

# Histological Studies on the Effects of Plant Extracts on the Greater Wax Moth, *Galleria Mellonella* L

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**Abstract:** Treatment of 7<sup>th</sup> larval instars of *Galleria mellonella* L. with LD<sub>50</sub> of ethanolic neem seed (*Azadirachta indica*) extracts, cause completely destructions in fat tissue, body wall, colon, Malpighian tubules and muscles just before death. These damages decreased in cases of treatment larvae after 12 and 24 hrs. There are simple damages in muscles when treated larvae with lethal dose of leave extracts of *C. antiquorum* and *V. rosea*. Lethal dose of leave extracts of *A. sativa* cause ruin in muscles, gonads and hindgut. Whereas the lethal dose of leave extracts of *M. azedarach* causes cracks in gonads. These results confirmed that the ability of using these plant extracts for control of this pest in a safety way for environment without pollution.

**Keywords:** - Histological, plant, extracts, wax, moth

## I. INTRODUCTION

The larvae of this insect cause great damages to honey bees comb by feeding on bee wax, pollen, nectar and immature stages of bees, (Sehna, 1966). This study to elucidate new methods for control of this pest by using plant extracts in safety way for bees, human and their environments. Tested plants are neem seeds of *Azadirachta indica* & leaves of *Melia azedarach* (Meliaceae), *Venca rosea* (Apocynaceae), *Allium sativa* (Liliaceae) and *Calcasia antiquorum* (Araceae).

## II. MATERIAL AND METHODS

This pest was reared on artificial diet, (corn & wheat flours, milk powder, bran, honey & bee wax and glycerin), under laboratory condition according to Sehna (1966) and Maraston & Cambell (1973). Ethanolic plant extracts were injected in the 2<sup>nd</sup> abdominal segment of the 7<sup>th</sup> larval instar of this moth. The tested doses were 0.5, 1, 1.5 and 2 μL/larva according to McMillian & Starks (1966) and Atwal & Pajni (1964) Some works on plant extracts were carried out e.g. Eid (1944), Abdel-Fattah & Mohammed, (2002), Hussein & Hamed (2004), Abdel-Fattah, (2005), Mohammed et al (2003), and Abdel-Fattah & Guneidy (2007), Tissue prepared for histological studies according to Hung et al (2000), Watson et al (2002) and Eid (1994).

## III. RESULTS AND DISCUSSIONS

Mortalities which caused by tested plant extracts were represented in table (1).

The histological changes were recorded after 12, 24 hrs and just before death after treatment with lethal dose of ethanolic extracts of seeds of neem and leave extracts of other tested plants as follows:

1- After 12 hrs:

- Hindgut & gonads: There is thickness in the hindgut epithelium, fig. (I-1 & I-2), than normal ones. The epithelial cells of the hindgut were elongated, simple cracks of body wall with destructions in their nuclei and cytoplasmic vacuolization of some cells, fig. (I-3). The gonads, muscle fibers and the body wall were relatively broken, fig. (I-4). Shoukry et al (2003) reported on the histopathological features of the ovaries of Indian meal moth, Mohammed et al (2003), studied the effects of other plant extracts e.g. peppermint and citronella on *Culex pipiens*

- Nervous tissue: There are vacuolization's in the nerve fibrous and appearance of certain granules among the nerve cells especially nerve ganglion with some cracks, (fig.I-5 & I-6). Bakr et al (2010) found damages also by using LC<sub>50</sub> of IGR which causes degeneration and necrosis in spermatids and spermatozoa in testis of desert locust. Gardiner et al (2002) reported on cellular destruction of high affinity glutamate transporter in the nervous system of cabbage Hooper.
- Watson and Schurmann (2002) studied the synaptic structure, distribution and circulation in the central nervous system of locust and related insects.
- Malpighian tubule: There are clumping in nuclear chromatin and considerable vacuolizations in the epithelium cells with some cracks in their walls, (fig II-7 & II-8).

