

Full Length Research Paper

Influence of the bioinsecticides, NeemAzal, on main body metabolites of the 3rd larval instar of the house fly *Musca domestica* (Diptera: Muscidae)

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The present study was designed and conducted to determine the effects of Neemazal on the general body metabolism of the house fly *Musca domestica*. The second instar larvae of the house fly *M. domestica* (L.) were reared on artificial diet treated with the botanical extract, NeemAzal. Five concentration levels of this compound (1000, 500, 250, 125 and 62.5 ppm) were used. The main body metabolites, carbohydrates, proteins, lipids and cholesterol were determined during the early and late third larval instar. Various degrees of almost significant ($p < 0.001$) reducing effects were recorded for proteins, lipids and carbohydrates during the early and late third larval instar. These decrements paralleled to the increments of the concentration levels. On the other hand, results indicated a slight, but non significant, reducing action of NeemAzal on cholesterol of early larval instar. This action followed by significant increase of the same metabolite at the end of this instar comparing with control congeners, except the highest concentration (1000 ppm) which exhibit a non significant ($p > 0.01$) reducing action on cholesterol. It is quite clear from these results that disturbances of nutrient contents might affect larval growth and development.

Key words: *Musca domestica*, *Azadirachta indica*, NeemAzal, neem, main metabolite.

INTRODUCTION

Phytochemicals from the neem tree, *Azadirachta indica* Juss, have been the most extensively studied in the late decades among the efficient alternatives of synthetic pesticides (Ntalli et al., 2009). Recent advances dealing with the activity of neem products were reported in the comprehensive reviews by Mordue and Blackwell (1993). Extracts from neem seeds have been reported to have wide ranging biological activities against insects (Isman et al., 1990; Schmutterer, 1990; Ghoneim et al., 2001). These include feeding deterrence, oviposition retarding, growth regulation, development impairing, against several insect species (Rice et al., 1985; Barnby and Klocke, 1990; Schmutterer, 1990; AliNiasee et al., 1997; Prakash and Roa, 1997; Ghoneim et al., 2000). The major active ingredient in the neem seeds is azadirachtin,

actually a mixture of seven tetranortriterpenoid isomers (Rembold et al., 1984). Pure azadirachtin is highly unstable, as well as the costly and tedious process of isolation, and the remote chances of synthesis of azadirachtin precludes the chance of utilization of the pure compound in insect pest management (Larson, 1987). The neem preparations are preferred over pure azadirachtin for field applications (Isman et al., 1990). NeemAzal is a neem seed preparation with the azadirachtin (the most active ingredient) content of 20%, and inexpensive production in addition to its safety toxicologically and environmentally (Kleeberg, 1992). The housefly *Musca domestica* is an endophilic eusynanthrope cosmopolitan insect, trophically and micro climatically related to man and his domestic animals. Houseflies are known as transmitters of human and animal diseases. In the present study, using the house fly *M. domestica* as an experimental insect, was undertaken to determine the effects of NeemAzal on the general

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Table 1. Effects of NeemAzal on total carbohydrate of the whole body tissue homogenate of early- and late-aged third larval instar of the house fly *M. domestica*, using feeding technique.

Concentration (ppm)	Total carbohydrate (mg/g fresh body weight)			
	Early third	Change (%)	Late third	Change (%)
1000	31.3 ± 1.2 ^{ns}	11.4	24.2 ± 2.5*	- 17.7
500	22.7 ± 1.8**	- 19.2	26.4 ± 2.6 ^{ns}	- 10.2
250	23.1 ± 1.6*	-17.8	26.1 ± 2.5 ^{ns}	-11.2
125	23.1 ± 1.2*	-17.8	25.6 ± 1.4 ^{ns}	-12.9
62.5	25.1 ± 0.5 ^{ns}	-10.7	23.7 ± 2.3*	-19.4
Control	28.1 ± 1.3	---	29.4 ± 2.3	---

ns: not significantly different (P >0.05), * significantly different (P <0.05), ** highly significantly different (P <0.01), *** very highly significantly different (P <0.001).

body metabolism to evaluate the potency of this new bioinsecticides in insect control.

