------J. Raf. Sci., Vol. 20, No.2, pp 8- 18, 2009------

Effect of *Melia azedarach* L. and *Ailanthus altissima* Swingle Extracts on the Larva Alimentary Tract and Growth of Black Cutworm, *Agrotis ipsilon* Hufn. (Lepidoptera: Noctuidae)

Atallah F. Mekhlif
Department of Biology
College of Education
Mosul University
dr_atallah1957@yahoo.com

(Received 15/10/2008 ; Accepted 26/1/2009)

ABSTRACT

The enriched methanolic extracts of seeds of *Melia azedarach* and leaves of *Ailanthus altissima* have had the same effect on the valves of the cardiac sphinector of *Agrotis ipsilon* larvae. At the low concentrations, the sphinector was expanded, and the valves degenerated at the high concentrations. *M. azedarach* extract was separated the peritrophic membrane and muscles of the midgut, while the extract of *A. altissima* was thicked the peritrophic membrane only. The application of extracts were effected on developed pupae and adults of treated larvae, this effect was represented by failure of adults emergence and degrees of wings malformations. Also, the extracts were significantly reduced the weight of *A. ipsilon* larvae fed on treated diets. For treated larvae, Lc_{50} of black cutworm larvae were 2.5 and 3.5 ppm for *M. azedarach* and *A. altissima* respectively.

Keywords: *Agrotis ipsilon*, *Melia azedarach*, *Alilanthus altissima*, malformation, mortality.

تأثير مستخلصا السبحبح وشجرة الجنة في القناة الهضمية ليرقة الدودة القارضة السوداء ونموها(Lepidoptera: Noctuidae) (*Agrotis ipsilon* Hufn.)

الملخص

يؤثر المستخلص الميثانولي المركز لبـذور الـسبحبح *Melia azedarch* وأوراق شـجرة الجنـة *Ailanthus altissima* تأثيراً متشابهاً في الصمام القلبي ليرقات الدودة القارضة السوداء، اذ توسع الصمام في المعاملات واطئة التراكيز وبدأ بالضمور والتلاشي في المعاملات عالية التراكيز. سبب مستخلص ثمـار السبحبح انفصال الغشاء حول الغذاء والعضلات للمعي الأوسط بينما مستخلص شجرة الجنة زاد مـن سـمك الغشاء حول الغذاء فقط. أثرت المعاملة بالمستخلصات في تطور العذارى والبالغات المعامل غذاء اليرقـات المتطورة عنها بالمستخلصات. وقد تمثل هذا التأثير بفشل بزوغ البالغات ودرجات من تشوه تلك البالغـات،

كما نقص وزن اليرقات المعاملة معنوياً، وقد بلغت قيمة Lc50 ليرقات الدودة القارضة السوداء 2.5 و 3.5 جزء في المليون لبذور السبحبح وأوراق شجرة الجنة على التوالي.

INTRODUCTION

Plants produce a variety of secondary metabolites, some of them in a significant amounts which are interfere with growth and development of phytophagous insects, therefore, application of the insect's diet with sublethal doses of these bioactive ingredients will cause mortality and abnormal progenies.

Chinaberry tree *Melia azedarach* L., is one of the important known species in the family meliaceae because it is rich in triterpenoids and limonoids (Srivastava and Gupta, 1985; Ascher *et al*, 1995; Huang *et al*., 1995; Nakatani *et al*., 1998). The extract of the different parts of *M. azedarch* have various effects on the developed insects of different orders, especially lepidoptera, when the larval diets treated with low concentrations of the plant extract, e.g. prolongation of egg, nymph and larval periods (Cespedes *et al*., 2000; De'Souze and vendramm., 2000; Mekhlif, 2004), antifeedant and failure of adult emergence (Carpinella *et al*., 2003), endocrine effects (Schmidt *et al*., 1998), and malformation of immature stages and adults (Schmidt *et al*., 1997 ; Al-Hamadani, 2002) and antimolting of immature stages (Cabral *et al*., 1996).

Tree of heaven, *Ailanthus altissima* (Mill.) Swingle (*glandulosa Desf*.) belongs to Quassia family (Simaroubaceae) (Sandor and Vera, 1979) was introduced to Mosul city in Iraq since about one decade as ornamental tropical tree, and cultivated in Mosul forest and other city areas. Extracts of *A. altissima* includes quassinoid triterpenoids, ailonthone, amarolide, acetyl amarolide and 2-dihydroxy ailanthone (Casinovi *et al*., 1983; Aono *et al*., 1999). The aqueous extract of *A. altissima* leaves have insecticidal and herbicidal activities (Heisey, 1996; Tsao *et al*., 2002) and have been used to control insects such as *Pieris rape* and *Platyedra gussyaella* (Yang and Tang, 1988).

