

Transgenic cotton expressing stacked *cry1A(c)* and *cry2A(b)* genes (BGII) : Effects on soil microflora and beneficial and non-target soil organisms.

Report

Submitted to RCGM

Date: April, 2003

mahyco[®]

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Study title:

Transgenic cotton expressing stacked *cryIA(c)* and *cry2A(b)* genes (BGII) : Effects on soil microflora and beneficial and non-target soil organisms.

Objective:

The objective of this study is to assess the safety of BGII cotton expressing stacked *cryIA(c)* and *cry2A(b)* genes (BGII) for soil organisms especially soil microflora and non-target insects.

Study conducted by:

Biotechnology R&D, Maharashtra Hybrid Seeds Co. Ltd., Jalna (Maharashtra).

Summary:

An important aspect of the biosafety assessment of genetically modified (transgenic) plants is to study their impact on soil ecosystem including changes in the plant-associated microflora. In the described research, evaluation of the effect of BGII cotton expressing *Bacillus thuringiensis* (Bt) stacked *cryIA(c)* and *cry2A(b)* genes (BGII) on soil microflora and soil organisms including earthworm and collembola has been carried out. BGII cotton of seven different hybrids viz., MRC-6160 BGII, MRC-6201 BGII, MRC-6221 BGII, MRC-6301 BGII, MRC-6322 BGII, MRC-6326 BGII and MRC-6703 BGII and their non-bollgard counter parts are being grown for experimental purposes in six different locations (Barwah, Coimbatore, Jalna, Ranebennur, Vadodra and Warangal) during 2002-03. MECH-62 Bt cotton expressing *cryIAC* gene, and NHH-44, a popular check, have been included as controls in these field trials. Soil samples were collected at set time points from these locations and were analyzed for the levels of BGII protein, and effect on microflora. Effect of BGII cotton on soil invertebrates including collembola and earthworms were also evaluated by monitoring their population in the experimental fields at different stages of plant growth. There were no significant differences between BGII and non-BGII control treatments in the quantity of the total culturable bacterial and fungal populations. Earthworms were observed at Jalna, Ranebennur, Vadodra and Warangal locations. No significant variations in soil insect populations were observed between BGII and non-BGII treatments. Whole nematode communities extracted from

