Studying the Effect of some Nanoparticles on the Percentage of Mortality of Larval Stages of Mosquitoes Culex Pipiens L.

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Abstract

The effect of different concentrations of silver nanomaterials and nano-titanium dioxide on the percentages of larval stages of Culex pipiens mosquitoes was studied. The results indicated that there was a direct relationship between each of the concentrations and exposure time on the one hand, and the mortality rates of larval instars on the other hand. Whereas silver nanoparticles outperformed titanium dioxide in achieving the highest rates of killing rates reaching (84.99, 74.16, %) respectively for both treatments at Concentration (2000) ppm after exposure period (48) hours. We also note an increase in the rates of killing rates after an exposure period of 48 hours (56.15 ,49.82) % compared to the rates of killing after an exposure period (24) hours, which amounted to 37.16%, 31.49) respectively for both treatments. Killing and the age of the larva, as the killing rates decrease with the increase in the age of the larva, so it was the highest killing rate in the first larval stage, reaching (50.33%) , (55.66%) respectively for both treatments.

1. Introduction

Mosquitoes are considered harmful insects to humans and animals and compete with house flies in terms of which of them poses the greatest danger to humans. Therefore, they are one of the important insects from a medical and veterinary point of view, as it constitutes a medium for transmitting many pathogens between organisms, as it is a mechanical vector for pathogens such as protozoa, viruses and nematodes. Which causes diseases to humans and animals, as well as being a source of anxiety and inconvenience, as it stings and sucks blood and leaves a profound effect as a result of injecting saliva, which contains highly toxic substances and substances against blood clotting Abul-Hab [1] . The mosquito genus *Culex pipiens* is spread all over Iraq. It follows the Culicidae family, and it is one of the important families of the Diptera order; the genus *Culex* has a global spread and is an important vector for many pathogens of humans and animals whose presence is associated with humans

And the animal, as it spreads in cities, suburbs and countryside, and it is one of the vital vectors of the causes Diseases such as malaria, the carrier of female mosquitoes of the genus Anopheles [2], filariasis (elephant disease), the carrier of female *Culex* mosquitoes, yellow fever, dengue fever

The carrier of them is a female mosquito, *Aedes aegypti*, who transmits meningitis or meningitis

Female mosquitoes of the genus *Culex* and Aedes in humans and Anopheles in horses

Balkhy et al. [3] Recent studies have shown that the increased use of chemically manufactured pesticides in mosquito control has exacerbated the danger signs represented by the physiological resistance (immunity) of mosquitoes against these pesticides, in addition to their high cost and being toxic to non-target organisms.

[4], as well as their residual effect for a long time and their impact on the genetic systems of living organisms and their pollution of the environment [5] Therefore, it is no longer acceptable to use chemical insecticides, even if they achieve effective results in insect control.

Kabaru et al. [6] which prompted researchers to search for new alternatives to pest control, including the use of nanotechnology as it does not pose negative effects in the environment and has less toxicity on milk [7] So this study aimed to use methods An alternative to chemical pesticides against *Culex* mosquitoes, using silver nanoparticles and nano-titanium dioxide, where their effect on mosquito larvae destruction was studied.

Culex pipiens and that the purpose of choosing the larvae is that the larvae are the most targeted in the field of insect control because it is easy to control them in their aquatic environment compared to controlling adults [8, 9].

2. Materials and methods

Preparing the permanent farm for Culex pipiens mosquitoes

Egg boats were collected from the marshes of Maysan Governorate by means of a long-arm strainer and placed in glass bottles with a perforated lid and transferred to the animal house laboratory in the Department of Life Sciences, College of Science, Dhi Qar University, and placed in plastic basins containing two liters of tap water (free of chlorine) , Adults were fed on a rat diet at a rate of (2) g per basin, as the containers were covered with tulle, and the pupae were transferred using a wide-mouthed dropper to plastic cans inside a wooden cage covered with tulle, and in the middle of it a hose of the same cloth so that we could extend the hand inside the cage and replace water from time to time to prevent rotting and after emergence into adults , they are fed on a sugar solution placed in dishes covered with medical cotton [10] and according to the method used by Mohsen and female mosquitoes were fed three days after emergence on the blood of a featherless pigeon was removed from the chest area and its wings were tied from