Black cuturom, *Agrotis ipsilon* Hufn. is one of notorious migrant pests in Iraq, it infest many plants in Iraq such as, cabbage, beet, lettuce, etc. It is multivoltine (Wiltshire, 1957). Application of enriched methanolic extract of *M. azedarach* with concentrations ranged between 10 and 100 ppm had been effected on larval growth parameters of *A. ipsilon* (Schmidt *et al*., 1997; Schmidt *et al*., 1998).

The purpose of this work was to evaluate the effect of enriched extracts of *M. azedarach* and *A. altissima* on some biological aspects and to investigate influence of the extract active compounds on functional tissues of treated larvae and other developed stages.

MATERIALS AND METHODS

Insects

The flying fertalized females of *Agrotis ipsilon* were captured by handle net around fluorescence light in the garden houses during early June, the time of passing migratory months in Mosul city in Iraq. The captured females fed on 15% honey solution in the suitable insetory. Filter paper strips were put for egg laying.

In order establishment the colony, the patches of the eggs were removed and incubated for two days, at 27±1 ºC, 60% relation humidity and 16:8 day time conditions the hatching neonate larvae fed on artificial diet (Al-Hamadani ,2002 ; Mekhlif ,2004).

After pupation, the pupae were sexually isolated for adults emergence.

Preparation of the extract

The unripe fruits of chinaberry were collected at the late October, washed and frozen. Leaves of heaven of china tree were washed and dried in the shade through June and ground with a coffee mill to a dry powder. The chinaberry unripe fruits were grounded by mortar.

To prepare the enriched extract, 50 gm of each *A. altissima* leaves powder and unripe fruits ground of *M. azedarach* were separately macerated with 150 ml 80% methanol for 24 hours. These extracts were transferred into a glass beaker, then, each extract was stirred for 6 hours and filtered under low pressure. The dry extract was redissolved in 80% methanol in separating funnel and washed with the same volume of petroleum ether (B.p. 30-35 °C) by hand shaking for half an hour. The extract was separated into two layers; the methanol extract was dried as formerly described, and washed with equal volumes of distilled water and ethyl acetate in a separating funnel. The ethyl acetate extract was released and dried. Then, the dry extracts were weighted and redissolved in 80% methanol to prepare a stock solution.

Preparation of the larvae for treatments

All the experimental applications were conducted with third instar larvae. The larvae were fasted for six hours before they fed on treated diets, by placing them in large container to avoid cannibalism behavior.

The third in star larvae were kept singly in a plastic container (d = 10 cm, h = 30 cm) with circular piece of about 20 gm of the treated diet, the diet piece was refreshed every two days.

Extract effect estimation

The effect of the extracts on treated larvae were estimated through adults malformations by sublethal doses less than 25 ppm and larval mortality thought 50 larvae for each treatment. Also, the effect of the extracts were investigated at the level of tissues deterioration of the treated larva digestive system. Tissues preparations and staining protocol were conducted after, Ewen (1962) and Mekhlif (2004).

RESULTS AND DISCUSSION

Extract effect on mortality and weight

Fig. (1) was showed the mortality of *Agrotis ipsilon* larvae which their diet treated with different concentrations of enriched *Melia azedarach* and *Ailanthus altissima* extracts. The effective concentration Ec_{50} (the concentration of the extract which causes mortality of 50% of treated larvae) of *M. azedarach* and *A. altissima* were 2.7 and 3.5 ppm respectively. The applied concentrations: 1, 5, 10 and 15 ppm were approximately caused similar black cutworm mortality either for unripe *M. azedarach* nor leaves *A. altissima*. But the later two concentrations: 25 and 50 ppm of *A. altissima* were more effective in relation to the same concentrations of *M. azedarach* extract.

The mechanism of larval mortality when the diets treated with chinaberry and tree of heaven extracts at the concentrations 1, 5, 10 and 15 ppm was attributed to the similarity of metabolic pathways of limonoid and quassinoid triterpenoids which extracted from chinaberry and tree of heaven respectively (Casinovi *et al*., 1983; Srivastava and Gupta, 1985; Ascher *et al*, 1995; Huang *et al*., 1995; Nakatani *et al*., 1998; Aono *et al*., 1999). It

was concluded from Fig. (1) the additional concentrations of quassinoids and other specific ingredients of tree extract of heaven would proportionally increased the mortality of treated larvae up to 98% at the concentration 50 ppm. In comparison between the larval mortality for the last two concentrations 25 and 50 ppm, the mortality was increased from 82 to 86% for *M. azedarach*, while that of *A. altissima* increased from 72 to 98%, so that, the mortality was multiplied more than six folds between the applied extracts at the concentrations 25 and 50 ppm (Fig 1).

