Effects of the Neonicotinoid compound, Emamectin on *Bracon brevicornis* (Hymenoptera: Braconidae) with parasitization on two lepidopteran hosts.

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Abstract: The objective of this study was to evaluate the direct and indirect toxicity of the Neonicotinoid, emamectin benzoate on *Bracon brevicornis* which is used in crop and vegetable production. Laboratory bioassays were done to investigate the immature and adult stages of *B. brevicornis* on the treated 2nd larvae instars of *Tuta absoluta* and *Ephestia kuehniella* from periods of zero time, 1st, 3rd and 5th days of parasitism. High toxic effect was recorded on the treated 2nd larvae instars of *T. absoluta* than that treated *E. kuehniella* larvae with contact method. For larval duration and pupal period of *B. brevicornis*, the difference between treated and untreated host individuals was statistically not significant within the same host and between two hosts at the same treatment. Both female and male longevities those derived from treated hosts were decreased gradually from at zero time to five day of parasitism on both hosts. Our results concluded that emamectin benzoate was found safer to *B. brevicornis* at all the tested periods of parasitism on *T. absoluta* and *E. kuehniella*. Therefore, emamectin is considered an important component within integrated pest management programs.
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1. Introduction:

Studying the side effect of insecticides on the natural enemies is highly required to exclude the detrimental effects on the natural enemies. The integration of chemical and biological control is often critical to the success of an integrated pest management (IPM) program for arthropod pests (Consoli *et al.,* 2001; Wakgari and Giliomee, 2003; El-Wakeil *et al*., 2006; Volkmar *et al*., 2008; Preetha *et al*., 2009). Emamectin benzoate, one of the newer compounds is synthesized from the naturally occurring insecticide/acaricide of avermectin family. This was discovered in 1984 as a broad spectrum lepidoptericide and produced by fermentation of a soil microorganism *Streptomyces avermitilis* Burg. It has been reported to possess excellent action against pests cotton and vegetables. It acts on the nerve cells to suppress muscle contraction thus inhibiting the larvae from feeding (Govindan *et al.,* 2010). Jansson and Dybas (1996) reported that emamectin benzoate is stored as a reservoir in plant parenchyma tissues and this accounts for its long residual activity against several phytophagous insects.

Ephestia kuehniella (Lepidoptera: Pyralidae) is the most serious and destructive storage pests to field and vegetable crops. The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a native microlepidopteron of South America (Torres *et al.,* 2001), which can affect all aerial parts of the plant (leaves, flowers, stems and fruit). This pest has the potential to cause damage which can be up to 100% losses (Moreira *et al.,* 2005). *T. absoluta* eggs and larval instars was examined under laboratory conditions (Consoli *et al*.,1998 and Urbaneja *et al*., 2009).

Integrated use of natural enemies particularity the larval parasitoid, *Bracon brevicornis* (Hymenoptera: Braconidae) for management of vegetable pests with different pesticides against pests appears possible by use of selective pesticides (Suh *et al*., 2000, Tillman and Mulrooney, 2000, Anne *et al.,* 2001, Dora *et al*. , 2004 and Luna *et al.,* 2007). Side effect of the insecticide was studied in the laboratory to maximize compatibility of chemical and biological control methods this will help minimize any negative impact on the natural enemies. (Pineda *et al*., 2007, Yu, 2008, Wang and Tian, 2009).

Therefore, the present work has been carried out to determine the toxicity of emamectin benzoate under laboratory conditions against the parasitoid, *Bracon brevicornis* for its selection in the IPM program of two lepidopteran pests; *Tuta absoluta* and *Ephestia kuehniella*.

2. Material and methods:

2.1. Tested pesticide:

The tested compound is Neonicotinoid, Emamectin (Proclaim® 5%) and was obtained from Syngenta Chemical Co. Ltd.

2.2. Tested insects:

2.2.1. *Tuta absoluta*

Culture of *T. absoluta* eggs and larvae was obtained from infested tomato plants at Kahaa Research Station, Qualiobia, Egypt. *T. absoluta* was reared on tomato leaf under laboratory conditions of 20 ±1°C, 65% R.H.

2.2.2. *Ephestia kuehniella*

Culture of *E. kuehniella* were obtained from Kahaa Research Station. Culture was stored and reared, under laboratory conditions of 20 ± 1°C, 65% R.H.

