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Effect of silver nanoparticles using *Melia azedarach* L. leaf extract on house fly *Musca domestica* L.

Zeena Zakaria Idrees [1], Muneef Abd Mustafa [2]
[1-2] Department of Biology, College of Science, University of Mosul, Iraq
Corresponding Author: **Zeena Zakaria Idrees**

Abstract
The current study aimed to study the effect of synthesized silver nanoparticles using *Melia azedarach* L.leaf extract by dipping and feeding methods at four concentrations (200, 100, 50,25) ppm in the stages of the house fly *Musca domestica* L.,the concentration of200 ppm caused an emergence inhibition rate of 100 %, while the lowest inhibition rate was at a concentration of 25ppm, which amounted to 53% by dipping method after ten days of treatment, while the value of LC50 (the concentration that kills 50% of insects) reached (24.23) ppm. While the percentage of inhibition by feeding method also reached 100% at a concentration of 200 ppm, and the concentration of 25 ppm gave the lowest inhibition rate of 48% and for the same time period, and the value of LC50 (27.06) ppm. For both methods, the results also showed that all concentrations had an effect on the natural development of the growth of the different stages and caused the appearance of morphogenetic abnormalities in the pupa and the adult and a decrease in the percentage of the appearance of the adult. The results showed that the effect of silver nanoparticles by dipping method was higher than by feeding method for all phases.

Keywords: house fly, silver nitrat, *Melia azedarach* L. morphogenetic abnormalities

1. Introduction
House fly *Musca domestica* L. (Diptera: Muscidae) is the most common and wide spread species of fly, exist a major pest of humans, livestock and poultry throughout the world (Rahual, 2013; Hussein and John, 2014) [23, 13]. House fly act as transporter of disease causing agents like bacteria (*Escherichia coli, Shigella, Salmonella spp.*) (Macovei *et al.* 2008) [8], Which spread more than hundred diseases in humans and animals like amoebic dysentery, helminthic and rickettisial infections etc. (Mian *et al.* 2002; Tian *et al.* 2011) [20, 28]. Therefore, there was need to search for ways to control the insect.

Chemical insecticides have been used to control many insect pests, including house fly, and due to the toxic effects of these pestiside on humans, animals, their negative effects on the environment, and the emergence of strains of insect that are resistant to action of pesticides, in addition to its high coast. So researchers were interest in revealing modren technique that effective in control insects and not polluting the environment to exploit them as alternative to chemical pesticides (Al-joary and Al-Iraqi, 2016) [4].

Nanotechnologies emerged by using very small size materials as a promising and developed field in controling insect pests (Khot *et al*., 2012) [17], one of these nanomaterials is silver nanoparticles Ag NPs, which possesses many lethal properties for some types of insects and other pests in addition to it has antimicrobial and antiviral activity, and it is non-toxic to humans and animals (Yeo *et al*., 2003) [30]. These properties have been used in agricultural, medical, pharmacy and biological applications (Chhipa, 2017) [9].

A number of plants containing different reductive groups use in the preparation of nanoparticles. The application of botanicals to prepare nanomaterials provides a number of benefits because the process does not use toxic chemicals in the synthesis (Borase *et al*. 2014) [8]. Furthermore, these green synthesized nanoparticles are more effective than pesticides, less costly, biodegradable, and safe for humans and the environment than their synthetic counterparts (Murugan *et al*. 2016; Sabbour & Abd ElAziz 2015) [26, 25]. It was found that treatment larvae with silver nanoparticles Synthesized from the extracts of *Moringa oleifera* showed significant reduction in the fecundity of female and the egg hatchability and larvicidal and pupicidal toxicity of housefly *Musca domestica* L. (Abdel-Gawad, 2018) [2]. RajaKumar (2011) [24] found that silver nanoparticles synthesized from the aqueous extract of *Eclipta prostrata* leaves showed a lethal activity to the larvae of *Anopheles subpictus Grassi* and *Culex quinquefasciatus* mosquitoes. While Kalimuthu, (2017) [15] noted that the biosynthetic silver nanoparticles from the extract of the white ginger lily plant *Hedychium corarium* and its rhizomes showed lethal activity against the larvae and pupae of *Aedes aegypti* mosquitoes after 24 hours of exposure, and many histological changes were observed, especially in Insect luminal epithelial cells.