2- After 24 hrs:

The muscle fibers and body wall were smashed into several parts and ruin, (fig.II-9). The nuclei of gonads cells have clumping of the nuclear chromatin. Naggar et al (2006) reported that *M. azedarach* cause reduction in sperm bundles in male which lead to vacuolation in *Agrotis ipsilon*, and cause abnormality of follicular epithelium cells of female. In the present studies, there are holes in colon wall with vacuolations and destructions in tissue, muscles and body wall, (fig.II-10).

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3- Just before death:

-Foregut: Clumping of the nuclear chromatin and vacuolization in epithelium cells, with complete destruction of gizzard, fat tissue and body wall. (Fig.III-11 & III-12). The muscle fibers and body wall of oesophagus were completely destroyed and broken into several parts, (fig III-13 & III-14). Mohammed et al (2004) reported also that the LC 50 of Jojoba oil because swollen, lysed and destruction in both peritrophic membrane and the brush border of gut epithelial cells of *Culex pipiens*

- Midgut and gonads: The epithelium cells of midgut were isolated from its basement membrane. Most changes are confined to the nuclei of epithelium cells which elongated. Small parts of different sizes of inner end of these cells are detached, (fig.III-15 & III-16). Some parts of peritrophic membrane were elongated, destruction of fat tissue and body wall. Ahmed (2010) studied the epithelial cells of mesentron and peritrophic membrane in mosquito. Hung et al (2000) studied the ultra structure of midgut of oriental fruit fly. Eid (1994) said that the extracts of *M. azedarach* detached the muscles from epithelium, boundaries of epithelial cells disappeared with vacuolation in the midgut of *Agrotis ipsilon*. He added that the extract of *V. rosea* destroyed the striated border of it. There are destructions in nuclei and cytoplasmic vacuolization of some epithelium cells of midgut. Their muscles degenerated and detached from the epithelium. The muscles fibers of midgut are broken into several parts, (fig.III-17, III-18 and III-19). Cells of Malpighian tubules are vacuolated and degenerated. There are many vacuolization in nerve fibers. The sarcolemma of muscles is completely destroyed. Khalil et al (2011) reported that the extracts of *Apium graveolens* L. and *Piper nigrum* L. were toxic to larvae of *Culex pipiens* after 96 hrs.

The effects of lethal doses of leave extracts of *C. antiquum* because simple effects on gonads and rectum, (fig.IV-20). El-Bokl et al (2010) reported that the IGR cause changes in gonads tissue of red palm weevil and disrupt female and male gamete productions. The extracts of *Venca rosea* cause simple damages in muscles and body wall, (fig.IV-21) and simple destructions also in gonads and fat tissue, (fig.IV-22). Bakr et al (2010) reported that the ovarian follicles of desert locusts developed from the treated nymphs with IGR showed vacuolization of cytoplasm and degenerations of the cell components of follicular epithelium. The lethal dose of *M. azederacht* cause damages in fat tissue, body wall and crakes in gonads, (fig.IV-23), and that of *A. sativa* cause ruin of hindgut (fig.IV-24).

**Table (1): Mortalities (%) of *Galleria mellonella* L. larvae injected by tested plant extracts.**

plants	Dose(µL/ larva )	0.0	0.5	1.0	1.5	2.0
C. <i>antiquum</i>	green leaves	0.0	16.0	34.0	46.0	58.0
	dry leave powder	0.0	22.0	37.0	50.0	74.0

M. <i>azedarach</i>	green leaves	0.0	28.0	38.0	50.0	66.0
	dry leave powder	0.0	30.0	44.0	58.0	96.0
A. <i>sativum</i>	green leaves	0.0	24.0	36.0	48.0	62.0
	dry leave powder	0.0	26.0	38.0	54.0	82.0
A. <i>indica</i>	seed	0.0	22.0	46.0	79.0	100.0
V. <i>rosea</i>	green leaves	0.0	25.0	37.0	46.0	63.0
	dry leave powder	0.0	30.0	45.0	56.0	92.0

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**Cross section in *G. mellonella* larvae:**

I- Untreated and after 12 hrs of treatment with lethal dose of neem seed extracts showing

