Study No. 13

Title : Effect of Bt cotton event MLS9124 on the beneficial fauna
Organization : Metahelix Life Sciences Pvt Ltd
Status : Completed Kharif 2005 and 2006

Objective:

To assess the safety of the transgenic *B.t.* cotton hybrids containing *cry1C* gene (event MLS9124) on the beneficial fauna as compared to the non-*B.t.* and *cry1Ac* based *B.t.* cotton hybrids.

Introduction:

B.t. technology controls certain insect pests in a targeted way and hence does not affect the beneficial fauna which belong to other groups. Cry1 group of *B.t.* proteins has been so far reported to be specific only to lepidopteran insects and does not affect any beneficial natural enemies (AAM, 2002; Romeis et al. 2006) like coccinellids (Coleoptera), Chrysopa (Neuroptera), Syrphids (Diptera) and the spiders belonging to class Arachnida. Wadhwa and Gill in 2007 reported more natural enemies on *B.t.* cotton than on non *B.t.* cotton and attributed it to less number of insecticidal spray on the same. The safety of the *B.t.* cotton expressing *cry1C* gene developed by Metahelix Life Sciences Pvt Ltd. was assessed in field conditions over two cotton cropping seasons.

I Year: Kharif 2005 (Extracted from Report on Strip Trial conducted and submitted by Metahelix Life Sciences, Bangalore on *B.t.* and Non-*B.t.* cotton hybrids and presented to RCGM meeting on 2 May 2006 at New Delhi)

Materials and Methods:

The observations were made in the strip trial conducted at the research facility of Metahelix Life Sciences, Bangalore, during the cotton growing season of kharif 2005. Counts of beneficial fauna like spiders, lady bird beetle (Coccinellids) and *Chrysoperla* were recorded and compared between the *B.t.* and non-*B.t.* cotton trial entries under both sprayed (to control lepidopteran pests) and unsprayed conditions

Results:

The results indicated that the non-target insects and beneficial fauna levels remained normal in the trial containing transgenic cotton. However the activity was more in unsprayed plot compared to sprayed plots.

Table 1: Population dynamics of natural enemies and pests in sprayed and unsprayed condition during Kharif 2005

Entries	Unsprayed condition		Sprayed condition	
	Spider	Coccinellids	Spider	Coccinellids
1)001YP				
2)136 X 0001 *B.t.* 3)136 NT		4		
4)0003 NT				
5)0001 X 136				
6)0001 X 0004			14	3
7)0004 X 0001				4
8)0001 *B.t.* X 0004	10			
9)0003 X 0001				
10)136 X 0001				
11)0001 X 0003	12			
12)133 NT	12			
13)0001 *B.t.*			10	
14)0004 X 0001 *B.t.*				
15)0004 NT				
16)0003 X 0001 *B.t.*	4			
17)00001 X 133			12	
18)0001 *B.t.* X 0003				
19)0001 *B.t.* X 133 20)MECH 162				
21)RASI 144				
22)0001 *B.t.* X 136				
23)133 X 0001 *B.t.*				
24)133 X 0001				

II Year : Kharif 2006 (Extracted from Report on Multilocation Trials conducted at all India level, including locations in north, central and south zones, during kharif 2006, and presented to MEC meetings).

Materials and Methods:

A randomized replicated multilocation trial was conducted during kharif 2006 at the following locations covering all the three zones

Location	State
Bathinda, Firozpur	Punjab
Sirsa	Haryana
Rajkot, Vadadora	Gujarat
Khandwa, Dewas	Madhya Pradesh
Aurangabad, Nanded, Yeotmal	Maharashtra
Adilabad	Andhra Pradesh
Dharwad, Raichur	Karnataka
Attur	Tamil Nadu

Observations were made on the 10 randomly selected and tagged plants in each plot and results were expressed as mean number per plant. At 30, 50, 70 and 90 DAS, the number of Coccinellids, Chrysopa, Syrphids and Spiders were counted on individual plants

Results:

