International Research Journal of Applied and Basic Sciences
© 2012 Available online at www.irjabs.com
ISSN 2251-838X / Vol, 3 (11): 2248-2251
Science Explorer Publications

Effect of Iranian Bt cotton on *Encarsia formosa*, parasitoid of *Bemisia tabaci*

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ABSTRACT: Transgenic Bt cotton produces the Cry 1Ab toxin of *Bacillus thuringiensis* that developing against *Helocoverpa armigera* may be affected on secondary pest such as *Bemisia tabaci*. The laboratory experiments were conducted to evaluate the effects of direct or indirect exposure on the biology of non-target species. Also, we compared the performance of the parasitoid *Encarsia formosa* developing in secondary pest on two cotton lines including Bt cotton and control cotton (non transgenic cotton). The results indicated that the parasitoid immature mortality was not significantly affected by Bt cotton. Host plants significantly influenced development time up to adult for *E. formosa*. Parasitoid reached the adult stage faster on control cotton than Bt cotton. The transgenic cotton effects on *Encarsia formosa* were complex but generally interpretable in terms of host whitefly quality variation among host plants used as food by the whiteflies during their development.

Key words*: Encarsia formosa*, Whitefly, Transgenic cotton, Bt

INTRODUCTION

Transgenic crops are planted on millions hectares worldwide. One of these crops is Bt cotton that was produced by transferring the cry (crystalline) gene from *Bacillus thuringiensis*. *B. thuringiensis* is a soil bacterium that produces Cry insecticide proteins (Zhang, 2005). Most of Bt cotton that express Cry1 protecting plants from Lepidopteran pest damages and have high level of resistance to primary pest (target pest) especially *Helicoverpa armigera*. Bt cotton was grown in nine countries including USA, Argentina, Brazil, India, China, South Africa, Australia, Mexico and Colombia (James, 2009).

Cultivating the transgenic plants is the best opportunity for limiting chemical insecticides use in pest control (Naranjo, 2005). By reduction in pesticide application, population of secondary pests may increase in Bt crops. Cotton secondary pests like *Bemisia tabaci* play important role in ecosystem and food chain because their honeydew is food source for many arthropods. Also the *B. tabaci* sever as host or prey for many of parasitoids and predators and transfer more than 100 plant viruses (Cock et al., 1993). In Bt cotton fields by reducing pesticides application, natural enemies can play a important role in controlling *Bemisia tabaci.* Several studies in recent years have examined the effect of Bt crops on natural enemies (Burgio et al., 2011). There are many efforts to determine the effect of Bt crops (transgenic plants that produce *Basillus thuringiensis* toxin) on non target organisms. And some negative effects have been reported (Hilbeck, 1998).

Previous studies show that the natural enemies can also be exposed to proteins expressed in transgenic plants (direct feeding on transgenic plant nectar or by their host organisms feeding on transgenic plants) (Arpaia, 2009). Parasitoids appeared to be more sensitive than predators to diets containing Cry toxins (Lovei et al., 2009). Negative effects occurred when parasitoids were feeding on hosts sensitive to the toxins expressed in transgenic plants. However, indirect effects do not fully explain the published results, and it seems that host quality have more effects on parasitoids biology (Naranjo, 2009).

Ramirez-Romero et al., (2007) observed negative effects were detected with respect to the *Cotesia marginiventris* developmental times, adult size, and fecundity that developing on aphids fed Bt maize. Ashouri et al., (2001) reported that the fitness and behavior of secondary pests may change when they feed on host plants. Biological control of the sweet potato *Bemisia tabaci* an economically important pest is frequently based on the use of aphelenid parasitoid especially of the genera *Encarsia*. Nothing is known about potential effects of *B.*

thuringiensis toxins on parasitoids. This study was conducted to evaluate the effects of host (*Bemisia tabaci*) quality that feeding on Bt cotton on growth and development of *E. Formosa*, parasitoide of *Bemisia tabaci*.