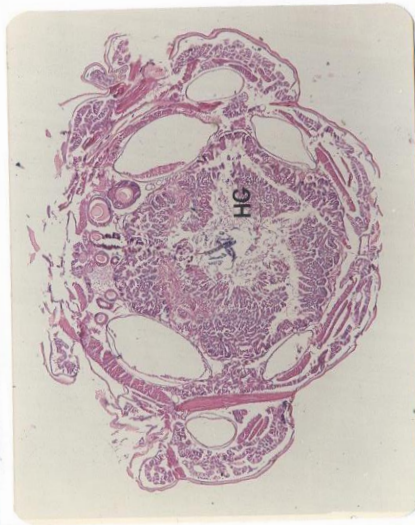


Fig (I-1): Untreated hindgut epithelium. (X40)

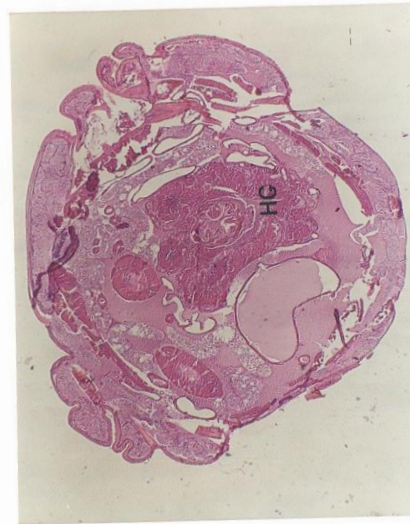


Fig. (I-2) :Thickness in hindgut layer by treatment. (X 35).

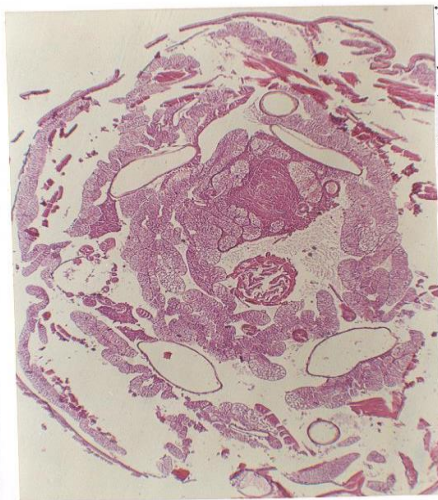


Fig. (I-3): Simple cracks in body wall and elongation of epithelial cells of hindgut by treatment. (X35)



Fig. (I-4): Simple broken in gonads, body wall and muscles fibers by treatment. (X 35).



II- Untreated and after 24 hrs of treatment with Lethal dose of neem seed extracts showing:

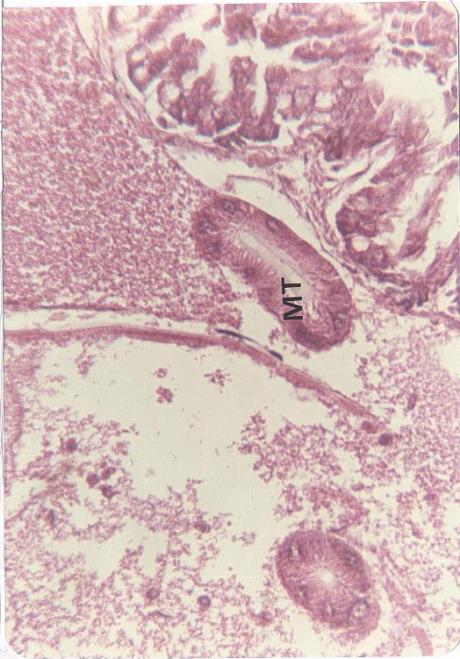


Fig (II-7): Untreated Malpighian tubules. (X 250).