There were no obvious differences between *B.t.* and non *B.t.* entries for natural enemy population viz., Coccinellids, Chrysopids, Syrphids and Spiders at Bhatinda (Table 2), Firozpur (Table 3), Sirsa (Table 4), Rajkot (Table 5), Vadadora (Table 6), Khandwa (Table 7), Dewas (Table 8), Aurangabad (Table 9), Nanded (Table 10), Yeotmal (Table 11), Adilabad (Table 12), Dharwad (Table 13), Raichur (Table 14) and at Attur (Table 15). Higher population of coccinellid and *Chrysoperla* population was observed at Raichur compared to other locations. In all the locations the uniform spread of Coccinellids, Chrysopids, Syrphids and Spiders was observed at different growth stages of the crop among the different *B.t.* and non- *B.t.* entries which shows the safe effect of *B.t.* gene on natural enemies.

Conclusions :

Populations of beneficial fauna viz., Coccinellids, Chrysopa, Syrphids and Spiders, were comparable among the test *B.t.* entries, the *B.t.* check and the non-*B.t.* checks indicating the safety of the Cry1C protein towards these organisms. Our

observations are in accordance with earlier reports that *B.t.* cotton is safe to non-target arthropods. Naranjo et al (2005) supported the earlier conclusions that *B.t.* cotton poses a relatively low risk to non-target arthropods. *B.t.* crops are safe and beneficial to farmers, human society, non-target organisms, biodiversity and environment in general (Ananda Kumar, 2006).

Table 2. Beneficial fauna population on cotton entries during Kharif 2006 at Bathinda (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0.03	0	0.2	0.2	0	0.13	0	0	0	0	0	0	0.13	0.1	0.27	0.53
T2	3134 *B.t.*	0	0.07	0.13	0.13	0.03	0.13	0	0	0	0.03	0	0	0.17	0.3	0.27	0.3
T3	5174 *B.t.*	0.03	0	0.13	0.2	0.03	0.07	0	0	0	0	0	0	0.1	0.16	0.1	0.13
T4	RCH-2 *B.t.*	0.06	0.01	0.2	0.03	0.03	0.03	0.07	0	0	0	0	0	0.23	0.1	0.33	0.1
T5	BUNNY	0.06	0.3	0.3	0.37	0.03	0	0.07	0	0.03	0.07	0	0	0.1	0.6	0.3	0.27
T6	NHH-44	0	0.13	0.07	0.2	0.07	0.13	0.03	0	0	0	0	0	0.13	0.3	0.13	0.27

Table 3. Beneficial fauna population on cotton entries during Kharif 2006 at Firozpur (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0	0.03	0.1	0	0	0	0	0	0	0	0	0.1	0.07	0.2	0.17
T2	3134 *B.t.*	0	0.06	0.1	0.07	0.07	0	0	0	0	0	0	0	0.03	0.07	0.13	0.2
T3	5174 *B.t.*	0.06	0	0.06	0.07	0	0	0	0	0	0	0	0	0.03	0.07	0.1	0.13
T4	RCH-2 *B.t.*	0.1	0.03	0	0.13	0.03	0	0	0	0	0	0	0	0.03	0.07	0.13	0.17
T5	BUNNY	0	0	0.03	0.13	0	0	0	0	0	0	0	0	0.07	0.07	0.17	0.07
T6	NHH-44	0.03	0	0.15	0.07		0	0	0	0	0	0	0	0.03	0.1	0.13	0.23

Table 4. Beneficial fauna population on cotton entries during Kharif 2006 at Sirsa (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0.17	0	0.03	0	0	0.07	0	0	0	0	0	0	0.4	0.33	0.17
T2	3134 *B.t.*	0	0.17	0.13	0.07	0	0	0.07	0	0	0	0	0	0	0.43	17	0.1
T3	5174 *B.t.*	0	0.1	0.13	0.03	0	0	0	0	0	0	0	0	0	0.4	0.23	0.1
T4	RCH-2 *B.t.*	0	0.1	0.07	0.03	0	0	0	0	0	0	0	0	0	0.3	0.2	0.13
T5	BUNNY	0	0.1	0.13	0.03	0	0	0.03	0	0	0	0	0	0	0.43	0.23	0.13
T6	NHH-44	0	0.03	0.1	0.03	0	0	0	0	0	0	0	0	0	0.43	0.27	0.1