The results in the table 1 shows that all the applied concentrations; 1, 10, 25 and 50 ppm were significantly reduced the weight of treated larvae of *A. ipsilon*. Also, the treated larvae were more affected by *M. azedarch* extract than *A. altissima* extract.

Malformation effect of the extracts

Generally, the normal newly emerged insect was characterized by curled wings, but with advanced time, the wings were expanded till they took usual appearance However, in the present study, after pupation, there was percent of emerged adults their wings varied between severely curled and folded with different degrees (Plate 1).

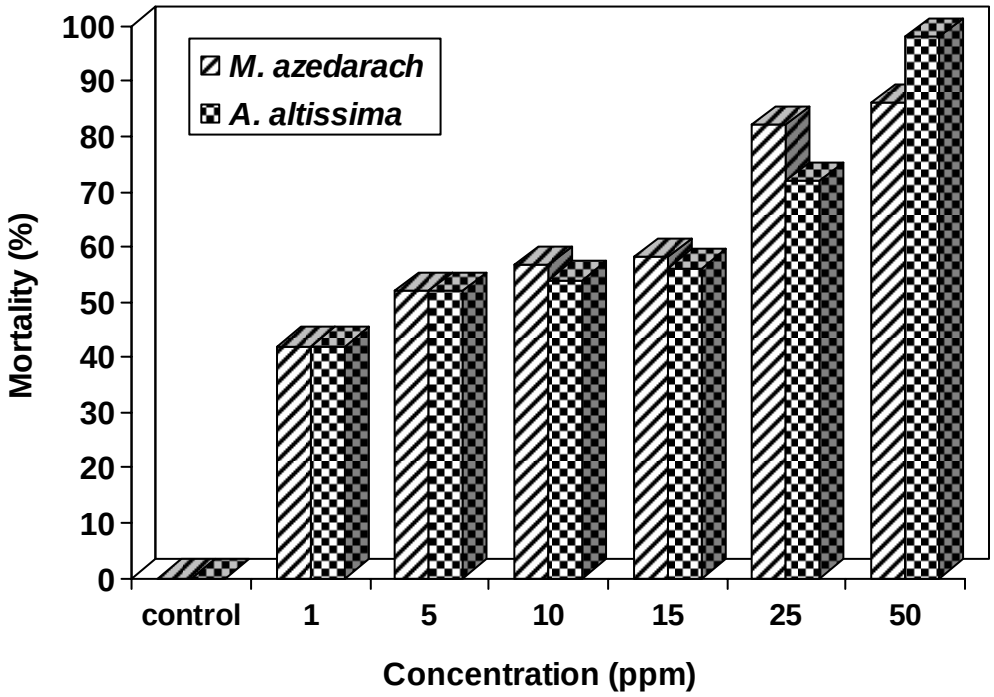
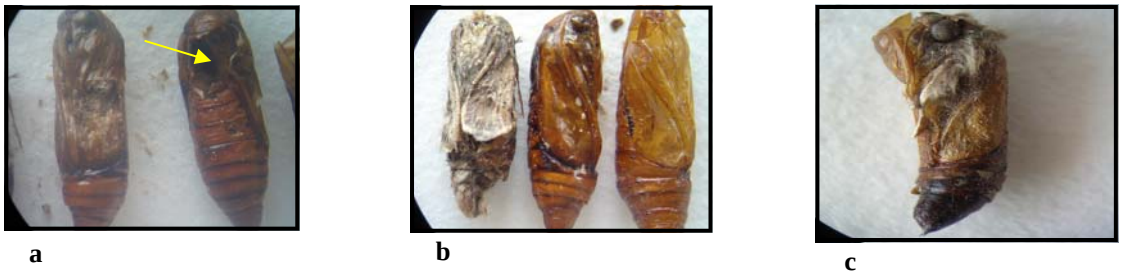


Fig. 1: Mortality of *Agrotis ipsilon* larvae, diet was treated with the extracts *M. azedarach* unripe fruits and leaves *A. altissima*

Table 1: Effect of *Melia azedarech* and *Ailanthus altissima* extracts on weight of *Agrotis ipsilon* treated larvae (mg)

