Sterilant and oviposition deterrent activity of neem formulation on Peach fruit fly *Bactrocera Zonata* (Saunders) (Diptera: Tephritidae)

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ABSTRACT

Impact of neem (600, 300, 150, 75, 37.5 and 18.7 ppm) on sterilant and oviposition activity was evaluated on the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) under laboratory conditions using filter paper dip method. Results revealed that neem formulation has a significant effect against eggs of *B. zonata* at all concentrations used, compared with control. However no effect of neem on the sterilant percent and adults emerged from treated eggs at all concentrations. Moreover, the rate of eggs sterility which produced from treated adults with the previous concentrations was concentration-dependent and significant differences were existed when mated (treated females with normal male and treated female with treated male). While no significant differences observed in treated male mated with normal female. When compared with control (normal male with normal female). The repellency effect of neem on egg deposited in orange fruits after being sprayed with previous concentrations was high and reach to 5.0 % eggs/puncture/fruit, compared with 65.8 % of control. Moreover, the percent of egg hatch decreased to 76.2 % at 600 ppm, compared with 89.0 % of control.

Keywords: neem, *Bactrocera zonata*, sterility, oviposition deterrent, bioassays.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders), is recognized as one of the most important and serious pests attacking fruit crops. It is established in South and South-East Asia (Allwood *et al.*, 1999). In Egypt, it has been established since the late 1990s, owing to the suitability of climate and the extension in planting favorable host fruits such as peach, guava, mango, citrus and apricot (1996). It causes serious economic losses, either by direct damage to fruit or by warranting the need for quarantines and insecticide treatments.

The control measures adopted rely mainly on contact poisons or baits (Lee, 1988). Contact poisons may have serious deleterious effect on health as fruits in Egypt and other developing countries are consumed raw, often unwashed. Besides, baits and sprays of conventional insecticides also have toxic effects on parasitoids of *B. zonata*. Sterile insect techniques (SIT) have been advocated (Wong *et al.*, 1984; Sheo *et al.*, 1990). However, because of the polyandrous and long distance migratory abilities of the flies, with high population densities throughout the year. SIT does not seem suitable for continental areas. This dilemma has made it essential to find effective control measures, which are safe to both human beings and non-target biological systems. These methods have a negative impact on the environment, and specifically on the phytoparasitic populations of beneficial organisms. Thus, environment friendly methods of control are much in need.

Neem (*Azadirachta indica* A. Juss) is native to India (Roxburgh, 1874) from where it has spread out to many Asian and African countries as well as Australia and South America (Srivastava *et al.*, 1997). In recent years, the bioactivity of Azadirachtin against insect pests has been investigated in detail (Huang *et al*., 2004; Senthil-Nathan *et al*., 2008). Large numbers of insect pests from different orders have been shown to exhibit different levels of susceptibility to Azadirachtin (Schmutterer and Singh, 1995).

Azadirachtin, a mixture of several structurally related tetranortriterpenoids, has attracted the greatest attention in recent years (Prakash and Srivastava, 2008) for modern pest control strategies. Mostly, three kinds of reaction are found: alternation of behavior leading to repellent and/ or antifeedant effects, disruption of insect development by inhibiting the release of prothoracicotropic hormones and allatotropins and sterillant effects of females caused mainly by alterations of ecdysteroid and juvenile hormone in the target organism (Mordue *et al.*, 1998; James, 2003). Azadirachtin are often described to have minimal toxicity to beneficial organisons such as parasitoids, predators and pollinators (Raguraman and Singh, 1999) and can degrade rapidly in the environment (Sundaram and Curry, 1996). Therefore, the objective of our study was to evaluate the neem formulation as sterilant and oviposition deterrent against the Peach fruit fly *B. zonata* under laboratory conditions.

MATERIALS AND METHODS

Rearing technique

The culture of peach fruit fly was collected from infested orange fruits and maintained in the laboratory conditions of 25 ± 2 ^{0}C, 65 – 75% RH and a photoperiod of L.D. 12h. Adult diet consisted of 1 part of protein hydrolysate: 3 parts of sugar by weight while the larval diet consisted of wheat bran 100g, brewer's yeast 17g, granulated sugar 33g, agar 3.5 g, nipagin 0.5 g, hydrochloric acid 20 ml and water 400 ml (Qureshi *et al.*, 1974).

Bio pesticide used

Commercial formulation of neem pesticide (Nimbecidene), active ingredients azadirachtin 0.03% was obtained from India (manufactures T.Stanes and company LTD, Tamil Nadu). For our experiments, we used it against the peach fruit fly (PFF) *B. zonata* at concentrations of 600, 300, 150, 75, 37.5 and 18.7 ppm.

