EFFECT OF AZADIRACHTIN ON EGG DEPOSITION AND ON PROTEIN CONCENTRATION IN THE GRASSHOPPER *HETERACRIS LITTORALIS* RAMB. (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT

Number of egg pods per azadirachtin-treated female decreased in a dose dependent manner from 6.47 ± 2.21 in control group to 2.00 ± 0.82. At higher concentrations (100 ppm and on words) no egg-pods were deposited. Nymphs treated at the beginning of the last instar suffered a significant decrease in the total protein concentration of the fat body and in the haemolymph. The present results suggest that azadirachtin may induce a depression in protein synthesis, therefore its release in the haemolymph and consequently, its uptake by developing oocytes. This decrease may also explain the significant decrease in the number of deposited eggs.

INTRODUCTION

Azadirachtin exhibits deterrent, antiovipositional, antifeedant, growth disrupting (growth regulating), fecundity and fitness reducing properties on many insect species. It is an effective sterilant: females of some insect pest species were sterilized to various degrees, sometimes completely (Steets, 1976; Schmutterer, 1987). So the aim of this work is to contribute some information of azadirachtin effects on egg deposition and protein concentration in the haemolymph and fat body of treated insects.

MATERIAL AND METHODS

Adults and nymphs of *Heteracris littoralis* were collected from Abou Rawash district (Giza Governorate), Egypt. A laboratory stock was reared in electrically heated wooden cages at constant temperature of 30±1°C with fluctuating relative humidity (50-70%). Insects were fed clover *Trifolium alexandrinum* L. from

November to May and then fresh leaves of *Sesbania sesban* L. Cages were supplied with suitable ovipositional pots for oviposition and were kept sufficiently moistened.

To test the effect of azadirachtin on egg production; a series of concentrations was prepared (50-1000 ppm). For each concentration, 20-30 insects were tested in five replicates, 5-6 grasshoppers each. Azadirachtin preparations (5 µl) were topically applied to the neck membrane of tested insects (at the beginning of the last nymphal instars). Control groups were treated with 5µl acetone.

The total protein was determined in the fat body and haemolymph of *H. littoralis* using Biuret reagent. The principle is that protein forms a colored complex with cupric ions in an alkaline medium. A standard protein reagent (albumin 60 mg/ml), was prepared and Biuret reagent was composed of: sodium hydroxide (0.2 N), potassium-sodium-Tartarte (18 mmol/l), potassium iodide (12 mmol/l) and cupric sulfate (6 mmol/l).

Insects were treated with 5µl at the beginning of the last instar with five concentrations of azadirachtin preparation. For each of the 5 concentrations used (25-200 ppm) a group of 25 insects (young instar nymphs) were treated and the last was repeated 3 times. The insects were dissected six days after treatment and the fat body was extruded. This time coincided with the vitellogenin synthesis in the fat body (Wigglesworth, 1970). The fat body was collected from last instar female nymphs (treated and control) by chilling insects in a fridge. Then they were cut opened and the fat body was collected in previously weighed Epindorf tubes (1.5 ml), each containing 20 µl of 0.9% NaCl. The Epindorf tubes were kept in ice during the collection process. Then these tubes were weighed again to calculate the fresh weight of the fat body in each tube. Fat body was diluted up to 1 ml with distilled water and homogenized. The fat body was centrifuged at 3000g for 10 minutes at 4°C. The resulting supernatant was used in the tests for protein concentration. Aliquots of fresh fat body were collected and weighed immediately, and were dried in hot air oven at 60°C. Water loss from these aliquots was used to calibrate the dry weight of the used fat body.

For the determination of total protein concentration in the haemolymph, groups of newly emerged adult females were treated with different azadirachtin concentrations (50-1000 ppm), and the test was repeated three times. The haemolymph was extracted six days after treatment. This time coincided with the appearance of vitellogenin in the haemolymph (Buhlmann, 1976). The haemolymph was obtained from both normal and treated adult females by cutting the hind coxae and the oozing haemolymph was collected by capillary tubes and then was poured

into Epindorf tubes (1.5 ml), centrifuged at 3000g for 10 minutes at 4°C and the supernataht was used. The working procedure for fat body and haemolymph preparations was started using three test tubes as follows:

1-A test tube contained one ml of Biuret reagent (blank).

