Evaluation Antifeedant and Larvicidal Effects Of water plant *Ceratophyllum demersum* Extracts, Against *Plodia interpunctella* Larvae

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Abstract

To producing bioinsecticides from environmentally friendly natural products .The present study was designed to producing bioinsecticide from water plant Ceratophyllum demersum extracts against the third and fifth larval instar of the Indian meal moth Plodia interpunctella based on LC50 values after 24, 48 and 72 hours of exposure, using the insecticide Nestor as a comparison . The results showed that the ethanolic extract of the C. demersum was toxic to the larvae, as its toxicity was superior to Nestor, as the LC50 values of the third larval instar were 0.051, 0.038 and 0.020 µl/insect, , , and they were 0.094, 0.038 and 0.48 µl/insect, after 24, 48 and 72 hours respectively. The results of testing the biological effects of ethanolic extracts on the external appearance or histological structure of the body wall and the mid gut as the most important target tissues for the resistant to insecticides in superficially treated insects exposed to feeding media treated with alcoholic extracts proved that these extracts It caused feeding inhibition, and consequently the death and death of treated insects after 10, 20 and 30 days of treatment. Accordingly, a number of effective phenolic compounds found in plant extracts were identified, the most important of which were Caffeic acid, catechin, Ellagic acid, Gallic acid, Kaempferol, p-Coumaric acid, Quercetin and Vanillic acid Using HPLC technology, the presence of these compounds in the plant was found, and the percentage of the two compounds was Quercetin and Kaempferol was the highest, as Quercetin appeared in the crude ethanolic extract (49.8%) and Kaempferol in the crude aqueous extract (66.2%)

1 Introduction

P. interpunctella is the first pest in flour mills (Subramanyam, 1996), it feeds on a wide range of grains including: barley, buckwheat, corn, oats, rice, and wheat, or on dried fruit meals such as: raisins, apricots, figs, prunes And plums, they also feed on nuts such as: almonds, peanuts, hazelnuts, and walnuts, as well as peas, beans, lentils, and various stored commodities (Kumar, 2017; Williams, 1964) .insecticides that are naturally produced or obtained from a biological source have a wide range of applications in plant crop protection, categorized into: phytobiological insecticides, biochemical biological insecticides, and microbial biocides (Marutescu et al., 2017), These biocides leave no residue on edible crops and also show similar efficacy to industrial insecticides (Kumar, 2012). Also, biocides have a different mode of action in eliminating pests such as inhibiting their metabolic activities (Mnifet et al., 2015), and it has been proven (Mahmoud et al., 2019) that extracts from Piper nigrum, Eucalyptus regnans and Neem oil *Azadirachta indica* was highly toxic against *Culex pipiens* mosquito larvae, while (Mekhlif and Muhammad, 2021) demonstrated a lethal effect of up to 100% of extracts of medicinal plants *Physalis angulate*, *Peganum harmala*, *Teucrium polium* and *Thymus vulgaris* on fourth instar larvae. *Culex mosquito*.

2 Ceratophyllum demersum

perennial; angiosperm; Leaves are circular stems, filamentous in shape, resembling filaments of algae, often crowded at the apex, fast growing, up to about 1 m long. The flowers (inflorescences) of both sexes are naked; stamens numerous; The pistil is one oval, slightly compressed, with one apical thorn. It grows in many places such as stagnant water, rivers, swamps, lakes, reservoirs, streams, and even in hot springs. This plant tolerates an unusual range of environmental conditions including fully open to shaded areas, extremely shallow to deep water (Les, 2018). It is now recognized as a submerged aquatic plant, and is popularly chosen for ornamental ponds in temperate gardens because it is a very good oxygenator, also helping to suppress grassy algae. Because the plant lacks a root system, all nutrient uptake occurs through the stems and leaves, which are filamentous and divided so much that it appears to be a species of the genus Chara, which is of the group of green algae. The plant also has the advantage that it stores energy in a starch-based system like that found in ferns, and this is not known in any other type of flowering plant. (Walker, 2020). It is not used as a food, but is used medicinally in many traditional medicines, including medicines to treat epilepsy, dermatitis, fever, jaundice, scorpion stings, and sunburn. Aqueous and methanolic extracts have shown to be potent analgesic, anti-diarrhea and anti-inflammatory, as well as antipyretic, as well as wound-healing activity in laboratory

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animals, while acetone extracts are an inhibitor of cyanobacteria (Les, 2018)The plant is characterized by its high ability to quickly absorb heavy metals and nutrients, and is widely used in the phytoremediation of water contaminated with sewage and various heavy metals, but in the case of excessive growth the plant interferes with the operation of hydroelectric power stations. (Pant et al., 2021). In a study conducted by (Al-Ani et al., 2019) on four selected sites along the Tigris River to know the effect of detergents on aquatic ecosystems, it has been proven that the pollutants resulting from washing activities that are released into the river are absorbed and accumulated very efficiently in the champlain plant, and these compounds that are absorbed by the plant as nutrients from the root or surface are used in all metabolic processes of the plant as energy.