Bioessays

Ovipositionol and ovicidal bioessay

The number of eggs laid was collected and 100 eggs of each treatment were used to determine the percent of egg hatchability. The collected eggs were placed on a black filter paper treated with different concentrations of neem (600, 300, 150, 75, 37.5 and 18.7 ppm) and placed on moist cotton pad in Petri dishes. After three days the percent of egg hatching was recorded for each treatment with 5 replicates each. The newly emerged larvae from each treatment transferred to larval diet to complete development. After complete pupation, the fine sand was sieved and the pupae were collected, and then placed in a Petri-dish. Eclosed adults were transferred to cages. Twenty five flies were collected from each sex and maintained in experimental cage. The percent of egg hatching was recorded to determine the adult sterility and estimated the total eggs/female.

Sterility bioassay

Newly emerged flies from control category separated for each sex and transferred gently to clean cage provided with adult diets (1 part of protein hydrolysate: 3 parts of sugar), treated wit different concentration of neem (600, 300, 150, 75, 37.5 and 18.7 ppm). Flies were fed on treated diet till sexual maturation. For each concentrate, 25 treated males were confined in a cage with 25 normal females, 25 treated females were confined with 25 normal males and 25 treated males were confined with 25 treated females. The sterility was determine after 3 days from egg laying and replicated 5 time for each treatment.

Ovipositional preference

The orange fruits were sprayed as described in experiment 1 and 2. Each treatment was arranged in rearing cage and maturity flies were released to each cage for oviposition on treated and untreated orange. After females puncture the orange fruits, the eggs were separated from each puncture and counted using a stereo-microscope. After 3 days, the eggs were checked again to record the percent of egg hatch.

Statistical analysis

Data obtained were statistically analyzed through ANOVA (SAS Institute 1999). When F-test was significant, means were separated using Tukeys test at the 0.05 level of significance.

RESULTS

The egg hatchability of *B. zonata* was estimated at 600, 300, 150, 75, 37.5 and 18.7 ppm of neem formulation and is presented in Table 1. Significant differences were found in egg hatchability at all concentrations tested (F= 102.290; P< 0.0000). While no significant differences were found in adult sterility emerged from treated eggs (F= 1.087; P< 0.3941) and in the total eggs laid by female (F= 0.231; P< 0.9627).

Data clearly indicate that the percent of egg hatchability of *B. zonata* decreased gradually by increasing the concentrations of neem. it decreased to 8.4% compared with control of 87.6%. Effect of neem on adult's sterility ranged from 8.6% to 14.0% at concentrations of 300 and 37.5 ppm, respectively compared with 9.6% in control. Total eggs per female also slightly decreased at all concentrations tested compared with control.

Table 1. Effect of neem formulation on egg hatchability, the sterile percent (in %) and fecundity of *B. zonata*

Concen trations (in ppm)	Egg hatchability	Adult sterility	Total eggslaid/ female
600	8.4 ^{e}	11.4 ^{a}	526.4 ^{a}
300	11.8 ^{e}	8.6 ^{a}	527.4 ^{a}
150	37.0 ^{d}	11.4 ^{a}	525.4 ^{a}
75	52.5 ^{c}	13.4 ^{a}	517.4 ^{a}
37.5	65.8 ^{b}	14.0 ^{a}	520.0 ^{a}
18.7	74.0 ^{b}	12.8 ^{a}	511.0 ^{a}
Control	87.6 ^{a}	9.6 ^{a}	550.0 ^{a}

Table 2. Effect of neem formulation on egg sterility of *B. zonata* emerged from treated adults

Concen-trations	Sterility %		
	T male × N Female[1]	T female × N male [2]	T male × T Female [3]
600	13.0 [a]	61.0 [ab]	46.4 [a]
300	14.4 [a]	57.4 [b]	37.6 [b]
150	15.4 [a]	70.6 [a]	43.6 [ab]
75	15.4 [a]	32.6 [c]	24.2 [c]
37.5	17.2 [a]	21.4 [d]	13.0 [d]
18.7	15.4 [a]	19.0 [de]	15.0 [d]
Control	9.6 [a]	10.0 [e]	9.4 [d]

1 Treated males mated with normal females, 2 Treated females mated with normal males, 3 Treated males mated with treated females

The sterility of eggs produced from neem treated adults of *B. zonata* is presented in Table 2. No significant differences were found in treated male when mated with normal female at all concentrations tested (F= 0.587; P< 0.5377) and the sterility ranged from 14.0% to 17.2% at concentrations of 600 and 37.5 ppm, respectively compared with 9.6% in control. Significant differences were found in treated females when mated with normal males (F= 49.129; P< 0.0000). It is noticed that the high percent of sterility was 70.6% at concentration 150 ppm and the sterility increased gradually by increasing the neem concentrations tested. At the last treatment when treated males mated with treated females (F= 34.646; P< 0.0000), it was recorded that the high percent of sterility at 600 ppm followed by 150 ppm, while the low percent of sterility was at 37.5 ppm compared with 9.4% in control.