MATERIALS AND METHODS

The Bt Cotton resistant to *Helicoverpa armigera* produced in Iran for first time by researches in Institute of Agricultural Biotechnology (Tohidfar et al., 2008). Cotton plants (Bt cotton cv. Cucker Line 61.12.29 and non-transgenic cotton cv. Cucker) were grown in 25 cm plastic pots filled with peat and perlit substrate. The pots were placed in net covered cages (75cm×75cm×75cm) in a climate controlled greenhouse conditions at 23±4˚C, 60% RH, 16 h L:8 h D. Plants with 4-8 true leaves were used for experiment. The whiteflies originally were collected from cotton field in Gorgan city, Iran, and were reared on cotton plants in cage and conditions explained above. Also the parasitoids were provided commercially (Koppert Co.) and reared on *Bemisia tabaci.*

Parasitoid immature survival, development time and dry weight

The 50 3rd instar larva of *Bemisia tabaci* that had developed since birth on each of the 2 cotton lines, were individually exposed a single attack by *Encarsia formosa*. After parasitization, they were returned to a host plant of the same line under a same temperature regime 23±4˚C, at 65 ± 10% RH and a 16L : 8D h photoregime, until emergence of an adult parasitoid. After 5 days (expected delay for egg eclosion at minimum temperature 19 ˚C), 10 presumably parasitized whitefly per line (1 whitefly per plant) were randomly selected for dissection to determine egg eclosion incidence. When whiteflies blacked indicating parasitoid survival to the pupal stage started, checking frequency was increased to 3 per day, and newly blacked pupa were placed in individual gelatin capsules. This allowed determination of emergence success, development time from egg to pupation, and from pupation to emergence. Newly emerged wasps were placed in an oven at 60 ˚C for 48 h to determine adult dry weight using a microbalance (sensitivity 0.001 mg).

Adult longevity, fecundity

We determined these indices of female fitness for parasitoids that developed from whiteflies reared on each cotton line. Forty (0–24 h old) female *Encarsia formosa* per line held in individual cages with sugar water and a cotton plantlet with 50 3rd instar *Bemisia tabaci*. The plants and aphids were replaced daily until females died. Parasitoid fecundity was measured for female aged 3, 6, 9 and 12 days after eclosion. After being exposed to the wasp for 24 h, whiteflies held at 23 ± 2 ˚C and 65 ± 10% RH under a 16L: 8 D photoregime, until black pupa formed. Numbers of black pupa formed were used to obtain age-specific fecundity and total fecundity was calculated as the sum of the 4 values, one of each age class sampled. The t-student test was used to detect significant differences for parasitoid development time, fecundity, dry weight and mortality% between Bt cotton and non Bt cotton.

RESULTS AND DISCUSSION

Results showed that the whiteflies exposed to one attack by *Encarsai formosa*, had 90–100% parasitism upon dissection 5 days after parasitation, that indicates high acceptability of 3rd instar whiteflies from Bt cotton and control cotton to *Encarsia formosa*. In control cotton, whiteflies mortality was 5 individuals out of 50 died in the course of the test. This test repeated in 20 replicates. There was no significant difference (t value= 1.57902; p= 0.11838) total parasitoid mortality from oviposition to adult emergence between the Bt cotton and control cotton (Table 1).

Table 1. Stage-specific mortality of the parasitoid *Encarsia formosa* developing in the *Bemisia tabaci* reared on Bt cotton and control cotton; the experiment started with 3rd instar whiteflies receiving one *Encarsia Formosa*.

Cotton line	Egg – prepupa			Prepupa – adult	
	N	n	%	n	%
Control cotton	50	5	10	0	0
Bt cotton	50	6	12	0	0

N is number of parasitized whiteflies. n is number of mortality for egg-prepupa and prepupa-adult stages.

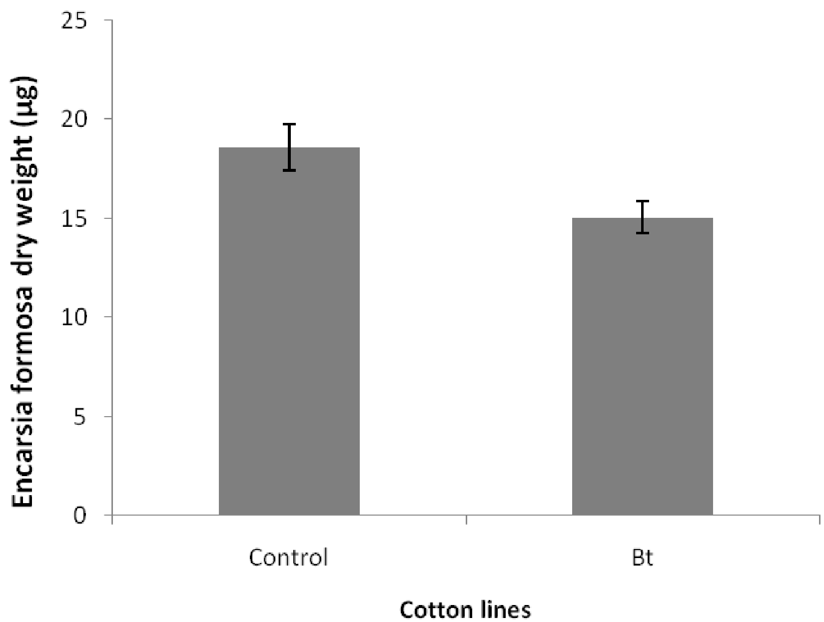

Figure1. Dry weight (mean ± SE) of newly emerged adult *Encarsia formosa* developing in *Bemisia tabaci* reared on the two cotton lines. Control: non transgenic cotton, Bt: Bt cotton.