The present study aimed to evaluate the toxicological activity of biosynthetic silver nanoparticles from *Melia azedarach* against larvae, pupae and adults of the house fly *Musca domestica* L. using dipping and feeding methods.

Materials and Methods

House fly rearing

The house fly was rearing in the Department of Biology, College of Science, and University of Mosul for several generations in the laboratory to obtain a pure colonies. Where the adult insect was reared in wooden cages with muslin cloth sides of dimensions (45 * 30 * 30 cm), and fed on cotton saturated with milk and 10% sucrose**.**

As for the larvae, they were fed artificial food consisting of 600 gm of cow manur (after sterilization in an electric oven at a temperature of 70 °C for 15 minutes), 22 gm of yeast, 200 g of barley, 20 cm^3 NaOH 5 N and 1200 cm^3 of distilled water and incubated at a temperature of 27 ±2°C and a relative humidity of 70±5% (Mustafa, 2008).

Preparation leaf of *M. azedarach* extract

The leaves of *M. azedarach* were collected from gardens of university of mosul, washed with distilled water, then dried in the oven at 60 °C for 12 h to remove the remaining moisture and then grinding into powder (Figure1). The aqueous M. azedarach leaf extract was prepared by adding 2 g of dried leaf powder in 50 ml of boiling distilled water for 5 min. Then the mixture was centrifuged at 5000 rpm for 15 min, and the supernatant was filtered in flasks to obtain a cell aqueous extract and stored at 4°C in refrigerator for further use.

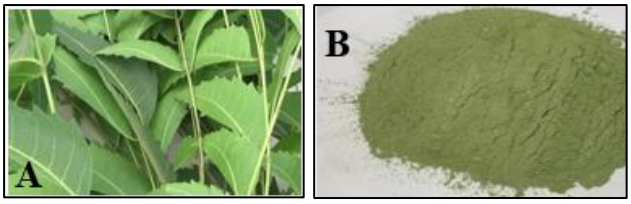

Fig (1): M. azedarach leaves **(a),** and M. azedarach powder **(b).**

Synthesis of silver nanoparticles

The synthsis of AgNPs by added 10 ml of silver nitrate solution (0.025 M) to the flask containing 40 ml of *M. azedarach* aqueous extract. The mixture obtained was heated to 40°C for 10 min until the color of solution changed from light yellow to brown which indicates the formation of AgNPs. Finally, the mixture was filtered by Whatman No.1 and dried (Jebril *et al.*, 2020).

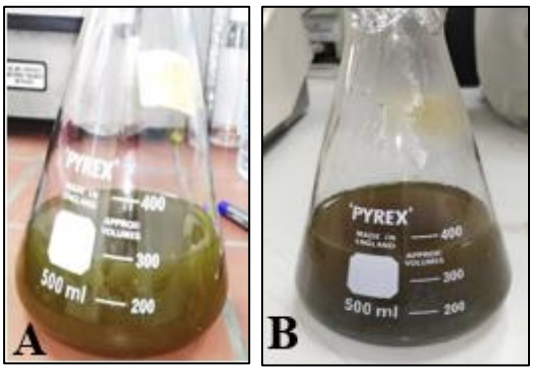

Fig (2): Color of M. azedarach aqueous extract before **(a)** and after **(b)** the addition of AgNO3

Characterization of biosynthesized AgNPs

1. UV-Visible Spectroscopy

The synthesis of AgNPs was determined by UV-visible spectrophotometer Unico S-2150 with a resolution of 1 nm in the range of 200 nm-800 nm. With pipette 2 ml of the sample in the cuvette and tested at room temperature. The UV-visible spectrum confirmed the formation of AgNPs by showing a typical silver surface plasmon resonance at a wavelength of about 442 nm.

2. Scanning Electron Microscopy (SEM)

The morphology and size of biosynthesized AgNPs were studied by SEM images at higher resolution with different magnifications. The SEM images showed the existence of small spherical nanoparticles with a size ranged from 18 to 30 nm (average size of about 23 nm).