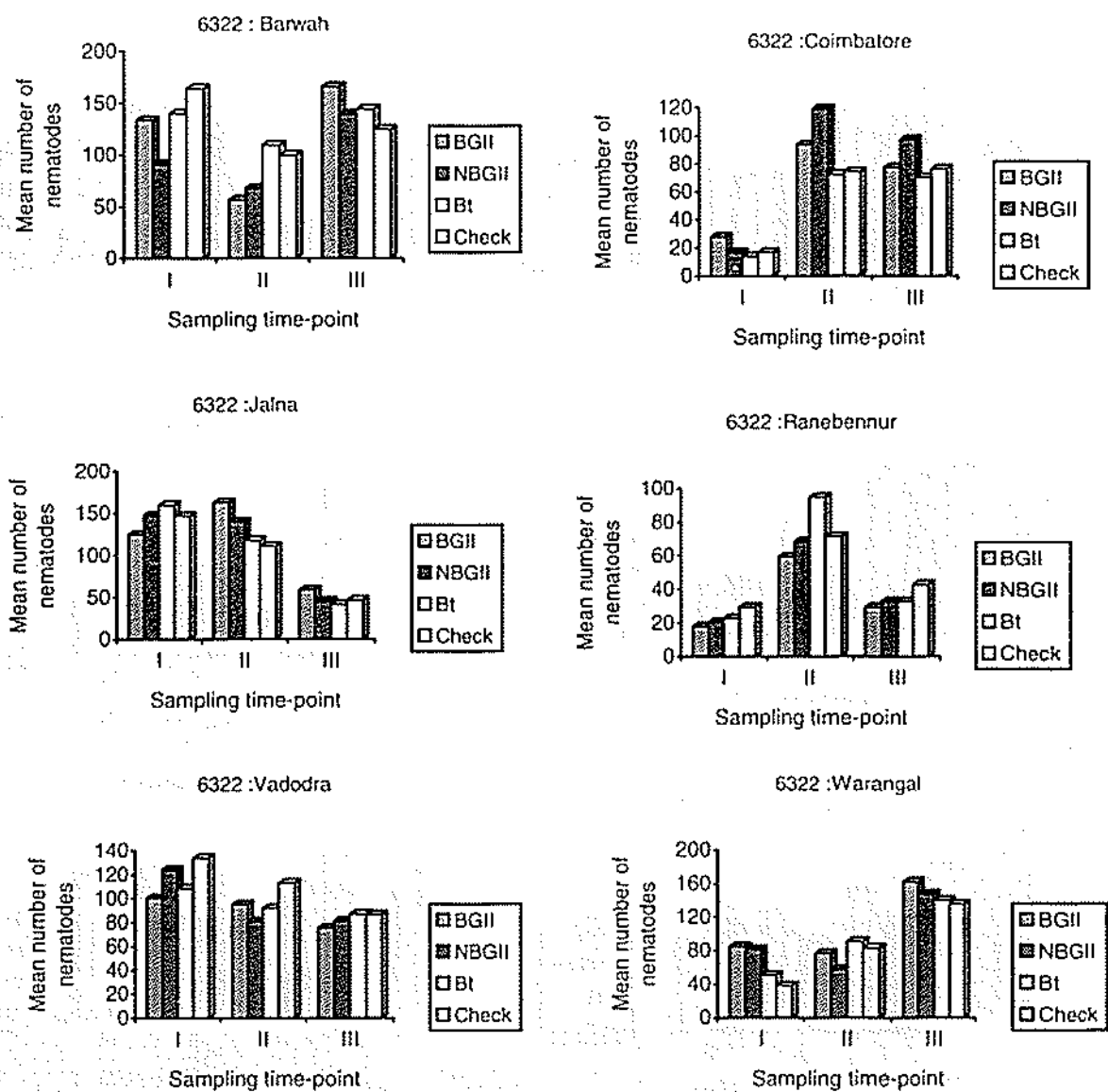


Figure 5. Measurement of nematode population in Bollgard II cotton fields. Sampling time point I and II refer to collection of soil samples for nematode analysis done at 60 and 120 days after sowing respectively. Sampling time-point III represents the post harvest analysis. Sampling time-point wise Least significant difference (LSD) values are as follows: a) Barwah: 14.75, 40.52 and 49.21, b) Coimbatore: 21.55, 40.02 and 30.39, c) Jalna: 85.33, 39.99 and 20.33, d) Ranebennur: 9.31, 65.08 and 13.30, e) Vadodra: 43.03, 41.31 and 28.55, and f) Warangal: 24.4, 32.37 and 22.24.