Table 5. Beneficial fauna population on cotton entries during Kharif 2006 at Rajkot (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0.07	0.03	0.07	0.13	0.03	0.03	0.03	0.07	0	0	0	0	0.17	0.10	0.23	0.23
T2	3134 *B.t.*	0.10	0.03	0.10	0.20	0.0	0.03	0.0	0.03	0	0	0	0	0.10	0.03	0.23	0.30
T3	5174 *B.t.*	0.03	0.03	0.10	0.23	0.03	0.0	0.03	0.10	0	0	0	0	0.10	0.13	0.27	0.27
T4	RCH-2 *B.t.*	0.07	0.03	0.10	0.17	0.0	0.0	0.0	0.10	0	0	0	0	0.10	0.10	0.27	0.30
T5	H-8	0.03	0.07	0.07	0.20	0.03	0.03	0.03	0.13	0	0	0	0	0.13	0.07	0.30	0.33
T6	NHH-44	0.07	0.03	0.13	0.23	0.0	0.0	0.0	0.07	0	0	0	0	0.10	0.10	0.27	0.30

Table 6. Beneficial fauna population on cotton entries during Kharif 2006 at Vadadora (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0.13	0.07	0.03	0	0.03	0.03	0	0	0	0	0	0	0.13	0.10	0.10
T2	3134 *B.t.*	0	0.10	0.10	0.0	0	0.03	0.0	0	0	0	0	0	0	0.17	0.13	0.07
T3	5174 *B.t.*	0	0.12	0.07	0.07	0	0.0	0.03	0	0	0	0	0	0	0.13	0.07	0.13
T4	RCH-2 *B.t.*	0	0.17	0.03	0.03	0	0.10	0.03	0	0	0	0	0	0	0.17	0.10	0.17
T5	H-8	0	0.13	0.07	0.03	0	0.03	0.03	0	0	0	0	0	0	0.13	0.07	0.07
T6	NHH-44	0	0.13	0.03	0.0	0	0.07	0.0	0	0	0	0	0	0	0.10	0.10	0.10

Table 7. Beneficial fauna population on cotton entries during Kharif 2006 at Khandwa (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0.07	0.0	0.0	0	0.0	0	0.10	0	0	0	0	0	0.0	0.13	0.10
T2	3134 *B.t.*	0	0.0	0.0	0.03	0	0.03	0	0.13	0	0	0	0	0	0.13	0.0	0.07
T3	5174 *B.t.*	0	0.0	0.10	0.0	0	0.0	0	0.0	0	0	0	0	0	0.03	0.0	0.13
T4	RCH-2 *B.t.*	0	0.03	0.0	0.0	0	0.0	0	0.07	0	0	0	0	0	0.0	0.0	0.10
T5	H-8	0	0.0	0.0	0.07	0	0.03	0	0.10	0	0	0	0	0	0.10	0.03	0.17
T6	NHH-44	0	0.0	0.0	0.10	0	0.0	0	0.0	0	0	0	0	0	0.17	0.17	0.13

Table 8. Beneficial fauna population on cotton entries during Kharif 2006 at Dewas (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0.0	0.03	0.13	0	0	0.17	0.0	0	0	0	0	0	0.07	0.0	0.10
T2	3134 *B.t.*	0	0.10	0.17	0.0	0	0	0.0	0.10	0	0	0	0	0	0.0	0.20	0.10
T3	5174 *B.t.*	0	0.0	0.0	0.07	0	0	0.07	0.0	0	0	0	0	0	0.2	0.10	0.20
T4	RCH-2 *B.t.*	0	0.20	0.0	0.0	0	0	0.10	0.0	0	0	0	0	0	0.0	0.03	0.03
T5	H-8	0	0.0	0.0	0.17	0	0	0.0	0.07	0	0	0	0	0	0.0	0.0	0.07
T6	NHH-44	0	0.0	0.0	0.0	0	0	0.0	0.03	0	0	0	0	0	0.0	0.07	0.07