3 Materials and Methods

Larvae of ***P. interpunctella*** (Indian Meal moth) of the order Lepidoptera were reared from prepared colonies. In industrial media prepared from a mixture of crushed nuts (almonds, walnuts and peanuts), equal quantities of culture media (250 g) were placed in sterilized plastic bottles with ethanol 70%, each of which had a capacity of 600 g, and insects were placed in each bottle of 50 random virgins. From the Indian meal moth, and in order to isolate the eggs of *P. interpunctella*, 25 pairs of pupae were placed inside a special box made by hand using a sieve with holes diameters of 800 µm. The sieve was fixed on a plate or bowl, and its opening was closed with a layer of reinforced plastic and incubated until the emergence of adults, and after mating Eggs were collected every 24 hours and placed on sterile food media in special containers. The first hatching of eggs was observed after five days. As for the larval stages, the insect recorded five larval stages, separated by six days. After 8-9 days, the fifth instar larvae turned into pupae, and these pupae needed 6-10 days, depending on the season of the year, to complete the morphological transformation into an adult. They survive for 10-14 days, while the majority of males live for half that time (Curts and Landolt, 1992)

3-1 Isolation and preparation of Plodia interpunctella larvae

The adults were first isolated from the pupae and larvae manually using a paintbrush, and the larval age was determined according to body length and head capsule width (Brindley, 1930) and distributed to each of them, the bottles were incubated under the previously mentioned conditions, and the isolation was re-isolated once a week to ensure survival The above phases are in their own bottles.

3-2 The insecticide Nestor®

In the current study, Nestor 20 sp insecticide was used, this insecticide belongs to the group of Neonicotinoides. It has the form of a water-soluble powder. Its active substance is acetamiprid 20% (w/w), and its manufacturer is Agri scinences.

3-3 Preparation of Extracts

C. demersum samples were washed well with running water to remove impurities and dust, then filtered and spread on aluminum foil and left to dry in the shade at room temperature 2±25 for a week to ten days, they were ground with a ceramic mortar until they turned into a fine powder and kept in cans. Sealed until use, according to the method (Ale-Grand et al., 1988) 10 g of powder was weighed and placed in a 500 ml flask, 100 ml of petroleum ether was added to it to perform the defatted process, the flask was closed tightly with aluminum foil and placed on Magnetic stirrer at a medium speed for 72 hours, after which the mixture was filtered using Whatman No.1 filter paper through a 250ml funnel, the precipitate was taken and put back into a 500ml beaker, then 200ml of 70-concentration ethyl alcohol was added to it. %, and after closing the mouth of the flask with aluminum foil and placing it again on the magnetic stirrer for another 72 hours, then filtered with filter paper and the extract was concentrated by a REV Rotary vacuum evaporator at a temperature of 40 °C. The remaining precipitate was soaked for the third time with 200 ml of distilled water inside a 500 ml beaker, and placed on the magnetic stirrer at a temperature of 60 ° C to obtain the hot water extract, then the crude extracts were placed in opaque glass bottles, closed tightly and kept in the refrigerator until use (Harborne, 1984).

3-4 Stock solutions

Depending on the results of the preliminary tests, stock solutions were prepared from the pesticide by placing the required weight of each Nestor pesticide or the extract in a graduated cylinder of 10 ml and completing the volume with distilled water to 10 ml, from which five concentrations were prepared (10, 25, 50, 75 and 100 micrograms). / ml), by adding 9 ml of distilled water to 1 ml of the concentration of the basic solution and in the same way the required concentrations (micro liters / ml) of the extracts were prepared using the appropriate solvents for each, and the solutions were kept in 30 ml glass Vial bottles with tight-fitting caps. Refrigerate at 4°C until use.

3-5 Vital tests

Experiments were conducted on the insect of the current study with 3 replicates for each concentration, and 10 larvae for each replicate, taking into account that the insects are active and homogeneous in size. Determination of the toxicity of aqueous and alcoholic extracts of plants.