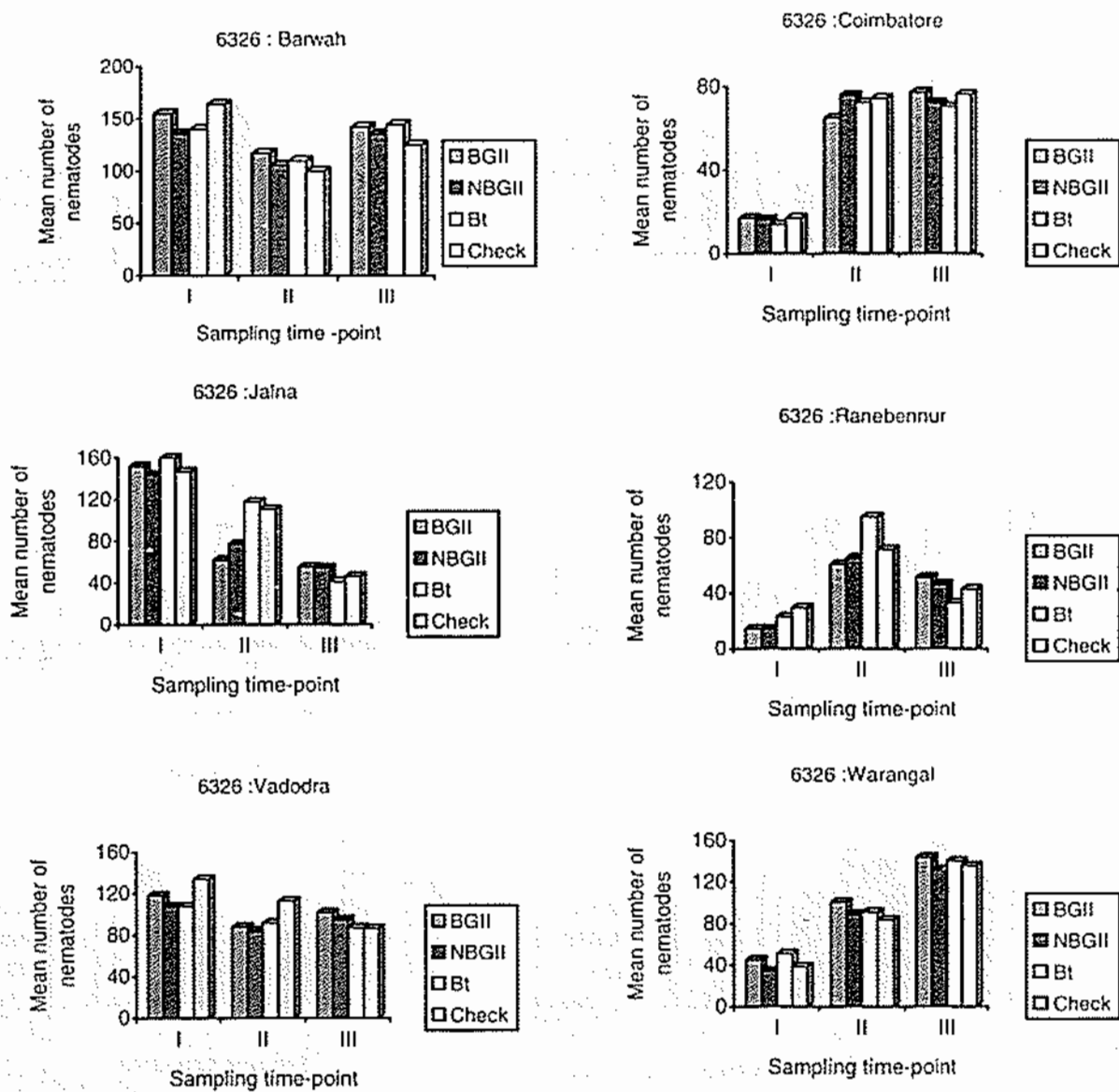


Figure 6. Measurement of nematode population in Bollgard II cotton fields. Sampling time point I and II refer to collection of soil samples for nematode analysis done at 60 and 120 days after sowing respectively. III time point represents the post harvest analysis. Sampling time-point wise Least significant difference (LSD) values are as follows: a) Barwah: 10.71, 49.72, and 57.6, b) Coimbatore: 9.44, 31.42 and 30.6, c) Jalna: 96.2, 36.77 and 17.62, d) Ranebennur: 25.8, 21.93 and 19.70, e) Vadodra : 77.56, 63.4 and 27.71, and f) Warangal : 4.68, 33.12, and 30.27.