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the back, as well as the legs. In the morning we notice the fullness of the abdomens of the females as a result of their feeding. Then small pots filled with water were placed inside the cage to be a place to receive eggs, then egg boats were transported It was moved by a small brush to new containers of water containing the food of the larvae and was followed up until the emergence of adults
The water is changed after four days to prevent rotting and preserve the vitality of the larvae
Al-Sharook et al. [11] Then samples were taken from adults for the purpose of diagnosis and according to the taxonomic characteristics mentionedby Abul-Hab [12] and and sent to the College of Science / University of Basra and diagnosed by Prof. Dr. Kazem Saleh / Department of Biology confirmed her diagnosis as *Culex pipiens* of the family Culicidae.

Preparation of nanoparticle concentrations

Four concentrations of ppm. (500, 1000, 1500, 2000) were prepared from silver nanoparticles and nano-titanium dioxide by dissolving (50, 100, 150, 200) mg respectively of each substance
In (2) ml of solvent (Hexane3: Ethyl acetate7) and then complete the volume to (100) ml of deionized distilled water, either the control treatment was using (2) ml of (Hexane3: Ethyl acetate7) and fill the volume to (100) ml with distilled water.

Effect of nanoparticles on the mortality percentages of larval stages of mosquitoes *Cx.pipiens*

The larvae of the first larval stage were obtained by isolating them from the newly laid egg boats and transferring them to (100) ml glass bottles, each containing (50 ml) of each concentration of the previously prepared concentrations with an amount of (500, 1000, 1500, 2000) ppm of silver nanoparticles and nano-titanium dioxide in an amount of (10) larvae per vial, and (0.05) gm of mice feed was added to each vial. As for the control treatment, (2) ml of (Hexane3: Ethyl acetate7) was used, and the volume was completed to (100) ml with water. Distilled and the same steps mentioned above were followed in the treatment of the second, third and fourth larval instars, where sufficient numbers of larvae of the previous instar were isolated and monitored until they reached the stage to be treated with the concentrations mentioned above. One hour of treatment with each concentration, and the percentages of mortality for the four larval instars were corrected according to the Abbott [13] shown below.

% mortality in treatment - % mortality in control
% corrected mortality =.
100 - % mortality in control

Statistical analysis

The mortality percentages are corrected according to the Abbott [13] and then converted to angular values
And then the results were analyzed statistically using the statistical program Spss
According to the Complete Randomized Design (C.R.D), the least significant difference (L.S.D) was used to compare the statistical differences and the interactions between rates and under a significant level (0.05) [14].

3. Results and Discussion

Table (1) shows the effect of different concentrations of silver nanoparticles on the rates of mortality percentages of the different larval stages of *C.pipiens* mosquitoes after 24 and (48 hours), as we note the difference in the rates of larval mortality according to the concentration and duration of exposure to nanoparticles on the one hand, and according to the different larval stage treated on the one hand. The results showed that the highest rate of mortality percentages for the different larval instars occurred when treated with the concentration (2000) ppm of the above-mentioned substance, which amounted to (71.25%), while the lowest percentage of mortality was (45.41) at the lowest used concentration (500). ppm compared with (0%) in the control treatment The results of the statistical analysis showed that there were clear significant differences in the effect compared with the control treatment, where all concentrations were highly superior to the control treatment, and the concentration (2000) ppm outperformed all other concentrations in causing the death rates for all larval stages.
The results also showed that the larval mortality rate was (37.16%) after (24) hours of exposure to the extract, while the mortality rate was (56.15%) after (48) hours, and this confirms what was shown by the results of the statistical analysis of the presence of significant differences between the period 24) and (48 hours), where the rates of larval mortality after (48) hours exceeded the rates of mortality after (24) hours of treatment.
We also note from the same table that the rates of mortality have differed according to the different larval stages. The results showed that the highest rate of mortality occurred in the first larval stage, reaching (55.66%), while the lowest rate of mortality was (37.99%) in the fourth larval stage, and this confirms what the results of the statistical analysis showed that there were significant differences between the larval instars, where the rates of mortality rates for the first larval instar were superior to all other larval instars.
The results of the binary statistical interactions between the concentrations and the time period of exposure showed that the highest rate of killing occurred at the concentration (2000) ppm It reached (84.99%) after (48) hours, where it outperformed all other concentrations after 24) and 48 hours. We note from the statistical overlap between the larval stages and the time period of exposure that the highest rate of mortality occurred in the first larval stage after 48 hours, reaching (65.33%), which led to its superiority over all other instars after 24) and (48 hours). Triple interaction between concentration, instar and time period, The results of the statistical analysis showed that there were statistically significant differences between the concentrations and between the different instars after 24) and 48 hours, where the concentration exceeded (2000 ppm) at the first larval instar and with an average mortality rate of (96.66%) after (48) hours. on all concentrations and other larval stages at 24 and 48 hours.