2.2.3. *Bracon brevicornis.*

The culture of larval parasitoid, *B. brevicornis* was reared under laboratory conditions of 20 ± 1°C, 65% R.H. on the 2nd larvae instars of *T. absoluta* and *E. kuehniella.* The culture was obtained from infested bollworm of the cotton plants at Kahaa Research Station.

2.3. Experimental design:

Four concentrations (12.5, 6.3, 3.1 and 1.5 ppm) of the tested compound, Emamectin and a control (distilled water) were tested against the 2nd larvae instars of *T. absoluta* and *E. kuehniella* with replication of 40 larvae/ treatment. Direct and indirect toxicity of the compound with concentration of 1.5 ppm was measured on the parasitoid; *B. brevicornis* under laboratory conditions of 20±1°C and 65% R.H. Direct toxicity to larval parasitoid was measured with spray method. While indirect toxicity was estimated by contact method to the 2nd larval instars of *T. absoluta and E. kuehniella* at zero time, 1st, 3rd and 5th days of parasitism. Direct toxicity on the larval parasitoid, *B. brevicornis* adults was measured by spray method on the inner surface of tubes. Contact method on the 2nd larval instars of *T. absoluta and E. kuehniella* was applied by spraying of emamectin solution using micropipette in petri dish in order to simulate of insecticide spray on plant leaves. Then, the larvae were deposited in these petri dishes for 5 minutes and then transferred to the test tubes. In each treatment, ten replicates of the parasitoid pairs were exposed to five hosts of both *T. absoluta and E. kuehniella* (2nd larval instars). Pupation, adult emergence, adult longevity and sex ratio were observed daily.

2.4. Statistical analysis:

Means and standard errors were calculated for each experiment and the data were compared using the ANOVA test and the significance between means was compared by LSD values at 0.05 level, using SAS program (SAS institute, 1988).

3. Results and discussion

Direct toxicity to 2nd larval instars of *T. absoluta* and *E. kuehniella*:

LC_{50} values of treated the 2nd larvae instars were plotted on probit paper and were recorded 0.142 and 1.105 ppm for *T. absoluta* and *E. kuehniella* larvae, respectively. Presented data in Table (1) indicated that the lowest pupation (%) were 33 and 37% at concentration of 1.5 ppm with *T. absoluta* and *E. kuehniella*, respectively while high adult emergence (%) were 30 and 32 %, respectively. In generally, emamectin was reduced pupation and adults emergence compared to those obtained by the untreated (100% for both tested insects).

Direct toxicity on *B. brevicornis*:

The mortality (%) of *B. brevicornis* adults by emamectin at 1.5 ppm with spray method was recorded 1.1 ± 0.2. This declining ratio is in agreement with Ishaaya *et al.* (2002) who indicated that emamectin is a macrocyclic lactone insecticide with low toxicity to non-target organisms and the environment.

Indirect toxicity on *B. brevicornis*:

Effects of emamectin with 1.5 ppm on larval duration of the larval parasitoid, *B. brevicornis* at all tested periods of parasitism indicated that larval durations were ranged from 5.1 to 5.2 days on *T. absoluta* and from 5.1 to 5.13 days on *E. kuehniella* (Table 2). These periods on untreated *T. absoluta* and *E. kuehniella* larvae were 5.15 and 5.20 days, respectively. Pupal periods were ranged from 5.10 to 5.32 days on *T. absoluta* and from 5.14 to 5.32 days on *E. kuehniella* (Table 2). These periods on untreated *T. absoluta* and *E. kuehniella* larvae were 5.20 and 5.35 days, respectively. The difference between treated and untreated individuals was statistically not significant within the same host and between two hosts at the same treatment. Different investigators stated that the pre-imaginal development stages within hosts appear to be well protected from many insecticides (Singh and Varma, 1986, Brar *et al*., 1991, Consoli *et al*., 1998).