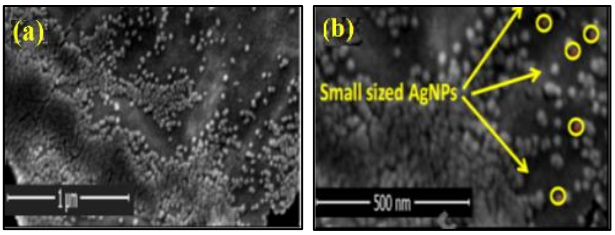

Fig (3): (a) The SEM images of biosynthesized AgNPs at X50 000 **(b)** at X100000(Jebril *et al.*, 2020).

Biosynthetic Silver nanoparticle bioassay

Dipping Method

The biological activity was tested the method mentioned by (Sinthusiri and Soonwera, 2010) [26], where the stock solution was prepared by dissolving 1 g of synthesized silver nanoparticles extract in (1000 cm^3) of distilled water to obtain a solution at a concentration (1000 ppm), from thes concentration prepare concentrations used in the test (200, 100, 50, 25 ppm). For the purpose of testing, 10 second instar larvae were taken and immersed in 10 ml of solution in the concentrations prepared above for 30 seconds. As for the control treatment, it was immersed in distilled water, then transferred to glass tubes (5 x 2 cm) containing 1 g of artificial food. The larvae were placed in the incubator at a temperature of 27±2°C and a relative humidity of 70±5%. The experiment was conducted with 6 replications for each concentration in addition to the control treatment. The growth and development of larvae was followed up on a daily until they reached the adult insect stage, as the number of larvae, pupae and adult dead insects were calculated.

Feeding Method

It was based on the method used by (Wright, 1971) [29] for the purpose of evaluating the biological activity of synthesized silver nanoparticles against the second instar larvae of the house fly, where a sample of 1 gm of artificial food contaminated with Synthesized silver nanoparticles was placed in glass tubes (5 x 2 cm) with the same concentrations used In the dipping method, as for the control treatment, not contaminated artificial food was used. 10 larvae were transferred to glass tubes and then placed in the incubator at a temperature of 27 ± 2 ° C and a relative humidity of 70 ± 5%, the experiment was conducted with 6 replications For each concentration and the same for the control treatment, the growth and development of the larvae was followed up a daily until they reached the adult stage, as the number of

larvae, pupae and adult dead insects were calculated.
The percentage of death in the coefficients was corrected by Abbott's equation (1925) in cases where death appeared in the control. The percentage of emergence inhibition at each concentration was calculated after ten days of treatment using the following equation:

$$100 \times \frac{100-T}{C} = \text{Emergence inhibition\%}$$

Where T = the number of insects from the treatment group.
C = the number of insects emerging from the control group (Darwazeh and Mulla, 1979) [10].

Results and Discussion
Table (1) shows the effect of synthesized silver nanoparticles on the different stages of the house fly by dipping method, as it was found that the percentage of emergence inhibition reached 100% at a concentration of 200 ppm after ten days of treatment, while the concentrations (100, 50) ppm caused the percentage of inhibition reached (98 and 73%) respectively, and the lowest inhibition rate was at the concentration of 25 ppm, which a reach (53%). Most of the dead insects were in the larval stage, then the pupa stage and the adults. The LC50 value (the concentration that kills 50% of insects) reached (24.23) ppm.

Table 1: The biological activity of synthesized silver nanoparticles against house fly larvae, pupae and adult by the dipping method

Concentrations (ppm)	Dead Larva	Dead Pupae	Dead Adult	Total	Percentage of emergence inhibition%
200	45	10	5	60	100
100	36	12	11	59	98
50	33	8	3	44	73
25	27	5	0	32	53

The results in Table (2) showed that synthesized silver nanoparticles had a significant effect on the different stages of the house fly by feeding method, as the concentration of 200 ppm caused the highest percentage of inhibition of adult emergence 100%, and the concentrations (100, 50) ppm showed the inhibition percentage reached (93and 70%) respectively after ten days of treatment, and the value of LC50 was (27.06) ppm.