Table No. (1) The effect of different concentrations of silver nanoparticles on the mortality rates of the larval stages of C.pipiens mosquitoes.											
Conc. Rate in exposure time		Conc. Rate	4th instar		3rd instar		2nd instar		1st instar		instars Conc. ppm
48 h	24 h		48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	control
54.99	35.83	45.41	46.66	30.00	50.00	33.33	56.66	36.66	66.66	43.33	500
64.99	43.33	54.16	50.00	33.33	63.33	40.00	70.00	46.66	76.66	53.33	1000
75.82	49.16	62.50	66.66	36.66	73.33	43.33	76.66	53.33	86.66	63.33	1500
84.99	57.49	71.25	73.33	43.33	80.00	53.33	90.00	63.33	96.66	70.00	2000
		46.66	47.33	28.66	53.33	34.00	58.66	40.00	65.33	46.00	Rate of instar in time
56.15	37.16	Rate of time	37.99		43.66		49.33		55.66		Instar rate
L.S.D. Time =5.29 L.S.D. Instar × time= 8.49 L.S.D. conc. × time =5.90 L.S.D. conc. × instar ×time =9.70 At instar L.S.D. P≤0.05 8.99 =											

Table No. (2) Indicates the effect of different concentrations of nano-titanium dioxide on the percentage mortality of *C.pipiens* mosquito larvae. The results showed that the highest percentage of mortality for the four larval instars was (62.08%) at the highest concentration used (2000) ppm. While the lowest rate of larval mortality (40.83%) at the lowest concentration (500) ppm compared with (0%) in the control.

The results of the statistical analysis showed that there were statistically significant differences between the concentrations and the control, where all concentrations were highly superior to the control, and the concentration (2000) ppm was significantly superior to all other concentrations. The results of the statistical analysis showed that there were statistically significant differences between the rates of larval mortality rates after 24) and 48 hours, as the rates of larval mortality exceeded during the exposure period.

48 hours, at a rate of 49.82%, over the mortality rate after (24) hours, which amounted to 31.49%. We note from the results that the rates of mortality have differed according to the different larval stages. The results showed that the highest rate of mortality occurred in the first larval stage, where it reached (50.33%), while the lowest rate of mortality was 33.33%) at the fourth larval stage, and this confirms the results showed by Statistical analysis that there were clear significant differences between the larval stages, where the rates of mortality rates of the first, second and third larval instars were superior to the fourth larval stage in the incidence of mortality percentages

The results of the statistical interaction between the larval instars and time showed that the average mortality rate for the first larval instar after (48) hours was (62.00%) over all other instars after 24 and (48 hours), while the lowest rate of larval kill was (26.00%) in the instar. Fourth larvae after (24) hours.

The binary statistical interactions between concentration and time also showed that the highest mortality rate occurred at the highest concentration (2000) ppm, which reached (74.16%) after (48 hours and outperformed all other concentrations after 24) and (48 hours), while the lowest mortality rate was (29.99%).) at the lowest concentration (500 ppm) after

(24) Hours. Also, the results of the triple interaction between concentration, instar and time showed that there were statistically significant differences between

Concentrations and between the different instars after 24) and 48 hours, where the concentration exceeded

(2000) ppm at the first larval instar with a mortality rate of (90.00%) after (48) hours on all concentrations and other larval stages after 24) and 48 hours.