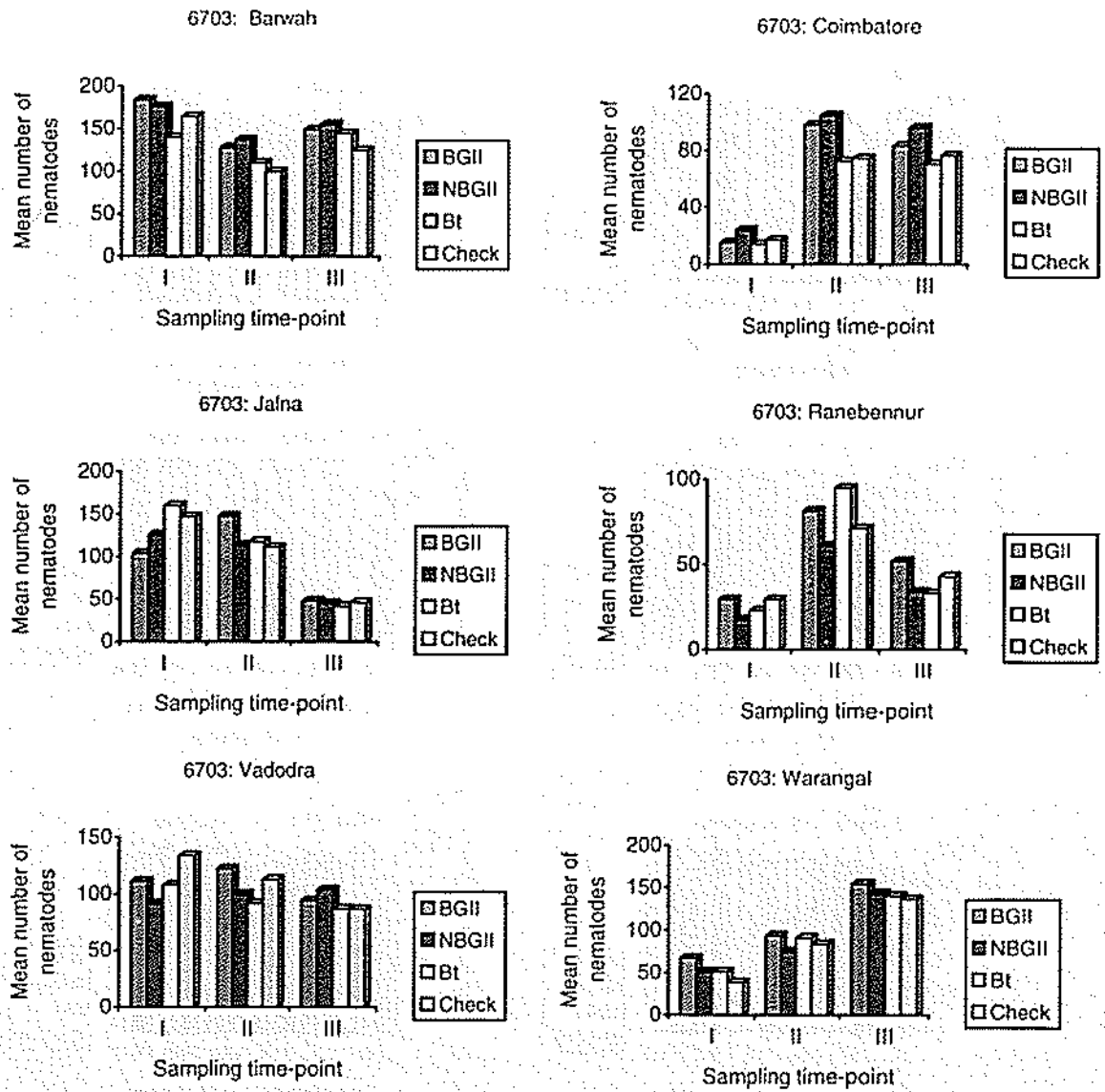


Figure 7. Measurement of nematode population in Bollgard II cotton fields. Sampling time point I and II refer to collection of soil samples for nematode analysis done at 60 and 120 days after sowing respectively. Time point III represents the post harvest analysis. Sampling time-point wise Least significant difference (LSD) values are as follows: a) Barwah: 60.17, 46.65 and 96.85, b) Coimbatore: 18.92, 36.02 and 26.46, c) Jalna: 97.88, 39.19 and 27.56, d) Ranebennur : 28.13, 33.53 and 15.32, e) Vadodra : 43.03, 32.05 and 25.0, and f) Warangal : 14.14, 41.35 and 27.07.

