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Impact of *Bt* transgenic cotton on population dynamics of aphids and natural enemies*

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Biological control programmes that are based on insect pathogens particularly bacterium, *Bacillus thuringiensis* (Berliner) have shown phenomenal feasibility and success mitigating the resistance problem. Since last 20 years several research organizations are engaged in exploiting the insecticidal properties of this gram positive soil bacterium in a meaningful way. Thus ultimately, GMO's (genetically modified organisms) were developed to which class *Bt*-(*Bacillus thuringiensis*) genotypes also belongs and have in built ability to express or produce crystal (Cry) protein toxic to the target pests. The largely exploited protein is Cry1 Ac having specific action against lepidopteron insects and most widely to Heliothine. Transgenic technologies have proven to be one of the fastest and most effective means of insect control ever developed and *Bt* is considered to be a natural choice for this role, as it produces a large variety of toxins very specific for certain orders of insect pests. Hence, there were continuous efforts to develop *Bt* transgenic crops, viz potato, rice, maize, canola, cotton, brinjal, tobacco etc to combat dreaded pests belonging to Lepidoptera, Diptera and Coleopteran orders. Past or present commercialized *Bt* crop and their respective genes include cotton (Cry1Ac, Cry2Ab2, Cry1Fa2), maize (Cry1Ab, Cry1Ac, Cry1Fa2, CryBb1, Cry9 c) and potato (Cry3Aa). Thus *Bt* technology has been regarded as the most significant development in the field of agricultural science after green revolution. Since loss of biodiversity threatens such benefits, transgenic *Bt* technology is increasingly scrutinized for its potential environmental impact despite fast spreading of *Bt* cotton world wide.

Adoption rates of *Bt* transgenic insect resistance cotton genotypes producing activated C*ry1Ac* gene are quite high in almost all cotton growing countries and at present it covers more than 90 million ha area in world. Further, with the approval of GEAC during 2002, India also resorted for *Bt* cotton cultivation based on demand for a fool proof and viable solution to the quite intense bollworm problem. Thus India gained status of mega biotech country with 7.8 million ha coverage under *Bt* cotton during 2006 (James 2006). At present more than 600 *Bt* cotton hybrids are being grown in the country. Transgenic plants produced Cry protein in high doses and in most of their tissues throughout the season. The insecticidal toxin could become available to the predatory insect in a modified form when it feeds on non-susceptible or sub lethally affected non-target herbivores that feed on *Bt* plants. This could constitute an important pathway for ecological impacts of *Bt* cotton and several experiments have sort to test such potential impacts (Lovie and Arpaia 2005). Therefore, the objective of the present investigation was to assess the impact of *Bt* cotton hybrids on predatory insects which are native and quite effective in natural as well as biological control of cotton pests, particularly aphids [*Aphis gossypii* (Glover)] and eggs/ neonates of bollworms.

The studies on natural incidence of insect predators, viz lady bird beetles/Coccinellids (*Cheilomenes sexmaculata* Fab. Coccinellidae: Coleoptera) green lacewing (*Chrysoperla carnea* Steph. Chrysopidae : Neuroptera) and hover flies / syrphids (*Ischiodon scutellaris* Fab. Syrphidae: Diptera) carried out through field experiments conducted during 2004–05 and 2005–06 at Agricultural Research Station, Dharwad, using RCH 2 *Bt* intraspecific hybrid. The crop was sown on 16 June 2004 and 24 June 2005 for respective seasons in un replicated block of 10.8 × 6.0 m^2 size with 90 cm × 60 cm spacing. Similarly a block of RCH 2 non-*Bt* was sown at the

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*Short note

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same time for comparative study. No plant protection measures were imposed on any block at any period of crop growth during both the seasons. Between *Bt* and non-*Bt* blocks an isolation distance of 10 m was maintained to avoid migration of insects. Observations were recorded from 10 randomly selected plants at weekly interval from July to December (28 to 51 ISW) for incidence of aphids (host insect) as well as predators. The population of aphids was recorded by counting the nymphs on three leaves, one each from top, middle and bottom portion of plant and then averaged as population per leaf. Similarly adults and nymphs of coccinellids, grubs of *C. carnea* and maggots of syrphids were recorded on whole plant basis and averaged to population per plant. The seasonal mean incidence of aphid as well as predators population in RCH 2 *Bt* and non-*Bt* was compared through paired t-test to assess the impact. The correlation between incidence of predators and aphid population was assessed in both *Bt* and non *Bt* crop.