Table No. (2) Effect of different concentrations of nano-titanium dioxide on the rates of mortality percentages of the larval stages of C.pipiens mosquitoes.											
Conc. Rate in exposure time 24 h		Conc. Rate 48 h	4th instar		3rd instar		2nd instar		1st instar		instars Conc. ppm
48 h	24 h		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	control

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51.66	29.99	40.83	43.33	23.33	46.66	26.66	53.33	33.33	63.33	36.66	500
55.83	34.16	45.00	46.66	26.66	53.33	30.00	50.00	33.33	73.33	46.66	1000
67.49	43.33	55.41	53.33	36.66	60.00	40.00	73.33	43.33	83.33	53.33	1500
74.16	49.99	62.08	60.00	43.33	66.66	46.66	80.00	53.33	90.00	56.66	2000
		40.66	40.66	26.00	45.33	28.66	51.33	32.66	62.00	38.66	Rate of instar in time
49.82	31.49	Time rate	33.33		36.99		41.99		50.33		Instar rate
L.S.D. instar = 8.90 conc L.S.D. 8.69 = .L.S.D time = 4.49 L.S.D. instar ×time = 7.18 L.S.D. conc. ×time =4.80 L.S.D. instar ×Time× conc. =7.62											

In general, the current results indicate that silver nanoparticles are more effective compared to nanoparticles of titanium dioxide in the percentage killing of the four larval stages of *C.pipiens* mosquitoes. What happened in feeding, and this was confirmed when he treated the stored date moth larvae *Cadra cautella* with silver nanoparticles, as its efficiency rate as a feeding inhibitor was (0.04%,) which differed significantly from the rest of the treatments used by the aforementioned researcher, which indicates its efficiency in combating the insect and this is consistent with

Also with what was Kitherian [15], as he showed that silver nanoparticles have an anti-nutrition effect

Significantly against the larva of the cotton worm *armigera Helicoverpa*, this usually affects

The particles negatively affect the digestive enzymes present in the insect's stomach, the researchers said insect body, as it distorts and changes the composition of digestive enzymes [16, 17].

It is also clear from the above tables that the rates of mortality rates after (48) hours are higher than the rates of mortality rates after (24) hours. Exposing (48) hours, or that the toxic substances in the treatment need more time for decomposition in the gut of the insect, leading to its effectiveness in the body of the insect. Silver nanoparticles have the ability to interfere with the vital activities of the insect due to its ability to easily penetrate the plasma membrane and destroy molecules vital enzymes such as enzymes as well as loss of plasma membrane function and coagulation of proteins, and thus lead to cell death [18].

Also, exposure of the insect to silver nanoparticles reduces the mobility of the larvae and makes

The body wall is stiff and swelling appears in the body, which becomes fragile and turns dark brown [19].

The results also indicate a difference in the sensitivity of the larval stages in the treatments

The first larval instar is the most sensitive and sensitive compared to the rest of the three larval stages

The other, as it reached the highest death rate for the first larval stage at concentration 2000 (p.p.m)

(96.66%) by treatment with silver nanoparticles after (48) hours, which outperformed all other concentrations and larval stages in all treatments, and this result is consistent with what was confirmed by

And, as the anti-insect effects of the treatment are directly proportional to the increase in the concentration used. The reason may be due to the different sensitivity of the instars to the toxic substances present in these treatments, or because the immune system of the larval stages (first and second) is incomplete and the body tissues are almost very thin, which leads to their penetration easily by the treatments or the effect of these substances on the ability to transfer food to the treated larvae. Its effect is most severe in the early larval stages

The reason for this is that it is more efficient in converting food, which shows its toxic effect, and in this regard it was mentioned by

that the high degree of resistance of larvae to pesticides with age is due to the increase in the thickness of the cuticle, which leads to poor penetration of the treatment during it, or the reason for the decrease in the effect of treatment with the age of the larvae is that the mechanism for eliminating the effectiveness of the treated substance is Weak at early ages, and this mechanism is complemented by the progress of growth, which allows the advanced larval stages to resist the impact of materials more than the early stages. Also mentioned that the early stages are often more sensitive to the disease than the advanced stages, which show a type of immunity called puberty immunity.

The last stages are usually a structural or physiological abnormality caused by the injury of the early stages 0

The current study indicates that nanoparticles will be suitable for developing a biological control method for mosquito larvae

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