- Surface treatment Topical Application

Preliminary experiments showed that the appropriate amount of solvent to carry the tested materials (microliter or microgram) for each larva was 10 microliter, so the insects were treated by adding 10 microliter of each of the

test solutions on the dorsal side (upper surface) of the insect's chest by means of a micro syringe (1000µl). As for the insects of the control group, they were treated with distilled water or ethanol used to dissolve the tested substance. After 15 minutes, the insects were inspected to ensure their safety. These treated insects were placed in Petri dishes, and the dishes were placed in the incubator. Mortality percentages were recorded after each of 24 hours and 48 hours. 72 hours from the start of the test.

- Exposure To Treated food media

In this method, only one concentration was adopted, which is 0.1 g, but in this method, 5 g of flour in equal proportions of the culture medium was weighed, and placed inside cans or containers of a capacity of 60 ml, where 1 ml of the ethanolic extract concentration was placed through an insulin syringe on the The food medium, leaving a few minutes for the medium to absorb the solutions, and then leaving it to dry completely. Insects were added to the food media treated with extracts after drying and the results and percentages of toxic effect were recorded after 24 hours, 48 hours, 72 hours, 10 days, 20 days, and 30 days.

Determination of the LC50% killer concentration value of the tested insects .The values of LCs for 50% of tested insects, Slope, and Upper and Lower Confidence Limits were determined using MSDOS by Probit software. Exe, Propit program) for the statistical analysis of the results of toxicity tests according to the (Finney, 1952) method.

3-6 Diagnosis using acid hydrolysis of raw extracts to detect phenols

There are phenols inside plants linked by a glycosidic bond with sugar, forming glycosides, and for the purpose of purification and identification of phenols, the process of acid hydrolysis is carried out to release phenols from sugar by breaking the glycosidic bonds based on the method of the researcher (Harborne, 1973), when 25 ml of hydrochloric acid (HCL) was added at a concentration of 2 molarity to 5 ml of the crude ethanol extract of the plant, they were put into a glass beaker and the mixture was heated in a Reflux Condensor at 70 °C for 1 hour, then the mixture was cooled down and 50 ml of Ethyl acetate was added to it in a 250 ml separating funnel over two phases, the first is 25 ml, and the mixture is shaken well, then left on a stand until two layers appear; An organic layer and an aqueous layer, the upper organic layer containing sugar-free free phenolic compounds was separated, and the above steps were repeated a second time with the lower aqueous layer, then concentrated using the rotary evaporator to obtain the phenolic extract, the process was also repeated for the aqueous extracts, and the phenolic extracts were preserved In opaque bottles in the refrigerator until use.

3-7 Quantitative and qualitative diagnosis of active phenolic compounds using High Performance Liquid Chromatography (HPLC)

A high-performance liquid chromatograph based on polarity and capillary action was used to separate separated phenolic compounds from plants, as most of these compounds are weakly acidic (ionize under basic conditions and dissolve easily in different polar solvents) (Al-Hayali, 2020). Research vehicles in the laboratories of the Ministry of Science and Technology / Department of Environment and Water using a high-performance liquid chromatography (HPLC) type (SYKAM) of German origin, with a separation column C18 with dimensions (4.6 x 250 mm), and a UV/VIS SPD-20A detector at The wavelength is 280 nm, and the carrier phase was used: methanol: distilled water: formic acid 5:25:70 (V:V:V) to separate the phenols, and the flow rate of the carrier phase was 1.3 ml/min flow rate.

4 Results and discussion

The results of biological tests to estimate the toxicity of Nestor and *C. demersum* extracts against Indian meal moth larvae depended on the values of concentrations and lethal doses for fifty percent of the tested insects (LC50 and LD50), and the minimum and upper confidence limits at the 95% upper and lower confidence limits 95%. As well as the regression values of the toxicity lines (Slope). The results showed the toxicity or non-toxicity of the test solutions, and how the toxicity of the poisonous ones varied significantly with the difference in the sex of the insect and its developmental stage, as well as the treatment method. The results also showed that the highest rates of toxic effect appeared in the treatment method. Surface Topical Application.

4-1 Toxicity of the Nestor to Indian meal moth larvae

The results in Tables (1) indicate that the pesticide Nestor is toxic to the larvae of the Indian meal moth, and that its toxicity varied significantly with sex, developmental stage and method of treatment. (Zhang et al., 2022) that exposing the soybean insect Aphis glycines to acetamiprid shortened the life cycles, parturition time and pre-reproductive period of adults, as confirmed (Wang et al., 2020) that acetamiprid is highly toxic against silkworm larvae. *Bombyx mori* after continuous exposure of the insect to a low dose of it, and its residues in the midgut did not degrade until after 96 hours of treatment. The results showed that the toxicity of the pesticide in the surface treatment method of third instar larvae of Indian meal moth is more sensitive to it than fifth instar larvae 1.8, 1.9 and 2.4 times, respectively, during 24, 48 and 72 hours.