MATERIALS AND METHODS

Tested insect

A culture of the house fly *M. domestica* was maintained for several generations under the conditions of 27 ± 2°C and 70 to 75% RH at entomology department, Faculty of Agriculture, Omar Al-Mukhtar University. The adult flies kept in 30 × 30 × 40 cm cages and provided with cotton pads soaked in 10% sucrose as food. Also, cotton pads were saturated with a milky solution in Petri dishes to serve as ovipositing sites. The larval diet used to rear is described in Bream et al. (1999).

NeemAzal treatments

The neem seed extract used herein was in the form of an emulsifiable concentrate containing 20% azadirachtin as the active ingredient. A concentration range of five levels was prepared using tap water as a solvent, 1000, 500, 250, 125, and 62.5 ppm. Four replicates (50 larvae or rep.) of early second instar larvae were continuously fed on an artificial diet treated with each of these levels of concentrations. The controls fed a diet free from NeemAzal.

Preparations for biochemical studies

At early and late third instar larvae age, approximately 20 individuals were pooled and weighed for estimating the total protein, lipid, carbohydrates and cholesterol content. These criteria were estimated in the whole body Homogenate. Three pools were used as replicates. The collected samples were hand-homogenized in a centrifuge tube containing 0.5 ml of saline solution. A further volume of 1.5 ml of the saline was added for washing to make a total volume of 2 ml of the saline in each concentration. After a 20 min centrifugation at 8000 rpm, (using cooling centrifuge) the clear supernatant was removed for use in the body metabolites using. A Pye Unicam SP6-450 Uv/Vis 50 spectrophotometer.

Metabolites assay

In the present study, the total protein content of the samples was assessed by the procedure of Stanbio LiquiColor kit No. 0250

(Proven Biuret methodology). The optical density was measured at 550 nm. Cholesterol content was determined at 500 nm according to the procedure recommended with Stanbio Cholesterol Kit No. 1010 (Cholesterol oxidase methodology). The total carbohydrate content was determined according to the method of Singh and Sinha (1977). Lipids were extracted and estimated according to the methods of Knight et al. (1972). The results were expressed in mg per g fresh body weight.

Statistical analysis

Data obtained were analyzed by the Student's t-distribution and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

RESULTS

Results of Table 1 showed that the total body carbohydrate content was increased with the age of control larvae. A similar result was obtained for larvae treated with NeemAzal, except those treated with low and high concentration levels. The treatment with NeemAzal resulted in significant reduction in the carbohydrate contents.

As indicated in Table 2, the total body protein content was increased with the age of control and treated larvae. It appears from Table 2 that treated early instar larvae showed decrements in protein content paralleled to the increments of the concentration level. The change percentage reached -49.8 at the highest concentration level. A similar trend was shown during the end of this instar. Lipid content of control larvae increased during the instar examined (Table 3). The same tendency was detected in treated larvae, except in the case of larva treated with the highest concentration level (1000 ppm). Generally, various degrees of reduction were recorded after treatment of second instar larvae with NeemAzal. The same tendency was detected in the larvae after treatment with NeemAzal. It is quiet clear from the results of Table 4 that NeemAzal had an inhibitory effect on cholesterol level in treated larvae, in early larval instar tested and vice versa for late-aged larvae examined.

Table 2. Effects of NeemAzal on total protein of the whole body tissue homogenate of early- and late-aged third larval instar of the house fly *M. domestica*, using feeding technique.