A laboratory study was done during 2004 for assessing the impact of Bt cotton on *C. carnea*. The seedlings of RCH-2Bt and non Bt cotton were raised by sowing seeds (without treatment of imidacloprid) in pots on 20 August 2004. Ten pots were maintained for both RCH 2 *Bt* and Non *Bt*. Late sowing was done to coincide the aphid incidence on the plants with optimum expression period for Cry 1 Ac, i e 50–80 DAS to have proper impact (if any) on aphids. The aphid colonies developed on these plants were used to feed *C. carnea* grubs in the laboratory. Feeding potentiality and impact on growth and development was assessed by rearing *C. carnea* from hatching to pupation on aphids infesting potted plants of RCH 2 *Bt* and non-*Bt*. All the instars were reared in specimen tubes (1.0 cm × 3.5 cm) till pupation by feeding known number of nymphs of aphids each day. For each treatment there were 10 grubs and each set was replicated five times. After every 24 hr, grubs were shifted with a fine brush to new specimen tube marked. The old specimen tube was taken for counting the aphids remaining in the tube to know the feeding potential each day. Host material, i e nymphs of aphids from RCH 2 *Bt* and non-*Bt* plants for respective treatments were increased gradually as instar advanced. After pupation and emergence of adults, pairs of males and females for each 10 treatment were isolated (two pairs from each replication) and released separately for mating and oviposition. Adult food (10% honey) was provided in each oviposition cage. The observations for days taken to complete different instar stage, prepupal stage, adult longevity, sex ratio, fecundity, incubation period and total aphid consumed were recorded and presented as comparative figures between two hybrids.

The relative abundance of aphids and three predators, viz coccinellids, *Chrysoperla* and syrphids did not vary much between *Bt* and non-*Bt* crops during 2004–05 as well as 2005–06 studies (Fig 1). In the pooled analysis (Table 1) the population of aphids ranged between 8.58/leaf (34 ISW) to 42.15/ leaf (50 ISW) with a mean of 23.82/leaf in RCH 2 *Bt*. Since beginning of the incidence at 28 ISW (July) population remained above ETL (>10.0/ leaf) till the end of the season and with increasing trend and heavy buildup was noticed during December. Population dwindled to below ETL during 31st, 34th and 35th ISW. This incidence appeared to be numerically more when compared to the incidence in respective weeks in non-*Bt* crop, which supported the buildup of aphids to thresholds and above from September onwards only. Prior to that, the incidence was found to vary much in weekly observations. The range of incidence was 7.43/ leaf (36th ISW) to 37.08/ leaf (46th ISW) with a mean of 21.37/ leaf. Despite numerical variations to limited extent, RCH 2 *Bt* supported the incidence of aphids as well as predators similarly to that of its non-*Bt* version and the difference between them was non-significant (Table 2). Thus the theoretic possible impact of Cry protein on predators through passive exposure was not supported by the two seasons and pooled data as far as the way in which naturally they occur in the cotton ecosystem. Similar to the present findings, *Bt* cotton did not affect the population dynamics of aphids (Wu and Guo 2003). Therefore, the likely adverse effect of *Bt* protein on aphid incidence and further on its predators was not evident in the present investigation.