Plant extract	Concentration (ppm)				
	0	1	10	25	50
M. azedarach	412 a	338 c	306 d	274 e	219 f
A. altissima	412 a	362 b	340 c	302 d	289 de

Note: Means followed by the different letters are significantly different according to Duncan's multiple range test ($P < 0.01$)



a **b** **c**

d **e** **f**

g **h** **i**

j **k** **l**

Plate 1: Pupae and adults of *Agrotis ipsilon* which had been treated at larvae stage with different concentrations of *Melia azedarach* and *Ailanthus altissima* extracts

a,b, and c- are pupae stage with different emergence failure.
d,e, and f- represent puparium adhesion.
g- Control.
h and i- are adults with curly wings.
j- conversion of wings.
k- hind wings vestigial.
l- malformed right wings.

The malformed adult wings when larval stage feeding on diet treated with *M. azedarach* and *A. altissima* extracts is apparently belong to unsufficient hemolymph hydrolyic pressure to ensure complete wings expandence.

Also, Plate 1 illustrates that complete or part of puparium was adhesived to adult abdomen or other its appendages. Eclusion is depend on the rate of polypeptide and ecdyson hormones (Begley *et al*., 2005), in this manner, the effective ingredients of the extracts were eliminate the role of eclusion hormones through denaturation of eclusion hormone or reducing the hormones titer in the hemolymph, other studies were found that triterpenoid azedarchtin group effected hormones titer in the hemolymph (Barynby and Klocke, 1990).

On the other hand, completed failure of emergence was found in spite of full grown pupae, the causative of this failure as well as previous resions, was the triterpenoids which multiplied the thickness of the integument (Mekhlif, 2004), so that, preventing the formation of ecdysial lines through pupation.

The dried liquid contents of the pupa means the applied extracts of chinaberry and tree of heaven prevents the pathways of histolysis and histogensis for the developed treated pupae (Plate. 1a).

For each treatment at the concentrations 10, 15 and 25 ppm of *A. altissima* extract, the percent of the eclusion failure parameter was more than 40% while that of undeveloped pupa was approximately one third of examined pupae. The curled and adhesived puparium parameters were only recorded for 1 ppm treatment. Also, it was not found significant differences between the effect of chinaberry and tree of heaven extracts on the studied parameters except for undeveloped pupae, which was less recording at the concentrations 10, 15 and 25 ppm for chinaberry extract (Fig. 2,3).

Effect on cardiac spninictor

Plate 2a shows the longitudinal section of control larva at the junction between crop and midgut. The valves between crop and midgut is control food passing between these two parts of digestive tract of Lepidoptera through interval opening of this valve (Richards and Davies, 1978). Treatment of the feeding diets with low concentrations (1, 5, 10 and 15 ppm) of *M. azedarach* was disrupted the valves function, and they were continuously still opened (Plate 2b). But the sections of the larvae which their diets treated with 25 and 50 ppm of *M. azedarach* were appeared significant reduction of the valves size and lost its ability to control food passing between crop and midgut (Plate 2c).