Concentration (ppm)	Total protein (mg/g fresh body weight)			
	Early third	Change (%)	Late third	Change (%)
1000	13.3 ± 2.7***	- 49.8	15.0 ± 1.4***	- 45.5
500	13.5 ± 3.1***	-49.1	17.1 ± 1.7***	-37.8
250	16.5 ± 3.3**	-37.7	19.5 ± 1.6**	-29.1
125	18.4 ± 2.2**	-30.6	21.0 ± 1.1*	-23.6
62.5	20.0 ± 2.1*	-24.5	22.3 ± 1.3*	-18.9
Control	26.5 ± 1.4	---	27.5 ± 1.9	---

ns: not significantly different (P >0.05), * significantly different (P <0.05), ** highly significantly different (P <0.01), *** very highly significantly different (P <0.001).

Table 3. Effects of NeemAzal on total lipids of the whole body tissue homogenate of early- and late-aged third larval instar of the house fly *M. domestica*, using feeding technique.

Concentration (ppm)	Total lipid (mg/g fresh body weight)			
	Early third	Change (%)	Late third	Change (%)
1000	23.3 ± 2.4***	- 47.0	23.2 ± 2.4***	- 58.6
500	25.9 ± 2.0***	- 41.1	20.4 ± 3.6***	-63.6
250	28.6 ± 2.4***	- 35.0	27.7 ± 1.2***	-50.5
125	28.6 ± 2.1***	- 35.0	36.9 ± 2.5***	-34.1
62.5	32.4 ± 3.3***	- 26.4	42.0 ± 2.6***	-25.0
Control	44.0 ± 1.2	---	56.0 ± 5.2	---

ns: not significantly different (P >0.05), * significantly different (P <0.05), ** highly significantly different (P <0.01), *** very highly significantly different (P <0.001).

Table 4. Effects of NeemAzal on total cholesterol of the whole body tissue homogenate of early- and late-aged third larval instar of the house fly *M. domestica*, using feeding technique.

Concentration (ppm)	Cholesterol (mg/g fresh body weight)			
	Early third	Change (%)	Late third	Change (%)
1000	0.82 ± 0.09 ^{ns}	-50.0	1.9 ± 0.23 ^{ns}	-9.5
500	1.05 ± 0.03 ^{ns}	-35.9	4.2 ± 0.29**	100
250	0.94 ± 0.03 ^{ns}	-42.7	4.9 ± 0.31***	133
125	0.94 ± 0.03 ^{ns}	-42.7	4.7 ± 0.42**	123.8
62.5	0.98 ± 0.05 ^{ns}	-60.9	3.8 ± 0.35*	80.9
Control	1.64 ± 0.15	---	2.1 ± 0.27	---

ns: not significantly different (P >0.05), * significantly different (P <0.05), ** highly significantly different (P <0.01), *** very highly significantly different (P <0.001).

DISCUSSION

Effect on the total body carbohydrate content of third larval instar

Results of Table 1 showed that the total body carbohydrate content was increased with the age of control larvae. A similar result was obtained for larvae treated with neemazal, except those treated with low and high concentration levels. The treatment with NeemAzal

resulted in significant reduction in the carbohydrate contents. At the early age, this reduction was significant except at higher and lower concentration levels. This reducing action of NeemAzal was extended to the late-age of these larvae. These results agree with that of Baker et al., (2002) after treatment of *Spodoptera littoralis* with different plant extracts; Abou El-Ela et al. (1993) who determined great reduction in carbohydrates during the pupal stage of the fly *Synthesomyia nudiseta* after larval treatment with some IGRs. Depending on the results of

Abo El-Ghar et al. (1995) on *Agrotis ipsilon* feeding of larvae on the petroleum ether extracts from *Ammi majus* and *Apium graveolens* and acetone or ethanol extracts from *Melia azedarach* and *Vinca rosea* caused a considerable reduction in the total carbohydrates of larval haemolymph. In addition, Khalaf (1998) estimated a significant reduction in the carbohydrate content in the whole pupal period of *Muscina stabulans* after larval treatments with volatile oils of *Cymbopogon citratus* and *Rosmarinus officinalis*. On the other hand, Abou El-Ela et al. (1990) found that treatment of *M. domestica* larvae with Altosid (ZR-515) resulted in decreased carbohydrates in 1 day old pupae and some increments in 3 day and 5 day old pupae. On contrast significant increases of carbohydrate content were observed in larvae of *S. littoralis* by the JHA (Isopropyl 3, 7, 11-triethyl-2, 4-dodacadiote) (Ismail, 1980).