The population of coccinellids, *Chrysoperla* and syrphids were also observed throughout the cropping season. The incidence of predators followed a similar trend in both the seasons.A density dependent variation with respect to prey was observed with respect to all the three predators. Mean population of coccinellids, *Chrysoperla* and syrphids was 0.89, 0.78 and 1.0/plant in RCH 2 *Bt* and 0.91, 0.75 and 1.04/ plant in RCH 2 non-*Bt* respectively. There was no significant difference between the predatory populations on *Bt* and non-*Bt* cotton crops (Table 2). The population of predator always depended on its prey. As there was no variation in the host population, between two types of cotton, the dependent predator also did not show any significant difference and there was a strong and positive correlation between aphid and predatory population (Table 2). Among the three predators, syrphids exhibited a high degree of host dependent, population buildup (r = 0.94 and 0.96), followed by chrysoperla and coccinellids. This clearly indicated that *Bt* toxin has no effect on major predators on cotton crop. The dynamics of major predators in *Bt* and conventional cotton fields were almost same in a similar study conducted by Xia *et al.* (1999), however there was reduction in eggs parasitisation of third generation noctuid eggs. As Cry1Ac has target specific action against selected lepidopteran pests the safety of insects belonging to other orders. There was no significant difference among *Bt* and non-*Bt* cultivars with respect to seasonal mean incidence of major insect predators, viz coccinellid, *Chrysoperla*, syrphids and spiders (Hegde *et al.* 2004 and Udikeri *et al.* 2003). *Bt* cotton hybrids expressing Cry 1 Ac could poses a negligible risk for aphid antagonists