Table (1): LC50 values of third and fifth instar larvae after 24, 48 and 72 hours of surface treatment with Nestor.

Larval stage	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.169	0.064	0.477	1.040	0.065	0.030	0.142	1.210	0.030	0.015	0.056	1.409
Fifth	0.305	0.118	0.785	1.261	0.126	0.064	0.249	1.603	0.074	0.039	0.139	1.585

4-2 Toxicity of ethanolic and aqueous extract of Ceratophyllum demersum to Indian meal moth larvae

The results of toxicity tests in Tables (2) indicated that the ethanolic extract was toxic to Indian meal moth larvae by surface treatment, and that its toxicity varied clearly with sex and developmental stage, and that its effect was not fatal as much as it was an anti-nutrition. The results of the experiments showed that the toxicity of the ethanolic extract was to the third instar larvae more than to the fifth instar larvae of the Indian meal moth 1.8, 2.1 and 2.4 times, respectively, during 24, 48 and 72 hours of treatment, while the aqueous extract had no lethal effect after 72 hours of treatment. Comparing the results of the toxicity of the ethanolic extract in these tables with the toxicity of the pesticide Nestor in Tables (1), it is clear that the toxicity of the ethanolic extract to Indian meal moth larvae was 3.2, 1.5 and 1.5 times, respectively, during the 24, 48 and 72 hours.

Table (2): LC50 values of third and fifth instar larvae after 24, 48 and 72 hours of surface treatment with ethanolic champlan extract

Larval stage	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.051	0.022	0.119	1.091	0.038	0.014	0.105	0.890	0.020	0.010	0.042	1.285
Fifth	0.094	0.048	0.182	1.560	0.083	0.039	0.178	1.276	0.048	0.024	0.098	1.327

The results of this study were in agreement with (Abbas, 2013) that the fourth instar larvae of the baffling flour beetle, the rusty red flour beetle and the long-headed flour beetle are more sensitive to the insecticides Aktar and Lannet than their adults, and this may be due to the fact that the activity of oxidative enzymes mixed the metabolic function of insecticides in the larval ages. Less than it is in adults, making it less susceptible to detoxification reactions, and this may also be attributed to size, because there is a relationship between toxicity and size, and this conclusion is confirmed by many studies in larvae and adults, in larvae it was found (Martin et al., 2000) that The smaller first instar larvae of the American cotton bollworm are more sensitive to cypermethrin than the larger successive larval instars. They also found (Al-Attar and Abbas, 2003) that the smaller third instar larvae of the red and bewildering flour beetle are more sensitive to the insecticides Coopex, Decis and Vapcocidin than the larger fifth and seventh instar larvae. The results of the experiments also proved that the ethanolic extract has nutritional and growth regulating properties, as many cases of predation were observed among the larvae, and their number was directly proportional to the increase in the concentrations of the treatment. To continue their life cycles, it was found that about 90% of the members of the new generation that it produced are characterized by fierce predation, as the larvae began to prey on each other a few minutes after they were removed from the feeding media.

4-3 Toxicity of ethanolic extract of champlan to Indian meal moth larvae by feeding media treatment.

The results of the food treatment in Tables (3) proved that the ethanolic extract of the *C. demersum* may have anti-nutritional properties, as the nutritional median weight treated with the extract of the *C. demersum* remained the same and there was no clear decrease in weight.

Table (3): Effects of Indian meal moth larvae on the method of treating the food media

Time	Third larvae stage				Fifth larvae stage			
	Control		Treated larvae		Control		Treated larvae	
	Percentage of toxic effect %	Loss in weight	Percentage of toxic effect%	Loss in weight	Percentage of toxic effect %	Loss in weight	Percentage of toxic effect%	Loss in weight
24 Hour	0%	0.02	25%	0.005	0%	0.03	30%	0.01
48 Hour	0%	0.02	80%	0.00	0%	0.03	90%	0.00
72 Hour	10%	0.02	100%	0.00	0%	0.03	100%	0.00
10 Day	50%	0.04	100%	0.00	35%	0.06	100%	0.00

The results of this study were in agreement with several studies in which plant extracts showed anti-nutritional properties, including what was mentioned by (Abo Arab et al., 2022) the effect of neem tree kernel extracts against some stored grain insects such as Callosobruchus maculatus and Khabra beetle