2-A test tube contained one ml of Biuret reagent +20 µl of protein standard 0.06g/ml (Standard).

3-A test tube contained one ml of Biuret reagent +20 µl of the sample (fat body/haemolymph preparation).

These tubes were shaken and incubated for 5 minutes at 25°C then they were read at 546 nm using a spectrophotometer (Shimadzu.UV-160). Total protein concentration was determined according to Oser (1979) as follows:

$$\text{Total protein concentration} = \frac{\text{A sample}}{\text{A standard}} \quad x \quad 6$$

RESULTS AND DISCUSSION

1. Effects of azadirachtin on egg deposition of *H. littoralis*

Table (1) shows that the number of egg pods per treated female decreased in a dose dependent manner, the average number of egg-pods/treated female decreased from 6.47 ± 2.21 in control group to 2.00 ± 0.82 in female treated with 25 ppm. At higher concentrations (100 ppm and on words) no egg-pods were deposited.

TABLE (I)

Effect of different doses of azadirachtin on the longevity and egg production of *H. littoralis* (mean ± SD).

Dose (ppm)	No. egg-pods/ female	No. eggs/pod
Control	6.47 ± 2.21[a] (4 - 9)	31 ± 6.00 [a] (26 - 45)
25	2.00 ± 0.82 [b] (1 - 3)	29 ± 4.00 [b] (21 - 33)
50	1.85 ± 0.69 [b] (1 - 3)	27 ± 3.60 [b] (24 - 31)
75	1.64 ± 0.67 [b] (1 -2)	19 ± 2.40 [c] (17 - 24)
100	0	0

Values followed by different letters in the same column are significantly different ($P < 0.05$).

2- Effects of azadirachtin on total protein concentration in the fat body

Nymphs of *H. littoralis* treated with azadirachtin (at the beginning of the last instar) showed a decrease in the total protein concentration of the fat body in a dose dependent manner. Total protein decreased sharply from 57.03 ± 5.56 mg/g dry weight in the control group to 38.4 ± 6.81 mg/g in treated insects with 25 ppm azadirachtin. This decrease was very highly significant ($P<0.001$). At 100 ppm azadirachtin, protein concentration significantly decreased ($P<0.001$) to 9.42 ± 4.62 mg/g dry weight. No significant decrease in protein concentration was obtained when azadirachtin concentration was raised to 200 ppm (Figure 1).

3- Effects of azadirachtin on total protein concentration in the haemolymph

Treatment of adult *H. littoralis* females during vitellogenesis period with 75 ppm azadirachtin has led to a highly significant decrease ($P<0.01$) of the total protein concentration in the haemolymph from 96.46 ± 20.10 mg/ml in the control to 65.59 ± 23.00 mg/ml (Figure 2). This sharp decrease continued to reach 32.41 ± 15.80 mg/ml at 100 ppm azadirachtin and was very highly significant against the control group ($P<0.001$). At higher concentrations of azadirachtin no significant changes in total protein concentration were observed.

It is well known that blood protein is produced in the cells of the fat body (Chapman, 1998). In female insects a sex specific protein (vitellogenin) appears in the haemolymph early in adult development. The concentration of vitellogenin increases rapidly as it is synthesized and released from the fat body, but subsequently it is absorbed by the developing oocytes and forms the principal yolk protein. Vitellogenin production is regulated by juvenile hormone (Buhlmann, 1976).

The importance of the hormone from the medial neurosecretory cells (NSCs) in the protein metabolism of locusts has been made very clear by the work of Hill (1962) and Highnam *et al.* (1965). Hill (1965) suggested that the neurosecretory products enhance the fat body to synthesize blood protein from the circulating amino acids. When isotopically labeled glycine was injected it appeared in the form of protein, first of all in the fat body cells, then in the haemolymph, and finally in the oocytes. It is the formed proteins of the haemolymph which are transferred by the follicular cells into the developing oocyte.