The cotton line significantly affected parasitoid development time to adult eclosion (t value= 7.005141; p= 0.00001). Parasitoid development was mostly affected during the prepupal stage (t value= 9.782314; p= 0.00001). In test course we did not any male parasitoid. On average, metamorphosis to the adult stage on whiteflies fed the non transgenic cotton tended to be relatively short. Parasitoid dry weight at emergence was significantly affected by the cotton line on which host whiteflies fed (t value = 7.05; p = 0.00001), Wasps were smallest when emerging from whiteflies on the Bt cotton and largest on the non transgenic cotton (Figure 1).

The fecundity of female *Encarsia formosa* provided with whiteflies cottons was strongly affected by cotton line. Parasitoid on control cotton significantly more whiteflies parasites (t value=8.91; p=0.00001). The fecundity of parasitoid was affected by the age and host plant interaction. Young parasitoid in both cotton lines parasite more whiteflies (Table 2).

Table 2. Fecundity of parasitoid *Encarsia formosa* developing in the *Bemisia tabaci* reared on two cotton lines.

Cotton line		Age of female(Days)			N	Total fecundity
Days	3	6	9	12		
Bt cotton	15.22±0.9	9.2±1.8	7±1.8	4.6±0.79	40	38.88±1.05
Control cotton	16.6±1.01	12.45±2.1	8.27±0.87	5.9±1.3	40	46.5±2.6

N is number of parasitized whiteflies

Comparing the above results with those of our previous study on *Bemisia tabaci* performance on the same cotton lines supports the hypothesis that the parasitoid was indirectly affected by plant resistance via its effects on whiteflise host quality for parasitoid growth and development. Variable host quality with Cotton line could have resulted from the nutritional quality of Cotton sap as whiteflies food (Brough and Dixon, 1989; Stadler, 1998; Salvucci et al., 1998). However, the specific causal relationships involved are not obvious, and will require further investigation. Whiteflies fed control cotton were high quality host for parasitoid wasps and supported normal parasitoid survival and fecundity, and relatively short wasp development. Another interesting observation was that parasitoid performance was better when the host whiteflies were feeding on control cotton than on the Bt cotton. Suggesting that expression of Bt proteins (Cry 1 Ab) in Cotton reduce nutritional value for the whiteflies.

However, these results do not means that Bt toxin was acquired by the whiteflies, nor that it directly affected the parasitoid, because There was no significant difference in parasitoid mortality from oviposition to adult emergence between the Bt cotton and control cotton. It seems that over-expression of the toxin in foliage reduces free amino acid availability in phloem sap (nutritional value for whiteflies) (Burgio, 2011). From the viewpoint of interaction of plant resistance with biological control, our results resemble those of Ashouri et al., (2001), Hilbeck et al., (1998), Guo et al., (2004) and Burgio, (2011) who found that plants expressing a Bt toxin negatively affected the natural enemies. The variation of *Encarsia formosa* adult weight with Cotton line correlates with the weight variation of the whiteflies host on these lines (Azimi et al., 2012, don't published data). *Bimisia tabaci*'s adult weight was smallest on the Bt. whiteflies size is a key factor that effect on *Bemisia tabaci* parasitoid fitness because parasitoid fitness depends on host quality (Ashouri et al., 2001).

Bt cotton developing against *Helicoverpa armigera* for reducing chemical insecticide. Reducing chemical insecticide is benefit for natural enemies but different unexpected effects of Bt cotton affected on the fitness of *Encarsia formosa* and *Bemisia encarsia* biological control. Our results and similar studies suggest that current knowledge of the transgenic crops is insufficient for making safe predictions. More empirical data are needed on specific crop systems such as the cotton and its pests.

ACKNOWLEDGMENTS

This study was supported by Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Iran and Agricultural Biotechnology Research Institute of Iran is gratefully acknowledged for financial support.

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