Effect on the total body protein content of third larval instar

Proteins are very complex and at the same time, they comprise most of the characteristics of living matter. They are present in all viable cells, in that they are the compounds which, as nucleoproteins, are essential to the process of cell division and, as enzymes and hormones, control many chemical reactions in the metabolism of cells. Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors have been implicated in the control of protein synthesis (Carlisle et al., 1987).

It appears from Table 2 that treated early instar larvae showed decrements in protein content paralleled to the increments of the concentration level. The change percentage reached -49.8 at the highest concentration level. A similar trend was shown during the end of this instar. These results agree with that of Bakr et al. (2002) using different plant extracts against *S. littoralis*. On the contrast, Amer (1990) determined increasing protein content in pupae of *S. littoralis* after larval treatment with mevalonic acid. Also, Basiouny (2000) estimated considerable increments of proteins throughout different developmental stages of *M. stabulans* by the chitin inhibitors IKI-7899 and XRD-473.

Effect on the total body lipid content of third larval instar

Lipids are important source of energy for insects. These are obtained from the diet or are synthesized by the insect itself (Gilby and Gilly, 1965). Lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Downer, 1985). Lipids are essential structural components of the cell membrane and cuticle. They facilitate water conservation both by the formation of an impermeable cuticular barrier and by yielding

metabolic water upon oxidation, and they include important hormones and pheromones (Gilbert, 1967).

Lipid content of control larvae increased during the instar examined (44.0 ± 1.2 and 56.0 ± 5.2 mg/g lipids of early- and late-aged third larval instar) (Table 3). The same tendency was detected in treated larvae, except in the case of larvae treated with the highest concentration level (1000 ppm). Generally, various degrees of reduction were recorded after treatment of second instar larvae with neemazal. This reduction was detected on early and late- aged larvae and it is found to be statistically significant with various degrees of change percentage. These results agreed with those of Baker et al. (2002) after treatment of *S. littoralis* larvae with different plant extracts and that of Bream (2002) after treatment of *Rhynchophorus ferrugineus* prepupae with Azadirachtin and Jojoba extracts. Synthesis of lipids by the fat body of *Choristoneura fumiferana* was diminished at all times examined during the 6 day experimental period after treatment with fenoxycarb (Mulye and Gordon, 1993). On the other hand, Ghoneim (1994) determined significant increments of lipid content throughout the pupal stage of *S. littoralis* by larval treatments with mevalonic acid and IKI-7899.

Effect on the total body cholesterol content of third larval instar

The synthesis of sterol from acetate has not yet been demonstrated in arthropods and, indeed, arthropods require a dietary sterol for several physiological functions and in some cases for life itself (Gilbert, 1967). The major functional sterol in arthropods is cholesterol. The roles of sterols in arthropods are likely manifold and include being precursors of the arthropods molting hormone (s) and components of subcellular membrane structures (Gilbert, 1967).

Cholesterol content of control larvae increased by the age (1.64 ± 0.15 and 2.1 ± 0.27 mg/g lipids of early- and late-aged larvae examined). The same tendency was detected in the larvae after treatment with NeemAzal. It is quite clear from the results of Table 4 that NeemAzal had an inhibitory effect on cholesterol level in treated larvae, in early larval instar tested and vice versa for late-aged larvae examined. This reducing effect was found to be not statistically significant. Lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Downer, 1985). This may explain the decrements in cholesterol, which is one of the precursors of molting hormone and other hormones, required at that instar to form the complete pupal stage.

Conclusively, various degrees of reducing actions were recorded for proteins, lipids and carbohydrates during the early and late third larval instar. Also, this reducing action of NeemAzal was detected on cholesterol during the early instar larvae but followed by significant increase at the end of the same instar.

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