Table 2: The biological activity of synthesized silver nanoparticles against house fly larvae, pupae and adult by the feeding method

Concentrations (ppm)	Dead Larva	Dead Pupae	Dead Adult	Total	Percentage of emergence inhibition%
200	**42**	**12**	**6**	**60**	100
100	40	11	5	56	93
50	30	9	3	42	70
25	26	3	0	29	48

The results appear that the different concentrations of synthesized AgNPs used in the current study (200, 100, 50,25) ppm caused an effect on the natural development of the growth of the different stages of the house fly, These effects led to the death of the larvae, pupae, and adult, but in different proportions increased with the increase in concentrations. They also caused in appearance of deformations in pupae and adult and a decrease in the percentage of appearance of natural adults in both methods of treatment (dipping and feeding). The results also showed that the effect of synthesized AgNPs by the dipping method was higher than the feeding method and for all stages of the house fly, as all concentrations had an effect on the different stages of the flies.
The larvae that could transform into pupae and pupae that were able to transform into adults were mostly deformed and in varying degrees and numbers (Table 4,3), which resulted in a large part of the pupae being small in size and light in color (Figure 4, Case a) and the emergence of an intermediate stage between the larva and the pupae. (Figure 4 case b), and it was also noted that most of the adult that started to emergence were attached to the capsule (Figure 4, case c),and appeared deformed adult unable to fly (Figure 4, case d), or the emergence of the head and thorax region from the capsule, or the emergence of the head region only from the capsule (Figure 4, case e, and f), and a large part of the dead adult appeared, but it is smaller in size than the normal adult (Figure 4, Case g).

Table 3: Morphogenetic abnormalities of house fly instars resulting from dipping of second instar larvae with different concentrations of synthesized silver nanoparticles

Concentrations (ppm)	a	b	c	d	e	f	g
200	5	3	2	1	0	0	2
100	4	7	5	3	2	0	1
50	3	5	1	1	1	0	0
25	3	2	0	0	0	0	0

Table 4: Morphogenetic abnormalities of house fly stages resulting from adding different concentrations of synthesized silver nanoparticles to the food of second instar larvae

Concentrations (ppm)	a	b	c	d	e	f	g
200	7	5	1	1	0	1	3
100	5	6	1	2	0	0	2
50	6	3	1	1	1	0	0
25	0	3	0	0	0	0	0

Fig 4: The different morphogenetic abnormalities of the house fly stages resulting from the treatment of the second larval instar with different concentrations of silver nanoparticles by dipping and feeding methods **(a)-** Small, light-colored pupa **(b)-** Intermediate stage between the larva and the pupa **(c)-** Adult attached to the capsule **(d)-** Deformed adult unable to fly **(e)-** Emergence only the head and thorax region from the capsule **(f)-**Emergence only the head region from the capsule and **(g)-** complete, small, dead adult.

The deadly effect of silver nanoparticles in the stages of the house fly is due to its small size and the speed of penetration into the body wall in the case of dipping, which affects the physiological processes in the body of the insect during its

development and this leads to a defect in the process of growth and development and the appearance of morphological deformations in all stages of the insect. These agreement with the mentioned by (Al-Qurashi *et al*., 2015) [5] when *Culex pipiense* mosquito larvae were treated with silver nanoparticles prepared from *Thevetia neriifolia* extract appearance of morphological abnormalities in the different stages of the insect.

Abinaya *et al*. (2018) [3]; Banumathi *et al*. (2017) [6] also found the application of ZnONPs nanoparticles led to several morphological, histological abnormalities and their accumulation in the thorax and abdomen of the insect. While Gul *et al*. (2016) [12] found that AgNPs synthesized from melon aqueous extract showed high mortality against housefly adults.

The effect of silver nanoparticles on housefly larvae by feeding method, it caused damage to the cells and tissues of the food channel and the appearance of cellular vacuoles, and this is reflected in the levels of carbohydrates and proteins in the insect's body, and this is what he observed by (Karthikeyan *et al*., 2014) [16] when exposing the fourth larval instar of *Culex quinquetasciatus* with silver nanoparticles. Foldbjerg *et al*. (2015) [11]; Sultana *et al*. (2018) [27] noticed that toxicity of nanoparticles may be due to partial lysis of the midgut epithelial cells; vesicles and damaged membranes at the apical side of epithelial cells. Earlier authors reported that the larvicidal effect of synthesized silver nanoparticles of *Mimosa pudica* showed that the highest mortality was found against the larvae of *Anopheles subpictus* and against the larvae of *Culex quinquefasciatus* (Marimuthu *et al*. 2011) [19]. It can be said that the use of synthesized silver nanoparticles with concentrations of 200 and 100 ppm may contribute to reducing the damage of the house fly and also to overcoming the problems related to the use of chemical pesticides in the control.

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