As seen in Table 3, significant differences were found in treatments, total eggs inside the puncture for one fruit after being sprayed was 5.0, 17.8, 17.4 28.2, 44.0 and 48.4 at concentrations of 600, 300, 150, 75, 37.5 and 18.7 respectively, compared with 65.8 in control (F= 16.176; P< 0.0000). Also, the percent of egg hatch slightly decreased than control and decreased gradually by increasing concentration of neem (F= 4.462; P< 0.0000).

DISCUSSION

The results obtained from the different treatments of experiments under laboratory conditions revealed a high effect of neem formulation in both sterility of eggs and oviposition repellent of *B. zonata*. Azadirachtin, a very complex tetranortriterpenoids, has been effectively used against more than 400 species of insects, including many key crop pests, and has proved to be one of the most promising plant ingredients for integrated pest management at the present time (Ma *et al*., 2000; Liang *et al.*, 2003). It displays an array of effects on insects, acting

Table 3. The repellency effect of neem formulation on egg layed and percent of egg hatching (in %) of *Bactrocera zonata*

Treatments (in ppm)	Eggs/puncture/fruit	Egg hatch
600	5.0 [d]	76.2 [c]
300	17.8 [cd]	73.2 [c]
150	17.4 [cd]	77.8 [bc]
75	28.2 [c]	81.2 [abc]
37.5	44.0 [b]	84.6 [ab]
18.7	48.4 [b]	85.0 [ab]
Control	65.8 [a]	89.0 [ab]

inter alia, as a phago-and oviposition deterrent, repellent, antifeedant, growth retardant, molting inhibitor, sterilant and preventing insect larvae from developing into adults (Schmutterer, 2002).

Steets (1976) found that colorado potato beette, *Leptinotarsa decimlineata* females fed with azadirachtin for 5 days recorded reduced fecundity (> 98%). Schmutterer (1987) also observed a highly reduction in the number of eggs of *L. decimlineata* after treatment of potato leaves by azadirachtin, a relatively slight egg sterilization effect was also recorded. Steffens and Schmutterer (1983) recorded a strong reduction in fecundity of medfly, *Ceratitis capitata* with methanolic neem seed kernel incorporated diet when compared with control. Burkhard (1989) observed a reduction in egg deposition, weight of the ovaries and free ecdysteroid in the haemolymph and the ovaries when applied the blow fly, *Phormia terraenovae* with azadirachtin. Similar effects on *Epilachna varivestis* were reported by Steets and Schmutterer (1975) and on other insects (Singh *et al*., 1996) too.

We confirmed our findings with neem formulation when applied as a commercial preparation named nimbecidine to *B. zonata* adults at different concentrations. A significant reduction in fertility was recorded in the treated famales mated with untreated and treated males compared with control and treated males mated with normal females and could have been due to either the absence of eggs or the production of sterile eggs. The observed immaturity of the ovaries and the maturity of the accessory glands in treated females indicate that neem should possess specific activity on the ovaries only, without affecting the whole female reproductive system (Vincenzo, 1999).

In our studies of the oviposition deterrence of *B. zonata* on orange fruits and the sterility of eggs showed differences compared with control. This is in conformity with the report of Chen *et al.* (1996), who suggested the role of non-volatile neem components detected by the ovipositor as a signal to reduce egg laying. Oviposition repllency has been reported for azadirachtin extracts against melon fly *Bactrocera cucurbitae* and oriental fruit fly *Bactrocera dorsalis* (Singh and Singh, 1998; Khan *et al* 2007). Singh and Srivastava (1983) reported that *B. dorsalis* required a much higher concentration of ethanolic extract of hexane extract for arrest of oviposition as compared with *B. cucurbitae*. Our results disagree with those of Saxena and Rembold (1984). Naumann and Isman (1995) and Saxena and Basit (1982) reported that azadirachtin had no oviposition deterrence effect.

The results clearly indicate that low concentrations of neem can be applied effectively as sterilant and oviposition deterrent for the peach fruit fly populations. Azadirachtin is a cheap, effective and renewable source of ecofriendly botanical insecticide. In comparison with synthetic agrochemicals, azadirachtin is safe to mammals (Niemann *et al*., 2002) and to non-targeted biological systems (Schmutterer, 2002; Anibal, 2007). Field use of neem may be cause problems because azadirachtin degrades rapidly after exposure to UV radiation (Barnby *et al*., 1989). However, the use of azadirachtin-based compounds in insecticidal baits appears possible especially when mixed with attractant substances such as protein hydrolyzate (Roessler, 1989).

As a result of this study, it is concluded that neem formulation gave significantly insecticidal efficacy against *B. zonata* with lower concentrations than control so that, higher concentrations of neem could provide suitable alternatives into an IPM program. Nevertheless, further research is needed to determine the antifeedant and the safety toward natural enemies of *B. zonata*. In addition, it should be assessed for field efficacy.

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