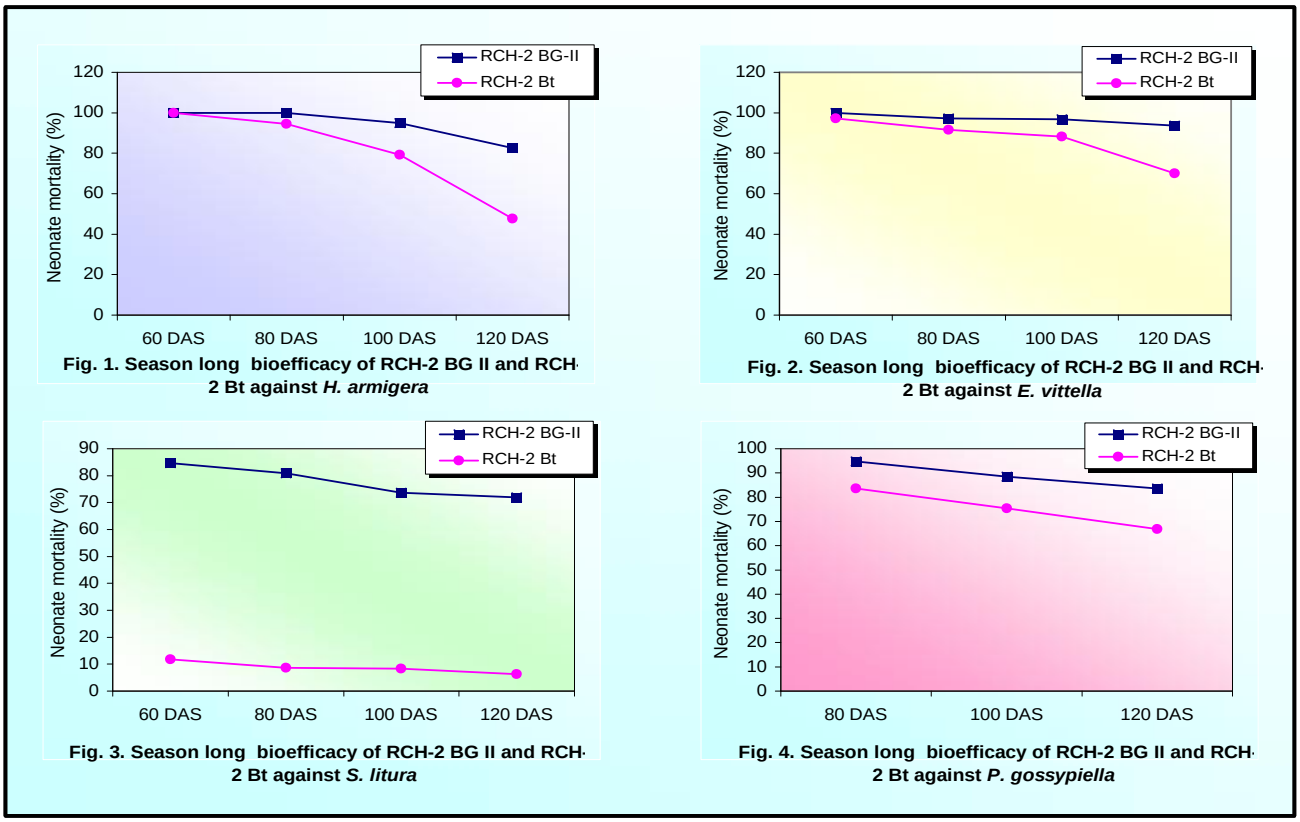

Fig. 1. Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *H. armigera*

Fig. 2. Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *E. vittella*

Fig. 3. Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *S. litura*

Fig. 4. Season long bioefficacy of RCH-2 BG II and RCH-2 Bt against *P. gossypiella*

Further the characterization of resistance to three bollworms and *S.litura* for all Bt transgenic events commercialized in India has been elaborated through neonate and late instars larval bioassays (Hallad *et al* ., 2011, Hallad *et al* 2014a) as well as field performance studies (Hallad *et al* ., 2014b) which strongly justifies significance present findings.

Thus through bioassay studies to different species of bollworms and *S. litura* which was considered a great threat to BG-I Bt cottons, significant advantage of second generation genotype has been unearthed critically. In Indian pest scenario perspective, the results of present findings could be considered very much significant.

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