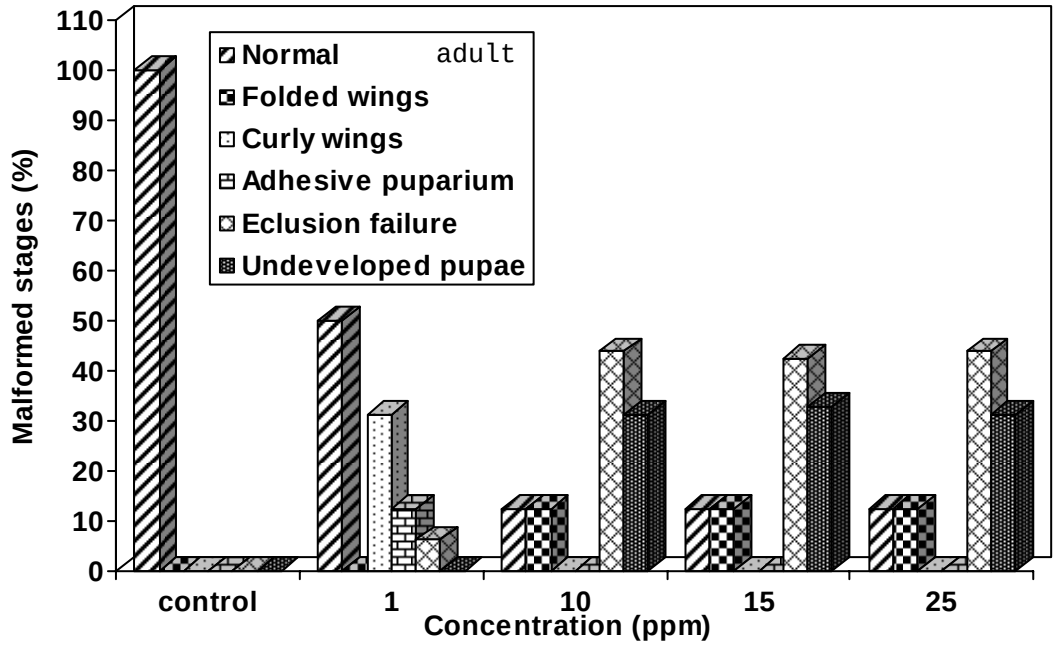


Fig. 2: Effect of the *M. azedarach* unripe fruits extract on emergence of *A. ipsilon* (%)

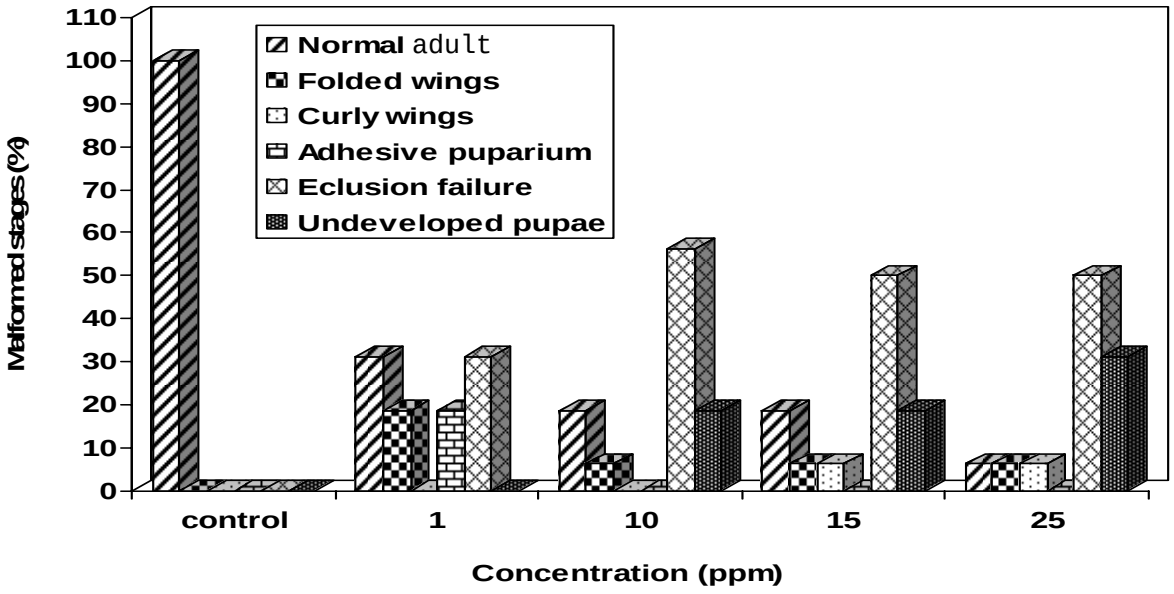
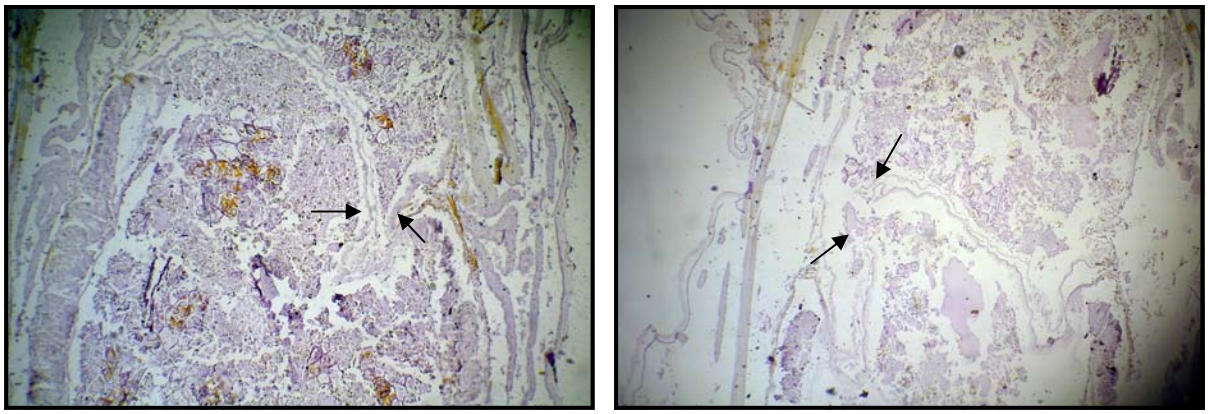
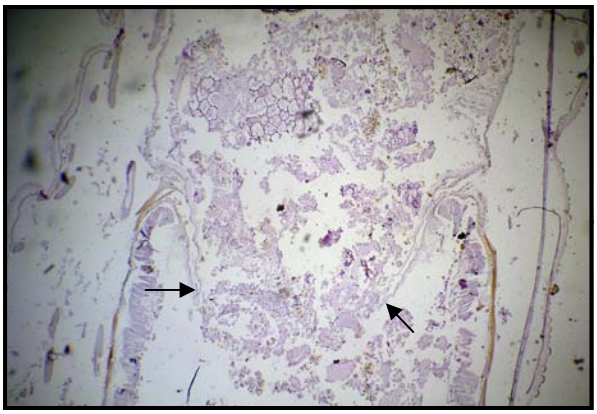