Trogoderma granarium, The study revealed a long residual toxicity when treating the grains as a result of Azadirachtin, which is the main chemical substance of the neem kernel, and that this substance has anti-nutritional properties. The study also agreed with (Ravi and Sundararajan, 2020), who studied the properties of extracts of a group of plants against the larvae of the American cotton boll worm, and proved that the Cassia.tora plant and the Balloon plant Cardiospermum halicacabum have preventative and feeding properties. The study also agreed with what was reported (Tebayashi et al., 2020) of the anti-feeding effect of the chemical (2-cyanoethyl-isoxazolin-5-one) present in sweet pea Lathyrus odoratus against the larvae of the tobacco worm Spodoptera litura.

4-4 Quantitative and qualitative diagnosis of active phenolic compounds using HPLC technology.

A number of phenolic compounds were diagnosed and separated from crude extracts, as well as from phenolic extracts and petroleum ether extract. Table (4) shows each of the standard phenolic compounds, and the standard retention time (minutes)

Table (4): Standard Phenolic Compounds and Standard Reten time (minutes)					
Standard phenolic compounds		Reten time	Standard phenolic compounds		Reten time
1	Caffeic acid	11.80	5	Kaempferol	6.82
2	Catechine	10.92	6	p-Coumaric acid	5.88
3	Ellagic acid	3.65	7	Quercetin	9.42
4	Gallic acid	2.58	8	Vanillic acid	13.88

Table (5) shows the phenolic compounds in *C. demersum* extracts. Quercetin appeared in the highest percentage (58.8%) in the phenolic ethanol extract, and the order of the compounds was as follows: Quercetin > catechin > p-Coumaric acid > Gallic acid > Vanillic acid in proportions (58.8 > 58.8). 56.2 > 54.8 > 40.5 > 30.5), while the compound Kaempferol appeared in the highest percentage (72.6%) in the phenolic water extract, and the order of the compounds was as follows: Kaempferol > Ellagic acid > Quercetin > Caffeic acid > Vanillic acid in proportions (72.6 > 55.0 > 54.7 > 47.8 >33.6), while the petroleum ether extract also showed the compound p-Coumaric acid with the highest percentage (21.4%), and the arrangement of the compounds was as follows: p-Coumaric acid > Quercetin > Caffeic acid in proportions (21.4 > 18.9 > 11.5), as studies indicated on Phenolic substances indicate that the phenolic content of the extracts depends on the different extraction conditions used, as well as the type of solvent used for extraction (ethanol, methanol, chloroform, hexane and ethyl acetate) (Machu et al., 2015)

Table (5): Comparison of the ratios of phenolic compounds in the extracts of the *Ceratophyllum demersum*						
	Extract Phenolic compounds	Aqueous extract		Ethanolic extract		Petroleum Ether Extract
		Before acid hydrolysis	After acid hydrolysis	Before acid hydrolysis	After acid hydrolysis	
1	Caffeic acid	36.9	47.8	-	-	11.5
2	Catechine	-	-	50.1	56.2	-
3	Ellagic acid	48.5	55.0	-	-	-
4	Gallic acid	-	-	33.6	40.5	-
5	Kaempferol	66.2	72.6	-	-	-
6	p-Coumaric acid	-	-	42.5	54.8	21.4
7	Quercetin	49.8	54.7	49.8	58.8	18.9
8	Vanillic acid	22.5	33.6	22.6	30.5	-

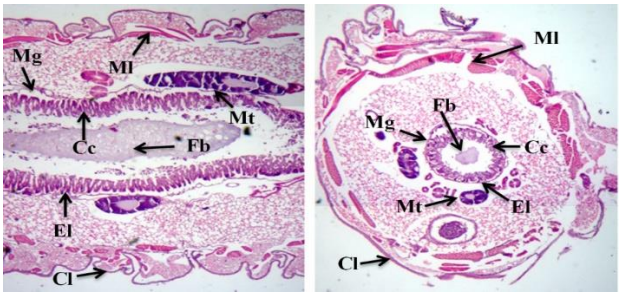
4-5 Histological effects of C. demersum extracts

Transverse and longitudinal histological sections were adopted for each of the midgut area and the body wall, and the results of sections of treated larvae were compared with sections of the control group, knowing that all histological sections were taken from the fifth instar larvae of the Indian meal moth treated by surface treatment, while sections were excluded. The larvae of the flour beetle, puzzled by the fact that they did not appear in the required shape and accuracy.