Table 9. Beneficial fauna population on cotton entries during Kharif 2006 at Aurangabad (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0.10	0.10	0.07	0.03	0.03	0.0	0.0	0.0	0	0	0	0	0.20	0.10	0.10	0.10
T2	3134 *B.t.*	0.07	0.07	0.03	0.03	0.0	0.03	0.03	0.0	0	0	0	0	0.17	0.13	0.13	0.07
T3	5174 *B.t.*	0.13	0.10	0.03	0.07	0.03	0.03	0.0	0.03	0	0	0	0	0.17	0.13	0.13	0.13
T4	RCH-2 *B.t.*	0.13	0.10	0.07	0.03	0.07	0.0	0.03	0.03	0	0	0	0	0.20	0.17	0.10	0.10
T5	H-8	0.10	0.10	0.07	0.07	0.07	0.07	0.03	0.0	0	0	0	0	0.13	0.13	0.17	0.13
T6	NHH-44	0.17	0.07	0.07	0.03	0.03	0.03	0.0	0.03	0	0	0	0	0.17	0.17	0.13	0.10

Table 10. Beneficial fauna population on cotton entries during Kharif 2006 at Nanded (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0.10	0.10	0.07	0.07	0.0	0.03	0.03	0.03	0.0	0.0	0	0	0.20	0.13	0.10	0.13
T2	3134 *B.t.*	0.03	0.17	0.10	0.03	0.03	0.10	0.03	0.07	0.0	0.03	0	0	0.17	0.17	0.07	0.10
T3	5174 *B.t.*	0.07	0.10	0.07	0.10	0.0	0.0	0.07	0.07	0.03	0.0	0	0	0.13	0.13	0.17	0.13
T4	RCH-2 *B.t.*	0.07	0.10	0.03	0.03	0.03	0.10	0.07	0.10	0.0	0.0	0	0	0.13	0.13	0.13	0.07
T5	H-8	0.03	0.17	0.13	0.07	0.0	0.03	0.03	0.10	0.0	0.03	0	0	0.20	0.10	0.20	0.10
T6	NHH-44	0.03	0.10	0.07	0.10	0.03	0.07	0.07	0.07	0.0	0.0	0	0	0.17	0.17	0.20	0.17

Table 11. Beneficial fauna population on cotton entries during Kharif 2006 at Yeotmal (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0.07	0.03	0.13	0	0.03	0.03	0	0	0	0	0	0	0.13	0.13	0.20
T2	3134 *B.t.*	0	0.03	0.07	0.10	0	0.0	0.03	0	0	0	0	0	0	0.13	0.10	0.23
T3	5174 *B.t.*	0	0.07	0.07	0.07	0	0.03	0.07	0	0	0	0	0	0	0.10	0.17	0.27
T4	RCH-2 *B.t.*	0	0.03	0.10	0.10	0	0.03	0.07	0	0	0	0	0	0	0.17	0.20	0.20
T5	H-8	0	0.07	0.07	0.10	0	0.03	0.03	0	0	0	0	0	0	0.17	0.10	0.17
T6	NHH-44	0	0.07	0.10	0.13	0	0.0	0.07	0	0	0	0	0	0	0.13	0.17	0.17

Table 12. Beneficial fauna population on cotton entries during Kharif 2006 at Adilabad (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0	0.17	0.10	0	0	0.03	0	0	0	0	0	0	0	0.23	0.30
T2	3134 *B.t.*	0	0	0.13	0.13	0	0	0.03	0	0	0	0	0	0	0	0.20	0.17
T3	5174 *B.t.*	0	0	0.17	0.17	0	0	0	0	0	0	0	0	0	0	0.27	0.27
T4	RCH-2 *B.t.*	0	0	0.17	0.17	0	0	0.03	0	0	0	0	0	0	0	0.30	0.27
T5	BUNNY	0	0	0.20	0.17	0	0	0	0	0	0	0	0	0	0	0.23	0.27
T6	NHH-44	0	0	0.17	0.17	0	0	0.03	0	0	0	0	0	0	0	0.23	0.27