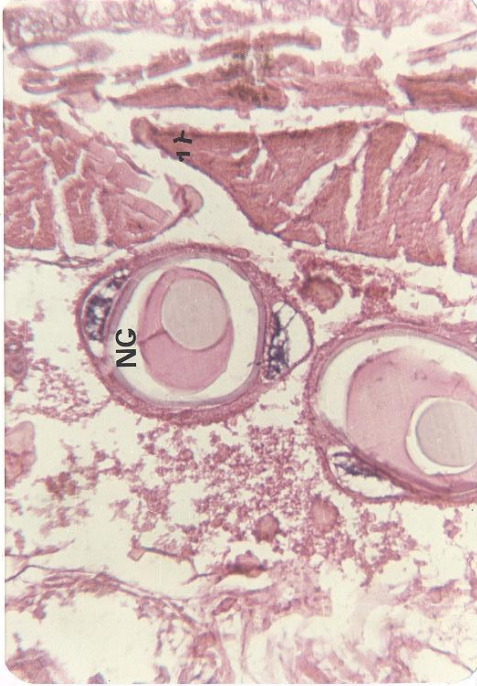


Fig.(I-5): Untreated nerve ganglia. (X 250).



Fig (II-8): Cracks in Malpighian tubules And body wall by treatment. (X 35).

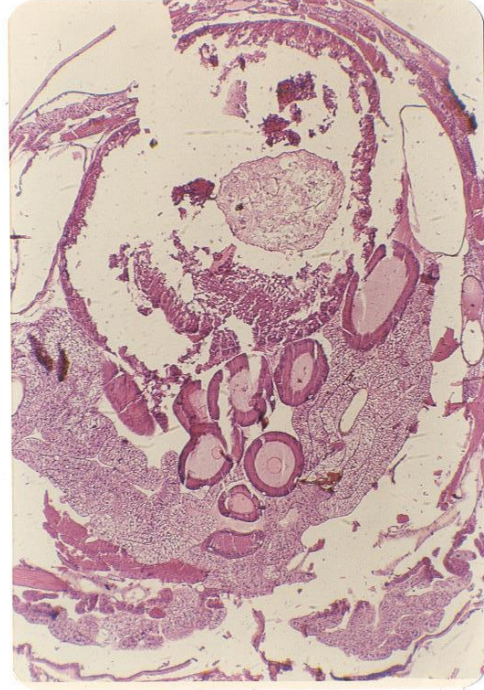


Fig. (I-6): Cracks in nerve ganglia by treatment. (X 60).



III- Untreated & just before death by treatment with  
Lethal dose of neem seed extracts showing:

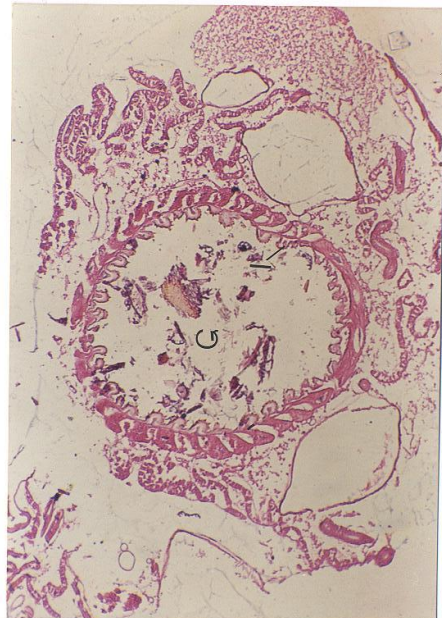


Fig (III-11): Untreated gizzard with internal intima. (X 60)

Fig (III-12): Complete destruction in gizzard, fat tissue  
And body wall by treatment. (X 60)

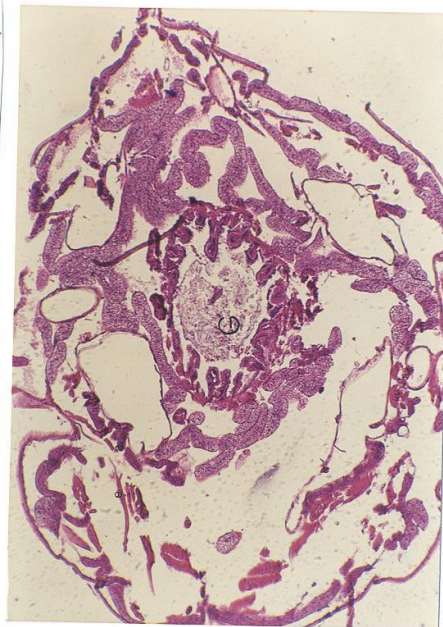


Fig. (II-9): Ruin in muscles and body wall by treatment. (X 35).