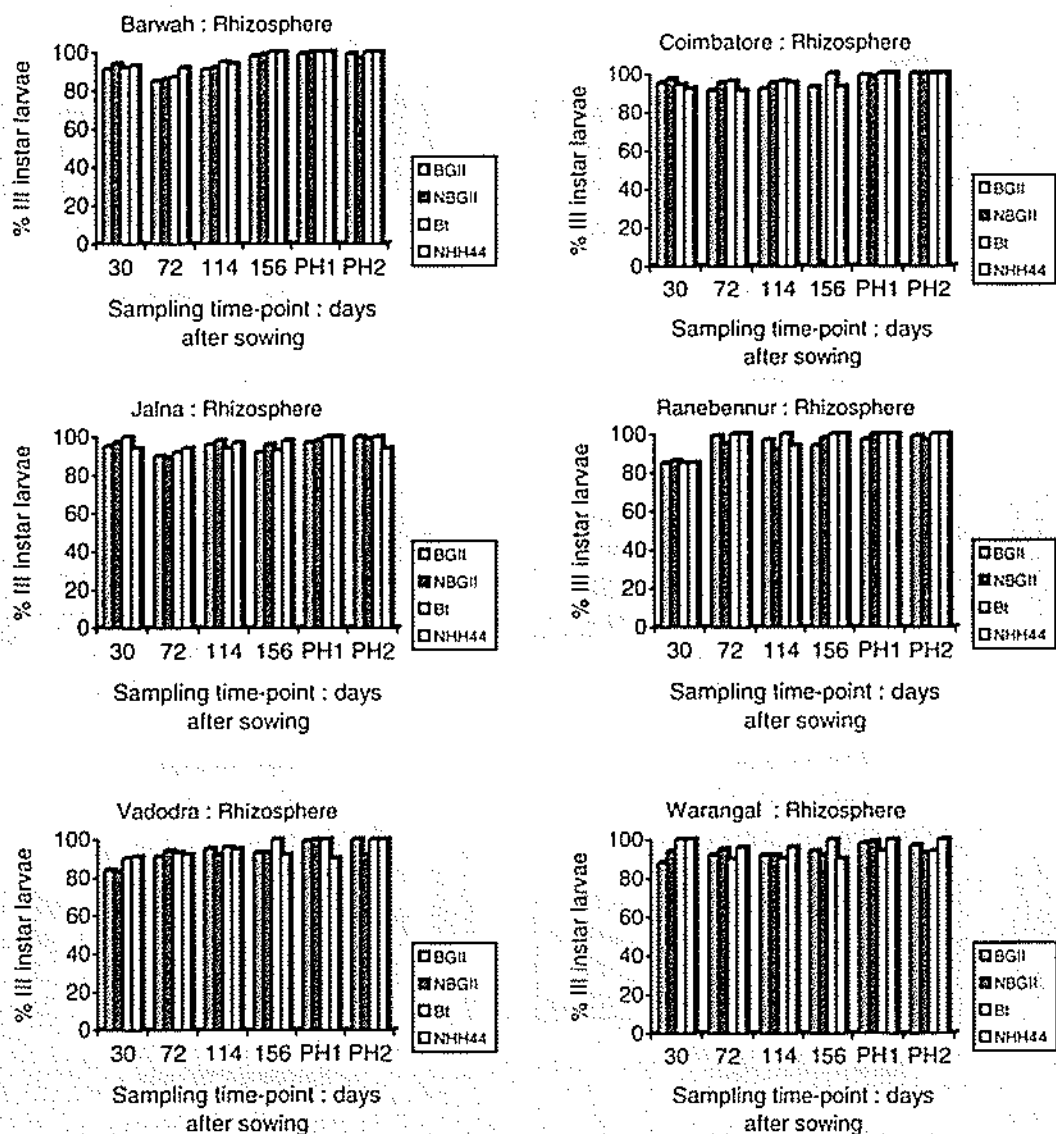


Figure 8. Insect bioassays with *Helicoverpa armigera* to detect the levels of CryX protein present in the rhizosphere soil samples collected from Bollgard II cotton field plots at different stages of plant growth. BGII, NBGII, Bt and NHH 44 represent the soil samples collected from respective field plots. Neonates of *H.armigera* were fed with artificial diet +/- soil samples to detect the BGII protein. After six days of incubation growth stages of introduced larvae were recorded. Each value is mean of 14 replications. In the sampling time-point PH1 and PH2 represent the first and second post harvest analyses.