Wigglesworth (1970) suggested that the corpus allatum hormone of *Schistocerca gregaria* had a direct effect upon the growing oocytes and the follicular cells of the ovary, stimulating the synthesis and release of proteins and other nutrients into the haemolymph and for the transport of these nutrients through the

follicular epithelium to the oocytes. Moreover, the neurosecretory system acted upon the protein metabolism of the body; when the neurosecretory system released its secretion into the haemolymph, the blood protein rose, when the secretory material was accumulated in the system, the blood protein fell.

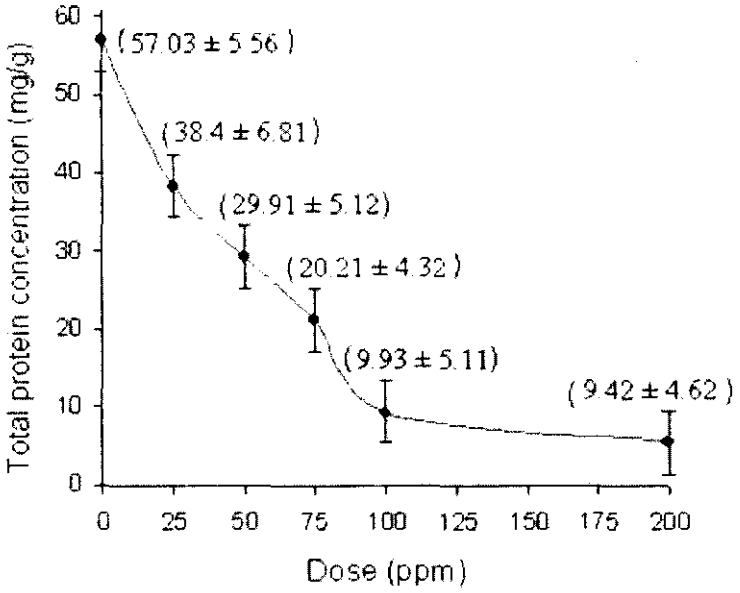

Fig. 1: Effect of different concentrations of azadirachtin on the total protein concentration (mg/g dry weight) in the fat body of the last larval instar *H. littoralis* females.

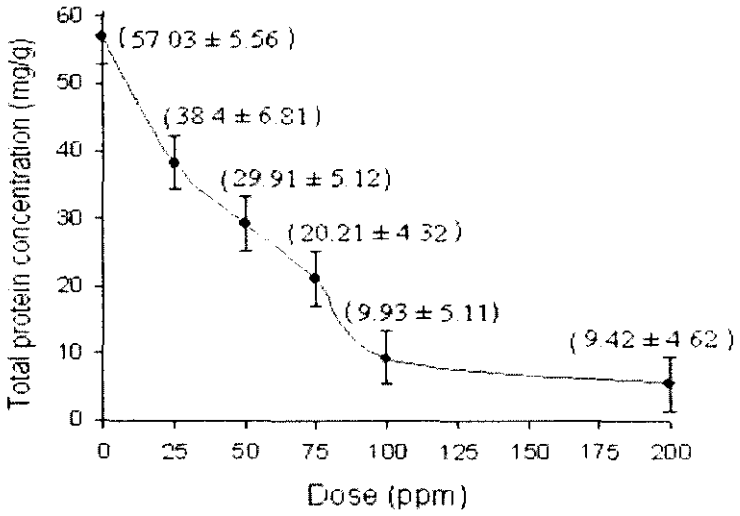

Figure (2): Effect of different concentrations of azadirachtin on the total protein concentration (mg/ml) in the haemolymph of *H. littoralis* adult females.

Rao and Subrahmanyam (1986) stated that a decrease in levels of haemolymph protein and amino acids was observed particularly in females of *S. gregaria* between fifth and eighth day after treatment with azadirachtin. Babu *et al.* (1998) showed that the azadirachtin treatment significantly decreased the levels of proteins and lipids in fat body, haemolymph and ovary of *Atractomorpha crenulata* (Orthoptera, Acrididae) during gonadotropic cycle. Schulz and Schlüter (1983) found that in neem treated individuals of *Epilachna varivestis*, not only the quantity of polypeptide bands was at first reduced but that later on qualitative differences appeared in protein composition.