Fig. 3: Effect of the *A. altissima* leaves extract on emergence of *A. ipsilon* (%)



a b



c

Plate 2: Longitudinal section through cardiac sphinictor region of last instar larvae, *Agrotis ipsilion*, diet of the larvae had been treated with *Melia azedarach* extract

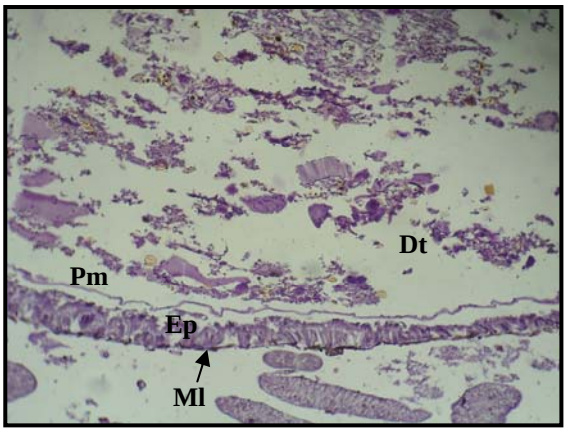
a- control.
b- treated with 1.0 ppm
c- treated with 50 ppm
arrows indicate to the spinictor valvae

Longitudinal sections of treated larvae with *A. altissima* extract were showed the same effect of *M. azedarach*.

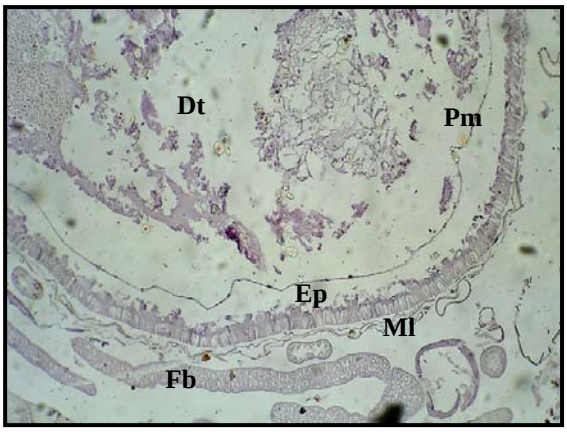
Effect on larvae midgut

In the application with *M. azedarach* and *A. altissima* extracts, the chinaberry extract was found more effective than the extract of tree of heaven. Sections of the midgut of black cutworm treated larvae explains the effect of *M. azedarach* extract which was took place by two ways: firstly, the extract was separated the peritrophic membrane and dissociated circulure and longitudinal muscles, therefore, *M. azedarach* was caused disruption of peristaltic and diastolic movement of the digative tract and the epithelia of the midgut was physically separated away than food materials (Plate 3). Secondly, *M. azedarach* was affected as antifeedant, especially at high the concentrations were used (Plate 3E). Schmidt (1997) and Al-Hamadani (2002) were studied the effect of *M. azedarach* on *Spodoptera exigua*, *S. littoralis* and *A. ipsilon*.