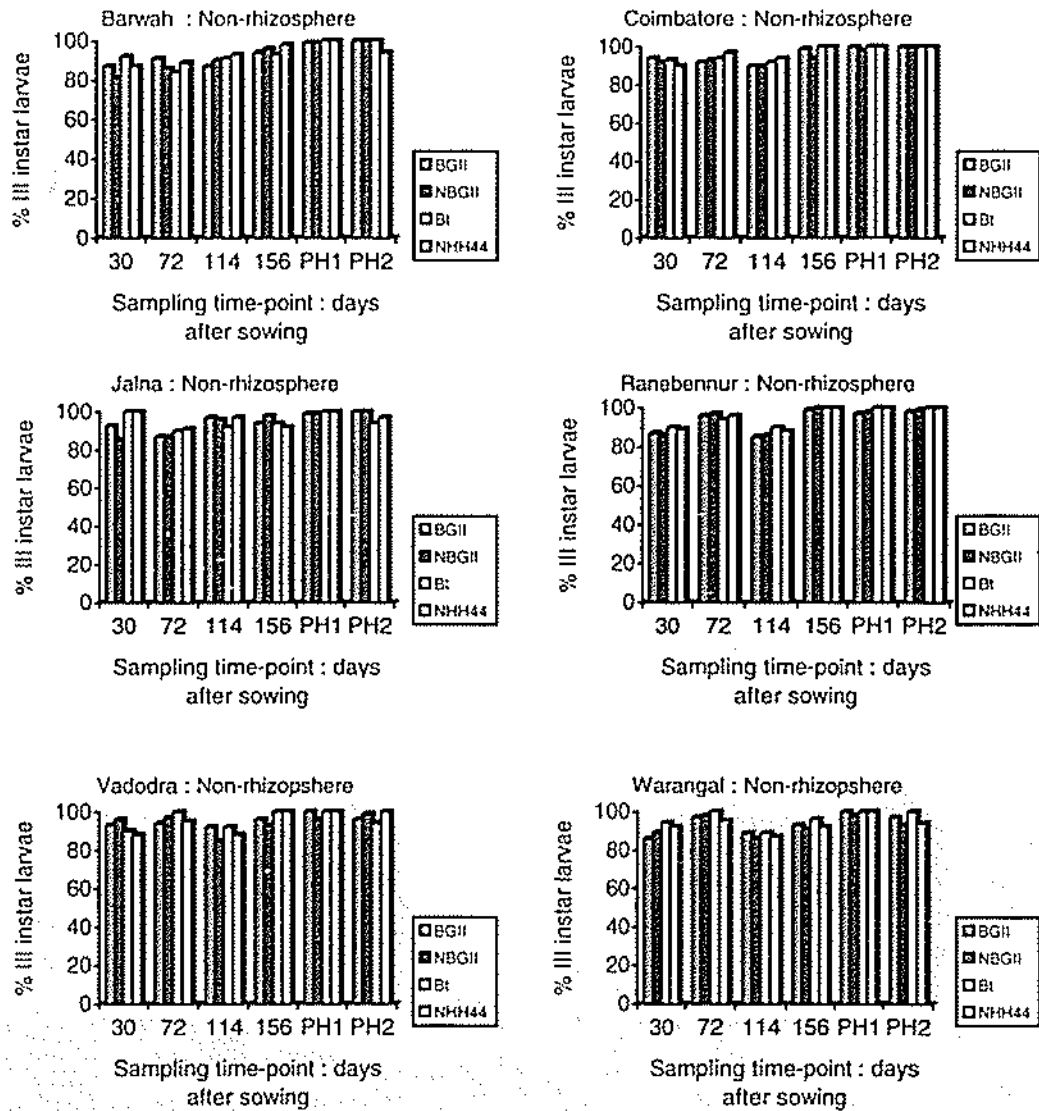


Figure 9. Insect bioassays with *Helicoverpa armigera* to detect the levels of CryX protein present in the non-rhizosphere soil samples collected from Bollgard II cotton field plots at different stages of plant growth. BGII, NBSII, Bt and NHH 44 represent the soil samples collected from respective field plots. Neonates of *H. armigera* were fed with artificial diet +/- soil samples to detect the BGII protein. After six days of incubation growth stages of introduced larvae were recorded. Each value is mean of 14 replications. In the sampling time-point PH1 and PH2 represent the first and second post harvest analyses.