The results also show great similarity to those obtained for juvenile hormone analogues. Ismail (1980) and Ismail and Fouad (1985) found that the topical application of (Isopropyl, 3,7,11-trimethyl, 2,4, Dodencadieonoate) on the pre-pupae of *Spodoptera littoralis* and *Chrysomia albiceps*, increased the total protein content all over the pupal period in the case of *S. littoralis* and decreased it in *C. albiceps* (Diptera, Calliphoridae). Khalaf (1993) showed that the treatment of the muscid fly, *Synthesiomyia nudiseta,* with dimilin, BaySir8514 and Altosid caused reduction in the protein content of pupae; this reduction was proportional to concentrations of the tested IGRs.

The present results suggest that azadirachtin may induce a depression in protein synthesis in the fat body, therefore its release in the haemolymph and consequently, its uptake by developing oocytes.

The decrease of protein concentration in the fat body and haemolymph of azadirachtin-treated insects may therefore explain some of our observations concerning the significant decrease in the number of deposited eggs.

It can be concluded that there is a further correlation associated with azadirachtin treatment between the inhibitory effects on oocytes development and the decrease of protein concentration in the haemolymph and oocytes. Differences in protein levels in fat bodies of treated and untreated insects support this contention. The lower protein levels in the fat body suggested interference of azadirachtin with fat body development and function.

Tanzubil and McCaffery (1990) showed that protein levels, as well as fat body development in females of *Spodoptera exempta* (Lepidoptera: Noctuidae) were suppressed by azadirachtin treatment due to interference of azadirachtin with vitellogenin synthesis and release by the fat body cells.

Azadirachtin causes many changes in the fat body cells resulting in interference with the sites of protein synthesis leading to disturbance of many biotic processes as shown by many authors: Schlüter (1985) showed that azadirachtin lead to molting inhibition in *Epilachna varivestis*; Schlüter and Seifert (1988) reported that azadirachtin interfered with premetamorphic events; Mitchell *et al.* (1997) and Mani and Rao (1998), showed that azadirachtin caused inhibition of the post embryonic development and reproductive cycle of fifth instar larvae of *Manduca sexta* and sixth instar of *S. littoralis* (Lepidoptera: Noctuidae), respectively. Josephrajkumar and Subrahmanyam, (2000) suggested that azadirachtin-treatment of *Heliocoverpa armigera* (Lepidoptera: Noctuidae) caused overt morphological abnormalities during the metamorphic molt.

Rembold and Sieber (1981) observed an inhibition of oogenesis in azadirachtin treated *L. migratoria* adults, so preventing any oviposition. Similar results have been documented by Dorn *et al.* (1987) who have showed that azadirachtin reduced fecundity in *Oncopeltus fasciatus* (Heteroptera: Lygaeidae). Tanzubil and McCaffery, (1990) reported that female *Spodoptera exempta* (Lepidoptera: Noctuidae), which emerged from larvae topically treated with azadirachtin, exhibited reduced fecundity but not fertility due to a failure of many oocytes to mature. Systemic application of azadirachtin was investigated by Stark *et al.* (1990) and Parkman and Pienkowski (1990) who have observed a reduction in fecundity of the fruit fly *Ceratitis capitata* (Diptera: Tephritidae) and the leaf miner *Liriomyza trifolii* (Diptera: Agromyzidae). VanRanden and Roitberg (1998) reported that azadirachtin prevented the western cherry fruit fly *Rhagoletis indifferens* (Diptera: Tephritidae) from maturing viable eggs and Di Ilio *et al.* (1999) showed that neem significantly reduced fecundity of *Ceratitis capitata* (Diptera: Tephritidae) which resulted in a complete and irreversible sterility of females when azadirachtin was added to the diet. Bruce *et al.* (2004) reported that neem oil significantly reduced oviposition in *Sesamia calamistis* (Lepidoptera: Noctuidae) and *Eldana saccharina* (Lepidoptera: Pyralidae). The fecundity of some non-target insects was not spared by azadirachtin, as Medina *et al.* (2004) observed a reduction in fecundity of *Chrysoperla carnea* (Neuroptera: Chrysopidae) treated with azadirachtin. On the other hand, Paets and Isman (1998) reported that azadirachtin did not significantly reduce the fecundity of *Delia radicum* (Diptera: Anthomyidae) when its extracts were added to the diet.

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