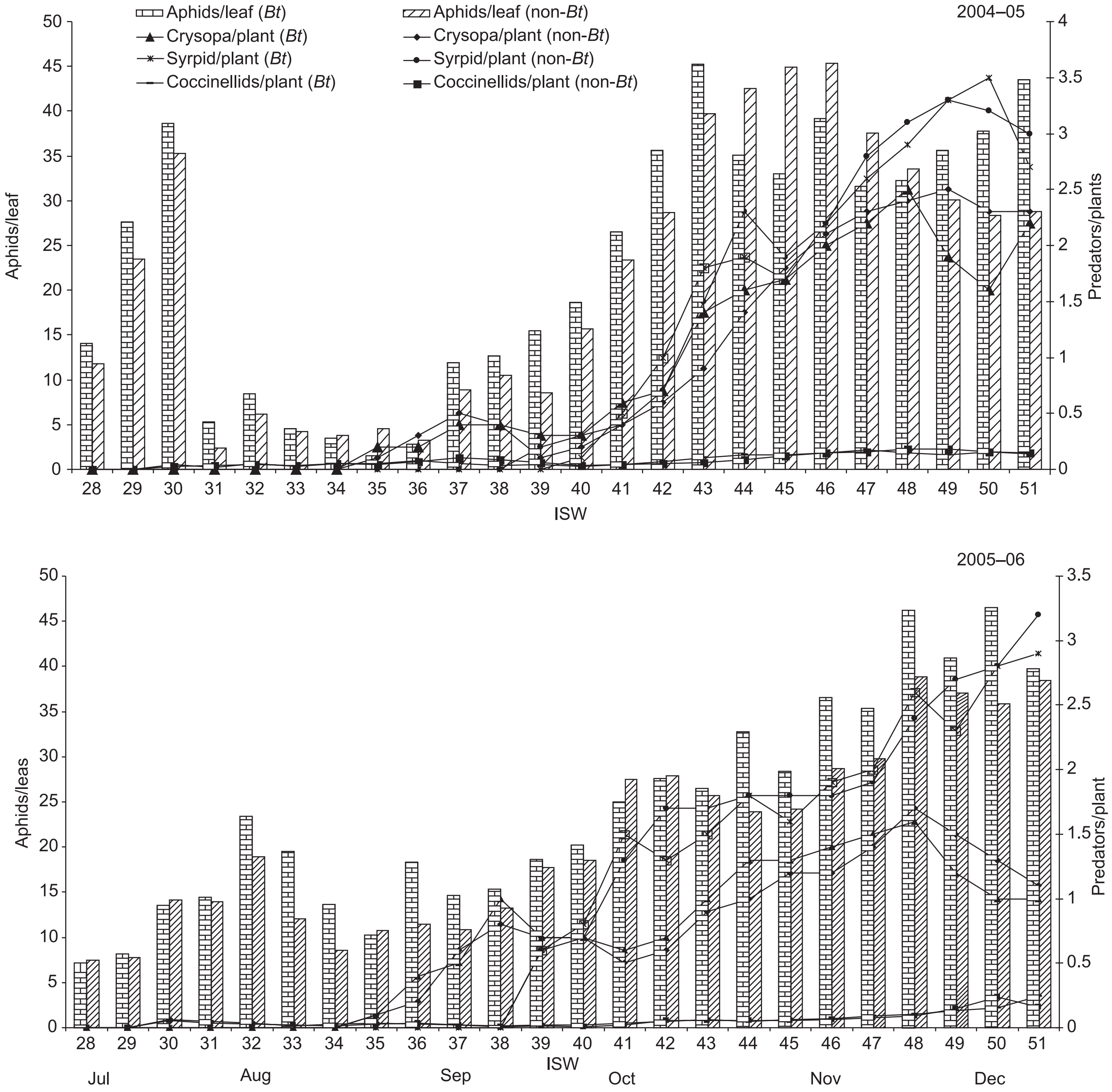

Fig 1 Dynamics of aphids and predatory insects in *Bt* and non-*Bt* cotton

(Lawo *et al.* 2009) in India. The predatory performance of chrysoperla could not vary between *Bt* and non-*Bt* cottons, instead it varied in accordance with host insect availability (Bahar *et al.* 2011). From the present study it was evident that the predatory action of green lace wing remain unaffected in even *Bt* cotton ecosystem. Among different hosts aphids are much preferred by chrysoperla in India (Tahmina and Akhtar 2010 Satpathy *et al.* 2012) and third instar grubs have greatest feeding potential ,which appeared true in case of *Bt* and non-*Bt* cotton infesting cotton also in this study. Thus, with untraceable bio activity of Cry toxin over aphids the safety of a promising polyphagous generalist predator appears to be assured even under widespread *Bt* cotton cultivation.

Further, the most potential predator *C. carnea* was found to remain unaffected in terms of its potentiality when fed with aphids infesting RCH 2 *Bt*. The figures of bio-potentiality

Table 1 Seasonal abundance of aphids and predatory insects on RCH 2 *Bt* and non- *Bt* cotton hybrid (pooled data of 2004 and 2005 *kharif* seasons)