**To assess the aggressiveness and Weediness of
BG-II and Non BG-II cotton hybrids**

**Report
2003-2004**

mahyco®

MAHARASHTRA HYBRID SEEDS COMPANY, LTD
Resham Bhavan, 4th Floor, 78 Veer Nariman Road, Mumbai 400 020, India

Study title: To assess the aggressiveness and Weediness of BG-II and Non BG-II cotton hybrids

Objective:

To confirm aggressiveness and weediness with regard to BG-II and Non BG-II cotton hybrids which were approved for RCGM trials during Kharif 2003.

Study conducted by:

Mahyco Ghanewadi Research Farm, Jalna.

Method:

The BG-II and Non BG-II cotton trial plots at Mahyco Ghanewadi Research Farm were uprooted after Kharif 2003 season and irrigated frequently to allow germination of any seeds that might have fallen to the ground after boll bursting. The trial plot will be maintained in this manner until sowing operations for Kharif 2004 begins i.e. May 25th 2004. Germination data was collected from 3 replications in both BG-II and Non BG-II plots each measuring 43.74 sq.m. Weeds germinated in these plots were identified and counted.

Data on Aggressiveness and Weediness in BG-I and BG-II trials :

PLOT NO.	AGGRESSIVENESS (GERMINATION OF COTTON SEEDS)				WEEDINESS					
	Number of Cotton seedlings				Name of Weed			Number		
	R-I	R-II	R-	Mean	R-I	R-II	R-	R-I	R-II	R-III
MRC-6160 BG-II	3	7	4	4.67	1,5,7,11	2,10	2,10,4	1=2 5=1 7=1 11=1	2=4 10=2	2=3 10=1 4=1
MRC-6160 Non- BG-II	2	5	9	5.33	2,7	2,5	Nil	2=3 7=1	2=1 5=1	Nil
MRC-6201 BG-II	9	7	6	7.33	2,11	2,14	6	2=4 11=1	2=1 14=2	6=1
MRC-6201 Non BG-II	11	5	7	7.67	1,2	14	2,4	1=2 2=1	14=1	2=1 4=1
MRC-6301 BG-II	10	8	3	7.00	3,6	Nil	2,7	3=1 6=2	Nil	2=1 7=2
MRC-6301 Non BG-II	7	5	9	7.00	2	8	2,7	2=1	8=1	2=2 7=1
MRC-6326 BG-II	4	4	7	5.00	11,2	6,2	12,1	11=1 2=2	6=1 2=1	12=1 1=2
MRC-6326 Non BG-II	6	5	7	6.00	Nil	5	2,6	Nil	5=1	2=2 6=1
MECH-162 Bt	3	7	7	5.67	10	2,8	2	10=1	2=1 8=1	2=2
Brahma	9	5	3	5.67	11,14	Nil	2,6	11=1 14=1	Nil	2=2 6=1
Bunny	4	2	7	4.33	Nil	10	2,7	Nil	10=1	2=2 7=1
NHII-44	7	5	4	5.33	1,2	4,10	1,8	1=2 2=1	4=1 10=2	1=1 8=1

1. Argemone mexicana
2. Parthenium hysterophorus
3. Chenopodium album
4. Solanum nigrum
5. Amaranthus viridis
6. Euphorbia hirta
7. Euphorbia thymifolia

8. *Trianthema monogyna*

9. *Ageratum conyzoides*

10. *Datura fastuosa*

11. *Cynodon dactylon*

12. *Corchorus acutangularis*

13. *Sorghum halpense*

14. *Celosia argentea*

Conclusion :

From the above results we found that there was no significant differences between BG-II and Non BG-II cotton with regard to aggressiveness as well as weediness.