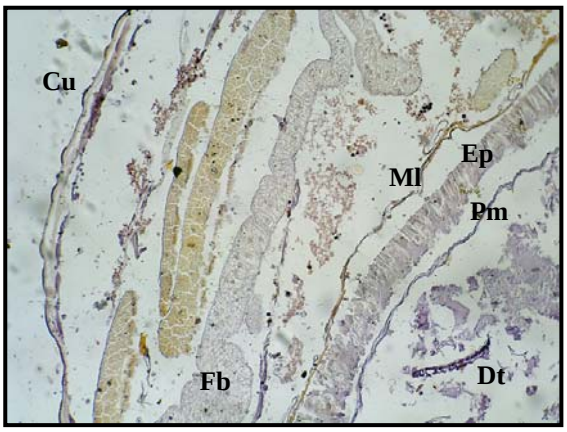
The peritrophic membrane of treated larvae with *A. altissima* was densely stained with AF stain (Plate 3D) in relation to peritrophic membrane of normal larva (plate 3A), therefore, the permeability and food absorption were significantly reduced.



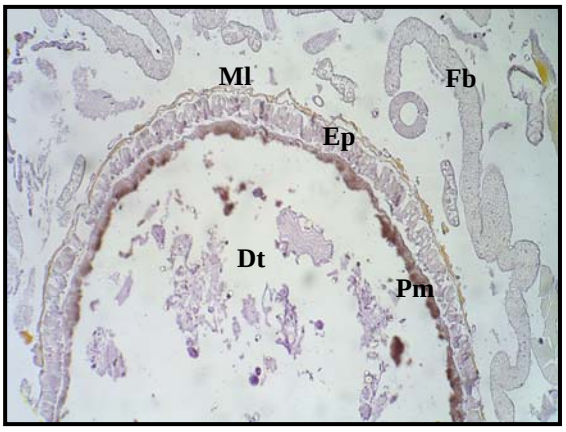
A



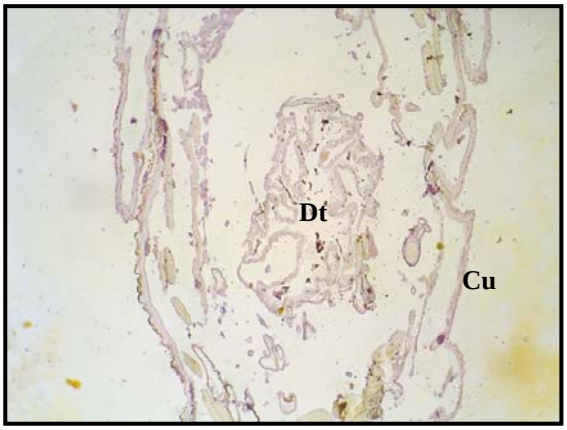
B



C



D



E

Plate 3: Effect of *Melia azedarach* and *Ailanthus altissima* extracts on midgut of *Agrotis ipsilon* larvae
A- Control, B- Diet treated with 5 ppm,
C- Diet treated with 10 ppm,
D and E- Diet treated with 50 ppm,
Note: B,C and E- The extract of *M. azedarach*. Extract for D was *A. altissima*.
Dt = digestive tract, Cu = cuticle, Ep = epithelia, Fb = fat bodies,
Ml = muscular layer and Pm = peritrophic membrane.

REFERENCES

Alche, L.E., Ferek, G.A.; Meo, M., Coto, C.E. and Maier, M.S., 2003. An Antiviral Meliacarpin from Leaves of Melia azedarach L. Z. Naturforsch, 58c: pp.215-219.

Al-Hamadani, M.A., 2002. Effect of some Neem Products, *Azadirachta indica* A. Juss and Fruit Extract of the Chinaberry Tree, *Melia azedarach* L. on some Physiological Aspects of the Beet Armyworm. *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae). Ph.D. Thesis, Mosul University, Mosul-Iraq, 134p.

Aono, H., Kanecko, J. and Ohmots, T., 1999. Alkaloids and Quassinoids from *Ailanthus malabariea*. Phytochem., 37: pp.579-584.

Ascher, K.R.S., Schmutterer, H.; Zebits, C.P.W. and Naqvi, S.N.H., 1995. The Persian Llilac or Chinaberry Tree: *Melia azedarach* L., pp.605-642. In: H. Schmutterer (ed.), The Neem Tree, *Azadirachta indica* A. Juss and other Meliaceous Plants. Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes. VCH, Weinhiem, Germany, 696 p.

Barnby, M.A. and Klocke, J.A., 1996. Effects of Azadirachtin on Levels of Ecdysteroids and Prothoracicotropic Hormone-Like Activity in *Heliothis virescens* (Fabr.) Llarvae. J. Insect. Physiol., 36(2): pp.122-131.