• Effect on the midgut (stomach) and the Malpighian tubes

The mid-gut consists of an epithelium layer resting outside on the basement envelope, while its free surfaces consisting of vertical cells inward facing the food, and covered with microvilillae, and the basal envelope resting on a serous layer composed of connective tissue And circular muscles, followed by the hemolymph, while the Malpighian tube is composed of one layer of secretory epithelial cells based on a basement membrane, and the membrane is surrounded by a circular muscle layer

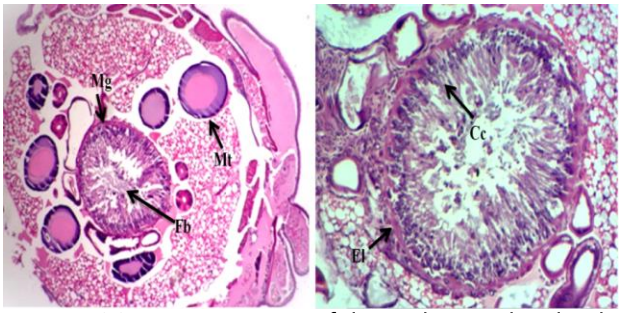
(Al-Mansour, 2021; Tawfiq, 1976), and the picture (1) shows the shape of the midgut in the control group.



Picture 1: Body section of an untreated 40X larva, A longitudinal section,B cross section : Mg (Midgut), Cl (Cuticle layer), Ml (Muscular layer), Cc (Colmunar cell), El (Epethelium layer), Mt (Malpighian tubule), Fb (Food bowls).

• The effect of Nestor on the midgut

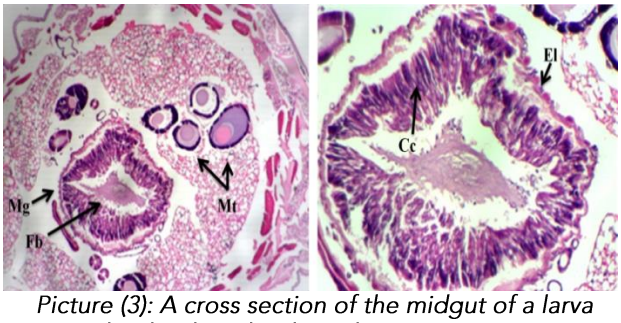
As shown in picture (2), the effect of Nestor's repeller on the midgut, as it was observed that a large elongation of the columnar cells and their separation from each other, also appeared in the muscular layer of the intestine and the decomposition of the nutritional mass, while the Malpighi tubes appeared very enlarged and the muscular layer atrophied to the extent of half Approximately.



Picture (2): A cross section of the midgut and Malpighi tubules in a larva treated with Nestor, (A) 40X, (B) 100X

• Effect of ethanolic extract of champignon on the midgut

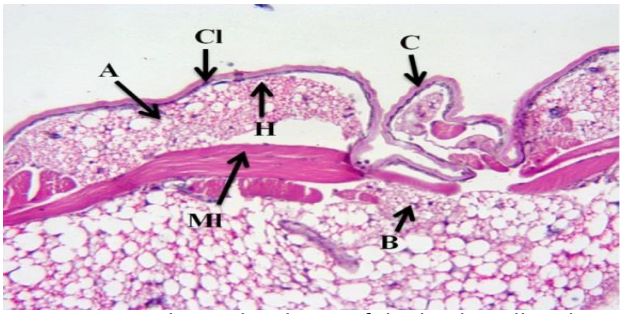
The picture (3) shows the histological effects of the ethanolic champlan extract of Indian meal moth larvae on the midgut, whose walls retracted and wrinkled. Also, there was a great elongation in the columnar cells and their separation from each other, and the separation of the intestinal epithelial tissue cells from the basal cover, while Malpighi tubes appeared. It is very enlarged and the muscle layer is loose and atrophied almost in half.