ANNEXURE 5.1(a)

Germination studies conducted by Maharashtra Hybrid Seeds Co. Ltd.
with Bollgard II™ cotton hybrids in India

1948

1949

1950

1951

1952

1953

1954

1955

1956

1957

Study title: Germination studies in Mahyco BG-II hybrids.

Objective:

To confirm germination of BG-II hybrids vis a vis its non BG counter parts under laboratory as well as soil conditions in the Green house.

Study conducted by:

Mahyco Life Sciences Research Center, Dawalwadi, Jalna-431 203.

Duration of study:

5th January 2004 to 27th January 2004

Method:

The germination test was conducted following the " paper towel method". The crepe used was having pH of 6.0-7.5. Two hundred seed in 4 replications of 50 seed each have been taken and spaced uniformly and adequately apart on the moist surface. Two sheets of germination paper (size-12" x 9") after wetting in water, have been placed on a polythene sheet of size 16" x 16" . The whole assembly, along with a polythene sheet cover and a descriptive label inside was rolled and tied with rubber bands. The samples were kept under controlled conditions at 25 ± 0.5 °C. After 4 days, the first count was taken and final count on 12th day.

During the final count, only normal healthy seedlings were counted. The counts were taken for all four replications and the percentage of normal seedlings was calculated.

Sr. No	BG-II Hybrid <i>Kharif- 03</i>	Category	Number of germinated seed (50 x 4 = 200)				Total germinated seed	Per cent seed germination
			R-I	R-II	R-III	R-IV		
1	MRC-6201 BG-II	Transgenic	43	41	44	45	173	86.5
2	MRC-6201 Non BG-II	Non-transgenic	44	40	39	45	168	84.0
3	MRC-6301 BG-II	Transgenic	44	47	45	45	181	91.5
4	MRC-6301 Non BG-II	Non-transgenic	48	43	44	45	180	90.0
5	MRC-6326 BG-II	Transgenic	48	48	47	45	188	94.0
6	MRC-6326 Non BG-II	Non-transgenic	46	46	47	45	184	92.0
7	MRC-6160 BG-II	Transgenic	48	47	47	48	190	95.0
8	MRC-6160 Non BG-II	Non-transgenic	45	44	44	43	176	88.0
Mean								
		Transgenic					183	91.8
		Non-transgenic					177	88.5

Inference :

In laboratory germination test, average germination percentage of BG-II transgenic hybrids was 91.8 per cent and average germination of non-transgenic hybrids was 88.5 per cent. This indicates that there was no substantial difference between BG-II and Non BG-II hybrid cotton germination in laboratory condition.

Sr. No	Hybrid	Category	Number of germinated seed (50 x 4 = 200)				Total germinated seed	Per cent seed germination
			R-I	R-II	R-III	R-IV		
1	MRC-6201 BG-II	Transgenic	42	38	41	45	166	83.0
2	MRC-6201 Non BG-II	Non-transgenic	39	38	41	44	162	81.0
3	MRC-6301 BG-II	Transgenic	43	43	42	40	168	84.0
4	MRC-6301 Non BG-II	Non-transgenic	41	38	43	39	161	80.5
5	MRC-6326 BG-II	Transgenic	42	42	40	42	166	83.0
6	MRC-6326 Non BG-II	Non-transgenic	41	38	42	41	162	81.0
7	MRC-6160 BG-II	Transgenic	42	43	39	41	165	82.5
8	MRC-6160 Non BG-II	Non-transgenic	42	38	37	43	160	80.0
Mean								
		Transgenic					166.2	83.1
		Non-transgenic					161.2	80.6

Inference :

In green house germination test, average germination percentage of BG-II transgenic hybrids was 83.1 per cent and average germination of non-transgenic hybrids was 80.6 per cent. This indicates that there was no substantial difference between BG-II and Non BG-II hybrid cotton germination in green house condition.

Conclusion :

The above germination test results indicate that there is no substantial difference between Bt and non-Bt hybrid cotton germination in the laboratory as well as in greenhouse conditions.