Begleg, T. P.; Means, A. R.; O'Malley, B.W.; Riddiford, L. and Tashjion, A. H., 2005. Vitamins and Hormones, Vol. 73, Elsevier Isc. 288p.

Cabral, M.M.; Garcia, E.S.; Rembald, H.; De Simone and Kelecom, A., 1996. Antimoulting Activity in Brazilian *Melia azedarach*. Mem Inst. Oswaldo Crug, Rio de Janeiro, 91(1): pp.117-118.

Carpinella, M.; Defago, M.; Valladares, G. and Palacios, S.M., 2003. Antifeedant and Insecticide Properties of a Limonoid from *Melia azedarach* (Meliaceae) with Potential use for pest Management. J. Agric. Food chem., 51(2): pp.369-374.

Casinovi, C.G.; Ceccherelli, P.; Fardella, G. and Grandolini, G., 1983. Isolation and Structure of a Quassinoid from *Ailanthus glandulosa*. Phytochem., 22: pp. 2871-2873.

Cespedes, C.L.; Calderon, J.S.; Lina, L. and Arada, F., 2000. Growth Inhibitory Effects on Fall Armyworm, *Spodoptera frugiperda* of some Limonoids Isolated from *cedrel* spp. Meliaceae, J.A. Food Chem., 48(5): pp.1903-1908.

De'Souza, A.P. and Vendramim, J.D., 2000. Effect of Aqueous Exctracts of Melaceous Plants on *Bemisia tabaci* B Biotype on Tomato Plants. Bragantia, Campinas, 59(2): pp.173-179.

Ewen, A.B., 1962. An Improved Aldehyde Fuchsin Staining Technique for Neurosecretory Products in Insects. Trans. Amer. Micros. Soc., 81: pp.94-96.

Heisey, R.M. 1996. Identification of an Allelopathic Compounds from *Ailanthus altissima* and Characterization of its Herbicidal Activity. Amer. J. Botany, 83: pp.192-200.

Huang, R.G.; Zhou, J.; Suenage, H.; Takezaki, K.; Tadera, K. and Nakatani, M., 1995. Insect Antifeeding Property of Limonoids from Okinawa and Chinese *Melia azedarach* L., and from *Melia toosendan* (Meliaceae). Biosci. Biotech. Biochem., 59(9): pp.1955-1757.

Mekhlif, A.F., 2004. Effect of Chinaberry Tree, *Melia azedarach* L. Extract on the Reproduction and Development of the Beet Armyworm *Spodoptera exigua* (Hub.) Lepidoptera Noctuidae. Ph.D. Thesis, Mosul University, Mosul-Iraq, 93p.

Nakatani, M.; Huang, R.C.; Okamura, H.; Iwagwa, T. and Tadera, K., 1998. Degraded Limonoids from *Melia azedarach*. Phytochem., 29: pp.425-427.

Richards, O.W. and Davies, R.G., 1978. Imms' Outlines of Entomology. Chapman and Hall, London and New York, 254 p.

Sandor, J. and Vera, C., 1979. Icongaphia Florae Parties Austro-orientalis Europae Centralis. Academia Kiado. Budapest, 704 p.

Schmidt, G.H., Ahmed, A.A. and Breuer, M., 1997. Effect of *Melia azedarach* on Larval Development and Reproduction Parameters of *Spodoptera littoralis* (Boisd) and *Agrotis ipsilon* (Hufn.) (Lep. Noctuidae). Anz. Schadlingskde ipfanzenschutz. Umweltschutz, 70: pp. 4-12.

Schmidt, G.H., Rembold, H., Ahmed, A.A. and Breuer, M., 1998. Effect of *Melia azedarach* Fruit Extract on Juvenile Hormone Titer and Protein Concentration in the Hemolymph of Two Species of Noctuid Lepidoptera larvae (Insecta: Lepidoptera: Noctuidae). Phytoparasitica, 26(4): pp.283-291.

Srivastava, S.K. and Gupta, H.O., 1985. New Limnoids from the Roots of *Melia azedarach* L. Indian J. Chem., 24B, pp.166-170.

Tsao, R., Romanchuk, F.E., Peterson, C.J. and Coats, J.R., 2002. Plant Growth Regulatory and Insecticidal Activity of Extracts of the Tree of Heaven (*Ailanthus altissima*). BMC Ecol., 2: 1p.

Wiltshire, E.P., 1957. The Lepidoptera of Iraq. Adlard and Son, Bartholomew Press, Dorking, 162 p.

Yang, R.Z. and Tang C.S., 1988. Plants used for Pest Control in Chinai, Literature Review. Econ. Bat., 42: pp.379-406.