Picture (3): A cross section of the midgut of a larva treated with ethanolic champlan extract, (A) 40X, (B) 100X

• Histological effects of C. demersum extracts on the body wall

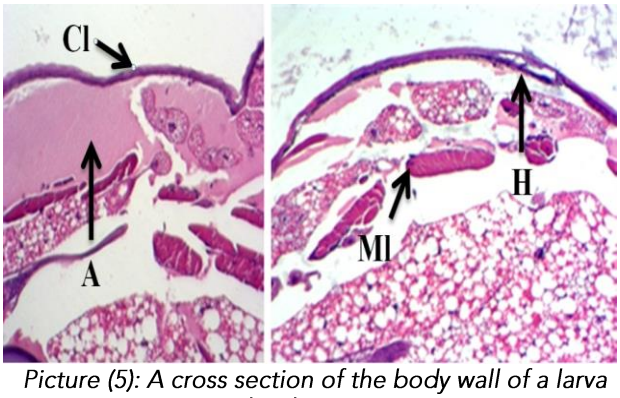
The body wall of insects consists of three layers arranged from the outside to the inside: 1 Cuticle or ice , It is a non-cellular layer secreted by the epidermal layer belo .2 The inner epidermis or hypodermis: a single layer of compact cells Basement membrane: It extends continuously below the inner epidermal layer around the muscle fibers attached to the body wall (Tawfik, 1976), and picture (4) shows the shape of the body wall in the control group.



Picture (4) shows the shape of the body wall in the control group. Mg (Midgut), Cl (Cuticle layer), Ml (Muscular layer), A wall fat layer Blayervisceral fat, C body cavityPicture (4): A cross section of thebody wall of a larva Untreated 100X

• The effect of the insecsticide on the body wall

As shown in picture (5), the effects on the body wall included partial separation of the epidermal layer from the cuticle layer, disintegration and necrosis of the connective tissue layer and its separation from the circular muscle layer, as well as the disintegration or fracture of the muscle layer itself, and the emergence of many cavities and voids between the body tissues resulting from The necrosis of cells was accompanied by cellular infiltration.

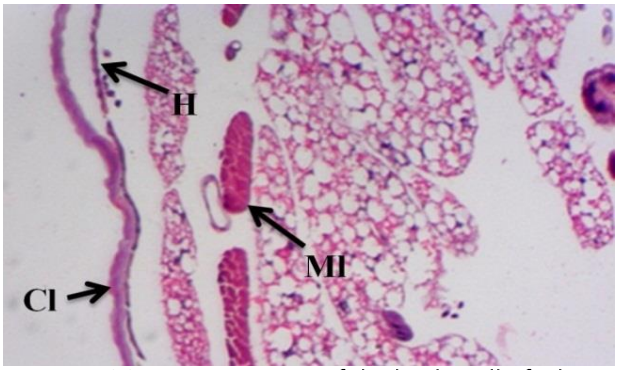


Picture (5): A cross section of the body wall of a larva treated with Nestor 100X.

• Effect of the ethanolic extract of the Ceratophyllum demersum on the body wall

Picture (6) shows the effect of the plant extract on the body wall of the larvae, as the separation of the epidermal layer from the cuticle occurred, a large disintegration accompanied by wide gaps in the connective tissue layer and its separation from the

circular muscles, as well as many gaps spread across the body tissues in general as a result of erosion and necrosis of tissue cells.



Picture (6): A cross section of the body wall of a larva treated with the ethanolic extract of Ceratophyllum demersum 100X.

5 References

Abbas, Lubna Yassin (2013). Synergistic effect of extracts of peel, juice and seeds of Citrus paradisi (Macf.) fruits in Aktara and Lannet pesticides and their interactions on three types of stored grain insects. PhD thesis, College of Education, University of Mosul, Iraq.

Abo Arab, R. B., M. El-Tawelh, N., Hamza, A. M., and Nasr, G. M. (2022). Comparative Efficiency of Selected Chemical and Bio Pesticides against some Stored Grain Insects. Journal of Plant Protection and Pathology, 13(7), 157–160. https://doi.org/10.21608/jppp.2022.145344.1083.

Al-Ani, R. R., Hameed, A., Al-Obaidy, M. J., and Hassan, F. M. (2019). Bioaccumulation of Anionic and Nonionic Surfactants in Ceratophyllum demersum Plant in Tigris River , Baghdad , Iraq. Indian Journal of Ecology, June.

Al-Attar, Hani Jihad and Abbas, Lubna Yassin (2003). Effect of species, age, developmental stage and exposure period on the toxicity of three pyrethroid pesticides against the red flour beetle Tribolium castaneum and T. confusum. Journal of Education and Science Volume (16). Issue (3). : 28-38.

Ale-Grand, A., Wondergem, P. A., Vepoort, R., and Pousset, J. L. (1998). An ti-interactions Phytotherapies of The Tree Savannah of Senegal (West Afri ca) II. Anti-microbial Activity of 33 species, J. of Ethnopharmacol. Jan.,22 (1),25-31.

Brindley, T. A. (1930). The growth and development Ephestia keuhnilla (Zller). (Lepid).and under controlled conditions of temperature and relative humidity. Ann. Entomol. Soc. Am. 23(4): 740-757.