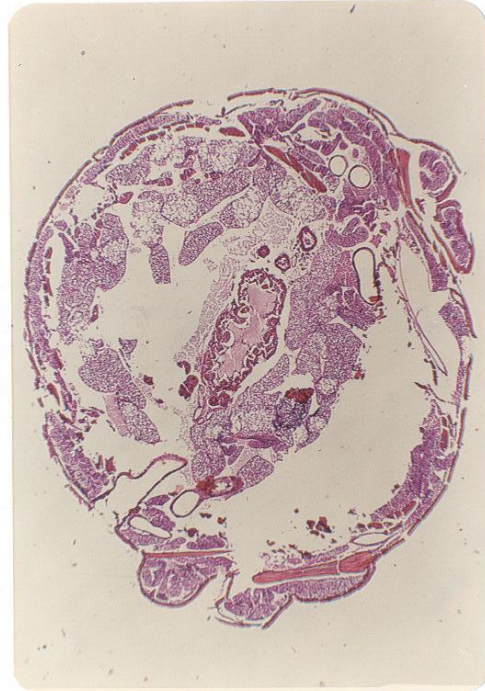


Fig. (II-10): Destruction in f fat tissue by treatment . (X35)





Fig (III-13): Untreated oesophagus and dilator muscles. (X 80)

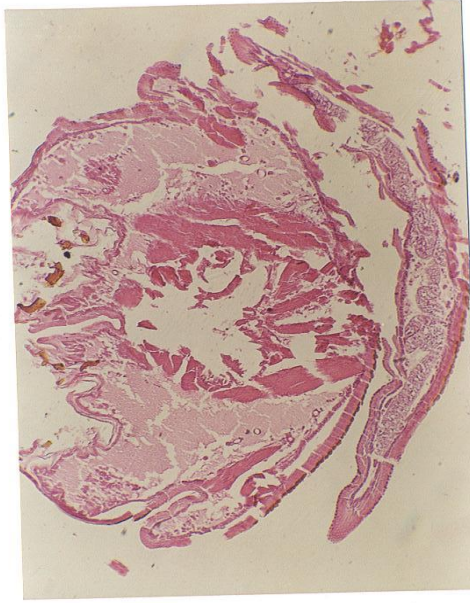


Fig (III-14): Complete destruction in muscles and Body wall by treatment. (X 80).



Fig (III-15): Untreated midgut and regeneration cells. (X60)

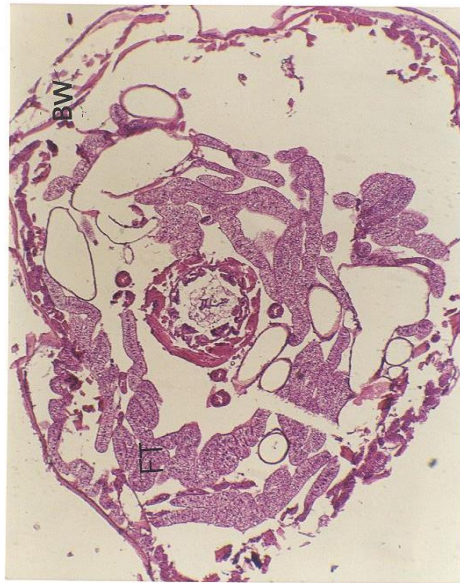


Fig (III-16): Cells Detached from basement membrane, fat Tissue & body wall destroyed by treatment(X60)





Fig. (III-17): Complete damages in colon and Muscles by treatment. (X 35).

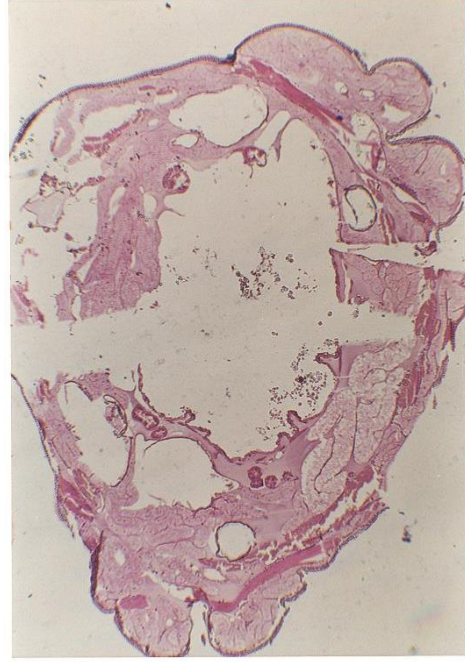


Fig. (III-18): Complete damages in midgut by treatment. (X 60).