In zero time old parasitized larvae, higher *B. brevicornis* longevity (Table 3) was recorded according to other treatments. Male longevity at zero time of parasitism were 12.6 and 13.5 days while female longevity were 16 and 16.2 days with parasitization on treated *T. absoluta* and *E. kuehniella* larvae, respectively. Both female and male longevity was decreased gradually from at zero time to five day of parasitism on both hosts. This finding is in agreement with the results reported by Anne *et al.* (2001) who mentioned that longevity of parasitoids surviving a sub lethal dose was reduced. Male longevity of the parasitoid that derived from untreated host was significantly differed with all treatments for both hosts while female longevity of the parasitoid that derived from untreated host was not significantly differed with

at zero time of parasitism for both hosts. Adult longevity that derived from two treated hosts larvae indicated that the males affected more than females.

Sex ratio affected with these compound at all periods of parasitism, especially in earlier old of parasitism (Table 3). These ratios at zero time, 1st, 3rd , 5th days after parasitism and untreated on *T. absoluta* were 1: 1.11, 1: 1.15, 1: 1.18, 1: 1.22 and 1: 1.28 (male: female), respectively. While on *E. kuehniella* were recorded as 1: 1.12, 1: 1.13, 1: 1.20, 1: 1.22 and 1: 1.25, respectively. These slightly differences in sex ratio are in confirmation with the results reported by (Vahid *et al.,* 2008, Temerak, 2009, Thanavendan and Jeyarami, 2010).

The results concluded that emamectin benzoate was found safer to *B. brevicornis* at all the tested periods of parasitism on *T. absoluta* and *E. kuehniella*. Therefore, emamectin with its low toxicity to this parasitoid and the environment is considered an important component within integrated pest management programs for controlling field crop pests.

Table 1. Effect of contact method by Emamectin on pupation and adult emergence with the treated 2nd larval instars of *T. absoluta* and *E. kuehniella*.

Treatment (ppm)	Pupation (%±SE)		Adult emergence (%±SE)	
	T. absoluta	*E. kuehniella*	*T. absoluta*	*E. kuehniella*
12.5	26	28	23	25
6.3	28	32	26	29
3.1	30	33	26	29
1.5	33	37	30	32
Mean±SE	29.25±0.05	32.5±0.02	26.25±1.07	30.03±1.02
Untreated	100	100	100	100

Table 2. Effect of contact method by Emamectin on pre-imaginal stages of *B. brevicornis* with the treated 2nd larval instars of *T.absoluta* and *E. kuehniella*.

Treatment	Larval duration (days±SE)		Pupal duration (days±SE)	
	On *T. absoluta*	On *E. kuehniella*	On *T. absoluta*	On *E. kuehniella*
Zero time	5.20±0.10	5.13±0.11	5.32±0.09	5.20±0.10
One day	5.16±0.12	5.11±0.12	5.18±0.10	5.14±0.11
Three days	5.10±0.12	5.10±0.12	5.10±0.12	5.22±0.10
Five days	5.15±0.11	5.12±0.11	5.30±0.09	5.32±0.09
Untreated	5.15±0.12	5.20±0.10	5.20±0.10	5.35±0.09

At the same column, means are not significantly different (P≤0.05).

Table 3: Effect of contact method by Emamectin on adult longevity and sex ratio of *B. brevicornis* with the treated 2nd larval instars of *T. absoluta* and *E. kuehniella*.

Treatment	Adult longevity (days±SE)				Sex ratio			
	On *T. absoluta*		On *E. kuehniella*		On *T. absoluta*		On *E. kuehniella*	
	Male	Female	Male	Female	Male	Female	Male	Female
Zero time	12.6±0.06^{b}	16.0±0.03^{a}	13.5±0.05^{b}	16.2±0.04^{a}	1.11	1	1.12	1
One day	11.6±0.08^{b}	14.3±0.05^{b}	12.6±0.06^{c}	15.5±0.04^{b}	1.15	1	1.13	1
Three days	12.2±0.08^{b}	12.8±0.06^{c}	12.4±0.06^{c}	14.5±0.05^{c}	1.18	1	1.20	1
Five days	10.5±0.08^{c}	12.2±0.06^{c}	11.1±0.07^{d}	14.3±0.05^{c}	1.22	1	1.22	1
Untreated	14.2±0.05^{a}	16.3±0.03^{a}	14.5±0.05^{a}	16.5±0.03^{a}	1.28	1	1.25	1

At the same column, means followed by similar letters are not significantly different (P≤0.05).

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