Curts, C. E., and Landolt, P. J. (1992). Development And Life History Of Pyralis Farinalis L. (Lepidoptera:Pyralidae) On An Artificial Diet. J. Stored Prod. Res., 28(3).

Finney, D. J. (1952). Probit Analysis, a statistical treatment of the sigmoid response curve. (2nd Ed), Journal of the Institute of Actuaries, 78 (3): 388-390.

Harborne, J.B. (1984). Phytochemical Methods. 2nd ed., Chapman and hall.

Harborne,J.B(1973). Phytochemical Methods:Aguide To Modern Technique Of Plants Analsis.1st Ed., Cox And Wyman, London, P.52-73.

Kumar S (2012), Biopesticides: a need for food and environmental safety, J BiofertilBiopestic., Vol.3, No.4, pp.107.

Kumar, R. (2017). INSECT PESTS OF STORED GRAIN: Biology, Behavior, and Management Strategies. Apple Academic Press, Inc, USA.

Les, D. H. (2018). Aquatic dicotyledons of North America: Ecology, life history, and systematics. CRC Press, Taylor and Francis Group. https://doi.org/10.1201/9781315118116.

Mahmoud, D. M., El-Bar, M. M. A., Salem, D. A., and Rady, M. H. (2019). Larvicidal potential and ultra-structural changes induced after treatment of Culex pipiens L. (Diptera: Culicidae) larvae with some botanical extracted oils. International Journal of Mosquito Research, 6(4), 1–09.

Martin, T.; G.Ochou; N. Hala; F. Kio; and M. Vaissayer (2000). Pyrethroids resistances in the cotton bollworm Helicoverl armigera (Hubner). West Africa Pest Manag. Sci. 56(6): 549-554.

Marutescu L, Popa M, Saviuc C, Lazar V and Chifiriuc MC (2017), Botanical pesticides with virucidal, bactericidal, and fungicidal activity, In New pesticides and soil sensors, Academic Press, pp. 311-335.

Mekhlif, A. F., and Muhammad, M. J. (2021). Larvicidal potentials of four medicinal plant extracts on mosquito vector , Culex pipiens molestus (Diptera : Culicidae). International Journal of Mosquito Research, 8(4), 01–05.

Mnifet I and Ghribi D (2015), Potential of bacterial derived biopesticides in pest management, Crop Protection, Vol.77, pp.52-64.

Pant, D., Bhatia, S. K., Patel, A. K., and Giri, A. (2021). Bioremediation using weeds. Springer Nature Singapore Pte Ltd. http://link.springer.com/10.1007/978-981-33-6552-0.

Ravi, G., and Sundararajan, G. (2020). Larvicidal and Antifeedant Activity of Some Plants Extracts Against the Larvae of Helicoverpa Armigera (Hubner). International Journal of Scientific Research and Management (IJSRM), 08(05), 67–76. https://doi.org/10.18535/ijsrm/v8i05.b02.

Subramanyam, B. (1996). Integrated Management of Insects in stored Products. Taylor and Francis Group, New York, London.

Tebayashi, S., Moriyama, R., Arakawa, R., and Sato, M. (2020). Induction of 2-cyanoethyl-isoxazolin-5-one as an antifeedant against the tobacco cutworm (Spodoptera litura) by jasmonic acid in sweet pea leaf. Bioscience, Biotechnology, and Biochemistry, 84(6), 1105–1112. https://doi.org/10.1080/09168451.2020.1724072.

Walker, T. (2020). POLLINATION: The Enduring Relationship between Plant and Pollinator. Princeton University Press.

Wang, H., Lu, Z., Li, M., Fang, Y., Qu, J., Mao, T., Chen, J., Li, F., Sun, H., and Li, B. (2020). Responses of detoxification enzymes in the midgut of Bombyx mori after exposure to low-

dose of acetamiprid. Chemosphere, 251, 126438. https://doi.org/10.1016/j.chemosphere.2020.126438.
Williams, G. C. (1964). The life-history of the Indian meal-moth, Plodia interpunctella (Hubner) (Lep. Phycitidae) in a warehouse in Britain and on different foods. Ann. Appl.Biol, 53, 459–475.
Zhang, A., Xu, L., Liu, Z., Zhang, J., Zhao, K., and Han, L. (2022). Effects of Acetamiprid at Low and Median Lethal Concentrations on the Development and Reproduction of the Soybean Aphid Aphis glycines. Insects, 13(1). https://doi.org/10.3390/insects13010087.