Fig. (III-19): Complete destruction in muscles and Body wall by treatment . (X 60).

VI- Just before death after treatment with lethal dose of other plant extracts showing:



Fig. (IV-20): Simple effects in gonads and rectum with *C. antioquium* extracts . (X 35).





Fig. (IV-21): Simple damages in muscles and body wall with *Venca rosea* extracts. (X35).

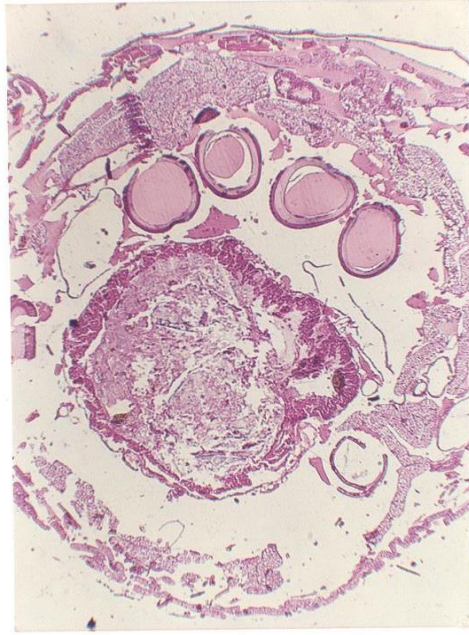


Fig. (IV-22): Simple destructions in gonads and fat tissue with *Venca rosea* extracts. (X 35).

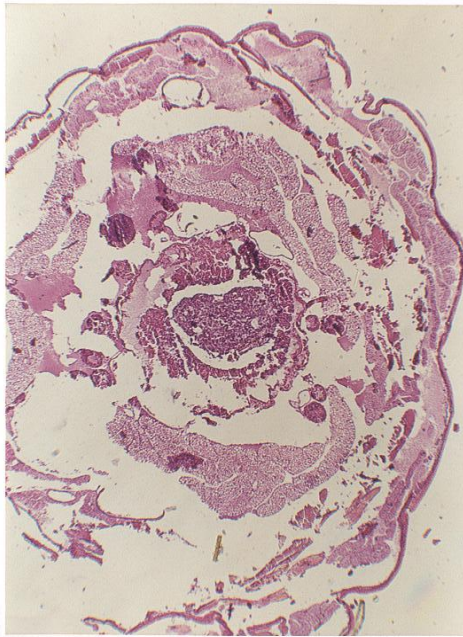


Fig. (IV-23): Damages in fat tissue and body wall with *M. Azedarach* extracts. (X 60).

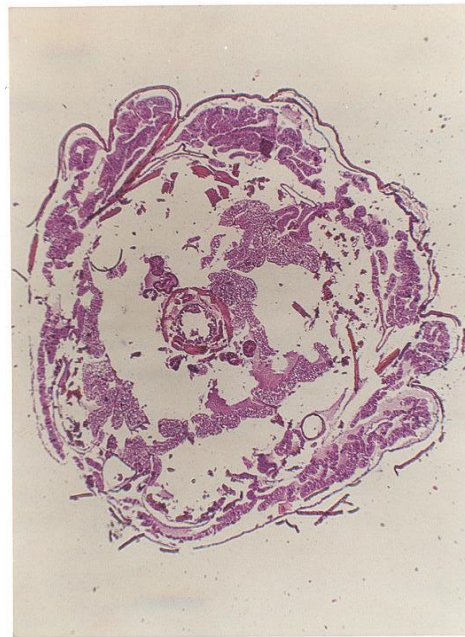


Fig. (IV-24): Ruin in the hindgut with *A. sativum* extracts. (X 35).