Table 13. Beneficial fauna population on cotton entries during Kharif 2006 at Dharwad (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 B.t.	0	0.73	0.17	0.03	0	0	0.1	0.03	0	0	0	0	0	0.33	0.23	0
T2	3134 B.t.	0	0.87	0.13	0.07	0	0	0.17	0.07	0	0	0	0	0	0.4	0.20	0
T3	5174 B.t.	0	0.83	0.13	0.07	0	0	0.17	0.07	0	0	0	0	0	0.37	0.23	0
T4	RCH-2 B.t.	0	0.83	0.17	0.10	0	0	0.13	0.03	0	0	0	0	0	0.37	0.17	0
T5	BUNNY	0	0.90	0.17	0.10	0	0	0.13	0.07	0	0	0	0	0	0.33	0.17	0
T6	NHH-44	0	0.77	0.13	0.13	0	0	0.17	0.03	0	0	0	0	0	0.43	0.23	0

Table 14. Beneficial fauna population on cotton entries during Kharif 2006 at Raichur (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	2.1	2.37	0	0	0	0.03	0.63	0	0	0	0	0	0	0	0
T2	3134 *B.t.*	0	1.9	2.07	0	0	0	0.07	0.67	0	0	0	0	0	0	0	0
T3	5174 *B.t.*	0	1.8	2.3	0	0	0	0	0.67	0	0	0	0	0	0	0	0
T4	RCH-2 *B.t.*	0	2.47	2.13	0	0	0	0.07	0.67	0	0	0	0	0	0	0	0
T5	BUNNY	0	1.67	1.63	0	0	0	0	0.43	0	0	0	0	0	0	0	0
T6	NHH-44	0	2.1	2.37	0	0	0	0.03	0.63	0	0	0	0	0	0	0	0

Table 15. Beneficial fauna population on cotton entries during Kharif 2006 at Attur (per plant)

T. No.	Hybrids	Coccinellids				Chrysopa				Syrphids				Spiders			
		30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS	30 DAS	50 DAS	70 DAS	90 DAS
T1	5125 *B.t.*	0	0	0.46	0.63	0	0	0.27	0.27	0	0	0.17	0.30	0	0	0.13	0.83
T2	3134 *B.t.*	0	0	0.40	0.60	0	0	0.23	0.20	0	0	0.17	0.13	0	0	0.50	0.76
T3	5174 *B.t.*	0	0	0.20	0.50	0	0	0.30	0.27	0	0	0.06	0.20	0	0	0.57	0.47
T4	RCH-2 *B.t.*	0	0	0.47	0.93	0	0	0.23	0.37	0	0	0.20	0.27	0	0	0.43	0.33
T5	BUNNY	0	0	0.40	0.83	0	0	0.37	0.23	0	0	0.06	0.17	0	0	0.63	0.47
T6	NHH-44	0	0	0.50	0.76	0	0	0.23	0.13	0	0	0.20	0.17	0	0	0.37	0.30

REFERENCES:

AAM. 2002. 100 years of *B.t.*: A critical Scientific Assessment. Report based on a colloquium, "100 Years of *Bacillis thuringiensis*, a Paradigm for Producing Transgenic Organisms: A Critical Scientific assessment," sponsored by the American Academy of Microbiology and held November 16-18, in Ithaca, New York.

AnandKumar, P. (2006) Safe Transition. Biotech News, Vol II (2), 3-5

Naranjo SE, Head G, and Dively GP (2005). Field studies assessing arthropod nontarget effects in *B.t*. transgenic crops: Introduction. Environmental Entomology, 34: 1178–1180.

Romeis J., Meissle M., Bigler F. (2006) Transgenic crops expressing Bacillus thuringiensis toxins and biological control, Nature Biotechnology 24, 63- 71

Wadhwa, S. and Gill, R.S. (2007). Effect of *B.t.*-cotton on biodiversity of natural enemies Journal of Biological Control vol. 21 (1) p.9-16