Month	ISW	Aphids/leaf		Coccinellids/plant		*Chrysoperla*/plant		Syrphids / plant	
		Bt	NBt	Bt	NBt	Bt	NBt	Bt	NBt
Jul	28	10.69	9.67	0.00	0.00	0.00	0.00	0.00	0.00
		(3.42)	(3.27)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
	29	17.92	15.65	0.00	0.00	0.00	0.00	0.00	0.00
		(4.35)	(4.08)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
Aug	30	26.04	24.73	0.70	0.62	0.00	0.00	0.00	0.00
		(5.20)	(5.07)	(1.30)	(1.27)	(1.00)	(1.00)	(1.00)	(1.00)
	31	9.87	8.17	0.45	0.47	0.03	0.02	0.00	0.00
		(3.30)	(3.03)	(1.20)	(1.21)	(1.01)	(1.01)	(1.00)	(1.00)
	32	15.93	12.57	0.60	0.50	0.01	0.03	0.00	0.00
		(4.11)	(3.68)	(1.26)	(1.22)	(1.00)	(1.01)	(1.00)	(1.00)
	33	12.00	8.18	0.25	0.40	0.02	0.01	0.00	0.00
		(3.61)	(3.03)	(1.12)	(1.18)	(1.01)	(1.00)	(1.00)	(1.00)
	34	8.58	6.22	0.55	0.48	0.06	0.04	0.00	0.00
		(3.10)	(2.69)	(1.24)	(1.21)	(1.03)	(1.02)	(1.00)	(1.00)
Sep	35	5.94	7.67	0.60	0.52	0.14	0.14	0.00	0.00
		(2.63)	(2.94)	(1.26)	(1.23)	(1.07)	(1.07)	(1.00)	(1.00)
	36	10.55	7.43	0.70	0.74	0.33	0.25	0.00	0.00
		(3.40)	(2.90)	(1.30)	(1.32)	(1.15)	(1.12)	(1.00)	(1.00)
	37	13.29	9.92	0.46	0.80	0.54	0.52	0.00	0.00
		(3.78)	(3.30)	(1.21)	(1.34)	(1.24)	(1.23)	(1.00)	(1.00)
	38	14.03	11.87	0.29	0.65	0.70	0.61	0.00	0.00
		(3.88)	(3.59)	(1.14)	(1.28)	(1.30)	(1.27)	(1.00)	(1.00)
Oct	39	17.05	13.10	0.41	0.52	0.46	0.53	0.32	0.40
		(4.25)	(3.75)	(1.19)	(1.23)	(1.21)	(1.23)	(1.15)	(1.18)
	40	19.26	17.13	0.33	0.30	0.47	0.50	0.46	0.53
		(4.50)	(4.26)	(1.15)	(1.14)	(1.21)	(1.22)	(1.21)	(1.23)
	41	25.77	25.45	0.49	0.44	0.48	0.56	1.01	0.91
		(5.17)	(5.14)	(1.22)	(1.20)	(1.22)	(1.25)	(1.42)	(1.38)
	42	31.43	28.30	0.84	0.76	0.67	0.68	1.15	1.27
		(5.69)	(5.41)	(1.36)	(1.32)	(1.29)	(1.30)	(1.47)	(1.50)
	43	35.91	32.73	1.09	0.83	0.95	1.12	1.65	1.64
		(6.08)	(5.81)	(1.45)	(1.35)	(1.39)	(1.46)	(1.63)	(1.62)
Nov	44	34.03	33.22	1.13	0.97	1.36	1.28	1.88	2.05
		(5.92)	(5.85)	(1.46)	(1.40)	(1.54)	(1.51)	(1.70)	(1.74)
	45	30.75	34.58	1.27	1.18	1.56	1.45	1.69	1.86
		(5.63)	(5.97)	(1.51)	(1.48)	(1.60)	(1.56)	(1.64)	(1.69)
	46	37.91	37.08	1.39	1.37	1.78	1.59	2.07	2.06
		(6.24)	(6.17)	(1.55)	(1.54)	(1.67)	(1.61)	(1.75)	(1.75)
	47	33.51	33.70	1.76	1.53	1.91	1.81	2.32	2.39
		(5.87)	(5.89)	(1.66)	(1.59)	(1.71)	(1.67)	(1.82)	(1.84)
Dec	48	39.23	36.17	1.71	1.83	2.04	2.10	2.75	2.77
		(6.34)	(6.10)	(1.65)	(1.68)	(1.74)	(1.76)	(1.94)	(1.94)
	49	38.25	33.62	1.75	2.26	1.88	1.71	2.80	2.99
		(6.26)	(5.88)	(1.66)	(1.81)	(1.70)	(1.64)	(1.95)	(2.00)
	50	42.15	32.17	2.06	2.71	1.66	1.47	3.17	2.99
		(6.57)	(5.76)	(1.75)	(1.93)	(1.63)	(1.57)	(2.04)	(2.00)
	51	41.62	33.62	2.62	2.06	1.65	1.67	2.80	3.10
		(6.53)	(5.88)	(1.90)	(1.75)	(1.63)	(1.63)	(1.95)	(2.02)
	Mean	23.82	21.37	0.89	0.91	0.78	0.75	1.00	1.04
		(4.98)	(4.73)	(1.38)	(1.38)	(1.33)	(1.32)	(1.41)	(1.43)

Figures in the parentheses are vx + 1 transformation; ISW, International standard week

Table 2 Test statistics and correlation matrix (r value) for relative abundance of aphids and insect predators on RCH 2 *Bt* and non-*Bt* cotton

Test of significance between *Bt* and non-*Bt* cultivar for incidence of												
Year	Aphids /plant			Coccinellids/plant			*Crysoperla* /plant			Syrphids/plant		
	RCH 2 *Bt*	Non-*Bt*	t-test	RCH 2 *Bt*	Non-*Bt*	t-test	RCH 2 *Bt*	Non-*Bt*	t-test	RCH 2 *Bt*	Non-*Bt*	t-test
2004	23.35	21.74	NS	0.95	1.02	NS	0.85	0.88	NS	1.02	1.05	NS
	(4.93)	(4.77)		(1.40)	(1.42)		(1.36)	(1.37)		(1.42)	(1.43)	
	Correlation (r)			0.62*	0.50*		0.72*	0.70*		0.74*	0.73*	
2005	24.28	21.01	NS	0.83	0.80	NS	0.68	0.66	NS	0.99	1.03	NS
	(5.03)	(4.69)		(1.35)	(1.34)		(1.29)	(1.29)		(1.41)	(1.42)	
	Correlation (r)			0.78*	0.79*		0.81*	0.85*		0.94*	0.96*	
Pooled	23.82	21.37	NS	0.89	0.91	NS	0.78	0.75	NS	1.00	1.04	NS
	(4.98)	(4.73)		(1.38)	(1.38)		(1.33)	(1.32)		(1.41)	(1.43)	

Table t = 2.08, Figures in the parentheses are vx + 1 transformation, * Significant at $P = 0.01$

Table 3 Comparative biology of *Chrysoperla carnea* reared on cotton aphids

Days									
						Adult longevity			
Aphids raised on	Egg period	I instar	II instar	III instar	Pupal period	Male	Female	Fecundity	Aphids consumed/ grub
RCH 2 *Bt*	3.22	2.95	3.73	4.10	10.27	45.30	48.13	97.50	510.80
RCH 2 Non *Bt*	3.72	3.27	3.51	4.35	10.65	47.23	50.17	102.90	523.22
t-test	NS	NS	NS	NS	NS	NS	NS	NS	NS

n = 20

(Table 3) parameters of *C. carnea* remained statistically on par for two batches reared on aphids that colonized on *Bt* and non-*Bt* plants. The incubation period was 3.22 and 3.72 days respectively when aphids host was RCH 2 *Bt* and RCH 2 non-*Bt*. The time spent in each instar stage was slightly more for the *C. carnea* reared on aphids with non *Bt* cotton host. Similarly pupal period (10.65 days), adult longevity (47.23 and 50.17 days for male and female respectively) was more in non- *Bt*.The fecundity was also high (102.90 / female) in *C. carnea* population reared on non-*Bt* crop colonized aphids. Thus total aphid consumption was more (523.22) in this treatment. However, none of the bio-potential parameter recorded significant variation. Thus bio-potentiality of *C. carnea* remained same irrespective of aphid host. Similarly, Hilbeck (2001) also demonstrated that the chrysoperla fed on intoxicated aphids survived and continued its progeny as good as chrysoperla fed on non-toxicated aphids. However, the negative effect of mortality and delayed development in predatory stages were observed when *C. carnea* was fed on intoxicated early instar larvae of lepidopteran pests. viz *H. armigera* and syrphids larvae was well documented Dutton *et al* (2002) and other researchers. It is essential to monitor the impact of *Bt* cotton on the natural enemies of crop pests continuously to rule out any non-target effect of this technology on beneficial insects.

SUMMARY

The dynamic aphids and its predator in RCH 2 *Bt* and non-*Bt* cotton hybrids was studied to know the effect of Cry toxin. The mean incidence of aphids was 23.82 and 21.37/ leaf in *Bt* and non-*Bt* cotton respectively indicating no significant variation. Mean population of coccinellids, *Chrysoperla* and syrphids was 0.89, 0.78 and 1.0/plant in RCH 2 *Bt* which was almost similar to the incidence on RCH 2 non *Bt*. There was strong and positive correlation between incidence of predators and aphid on both *Bt* and non- *Bt* cotton. The 'r' value for syrphids v/s aphids was 0.94 in RCH 2 *Bt* and 0.96 in non- *Bt*. The feeding assay with *Bt* intoxicated cotton leaves could not affect the biology and feeding potential of *Chrysoperla*.

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