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EVALUATION OF LOCAL *BEAUVERIA BASSANIA* ISOLATION AS A NANO-BIOCIDE (Bb-AgNPs) AND NON-NANO-BIOCIDE AGAINST THE FOURTH INSTAR LARVAE OF *CULEX QUINQUEFASCIATUS* PUT IN SWAMP WATER AND UNDER LABORATORY CONDITIONS

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Abstract

This experiment was carried out at the Plant Protection Laboratories, College of Agriculture, University of Baghdad for assessing the effect of local isolating *Beauveria bassania* as a biolarvicide (Bb-AgNPs) on the fourth instar larvae of *Culex quinquefasciatus* mosquitoesat different three times. *Beauveriabassania* wasisolated from the dead larvae of mosquitoes *C. quinquefasciatus*. *Beauveria bassania* used for convert $AgNo_3$ into nanoparticles in a biological way. Three concentrations of Bb-AgNPs (1000, 2000 and 3000 ppm) were present. They gave results of death in fourth stage larvae of *C. quinquefasciatu* (30.5, 37.6 and 47.8%), respectively after (24, 48, 27 hours). In addition to testing the effect three concentrations of *Beauveria bassania* (3×10^4, 3×10^5 and 3×10^6) spore /ml as a natural biolarvicide (non-nanoparticles) on the same larvae. The result of mortality was 19.1, 14.9 and 11.8%), respectively.

Key words : *Culex quinquefasciatus*, *Beauveria bassania*, nanotechnology.

Introduction

Mosquitoes are one of the most important medical insects for their ability to transfer many diseases to humans and animals. It is often considered necessary to biotransfer because to the pathogen can not continue to living or reach adulthood without mosquitoes. Anopheles, Aedes and Culex are most important species that transmit diseases (Briant, 2011). Mosquitoes are an annoying insect that negatively affects the lives of people in terms of stings and humming sound that cause insomnia and itching and possibly skin infections may occur skin bleeding accompanied by the wound and show symptoms of allergies because of the saliva of mosquitoes of chemical components cause redness and itching. *Culex quinquefasciatus* is the main carrier of nematodes that cause the disease of Filaria, it cases to infective about 120 million people annually in more than 83 countries of the world (WHO, 2012). After the increased risk of chemical pesticides that fight mosquitoes on the environment and its components live and non-living, taking the human thinking of the use of the principle of biological control to reduce the problems of chemical pesticides, where the fungi used in the field of biological control. *Beauveria bassiana* is one of the first fungus used in the control of the effectiveness of insect pests, it causes disease Museardine White, which infects many insect example Diptera, which includes families of mosquitoes. After the first spark of the idea of nanotechnology in 1959, when famous physicist Richard Feynman spoke to the American Physical Society in his famous lecture, "There's a lot of space at the bottom," he said that the material at the Nano volume showed new properties. The world took a step forward in this field after that date where, the nanoparticles used into industrial, medical, agricultural, physiological, biological and other applications (Adhikari and Ghoshand, 2013). In this study, we seek to convert silver nitrate into nanoparticles by fungal toxins and use them to control on fourth instar larvae of *Culex quinquefasciatus*.

Materials and Methods

Beauveria bassiana was obtained from dead larvae

B.bassiana **$AgNo_3$** **B.b-AgNPs**

Shape 1 : Stapes of converting B.bAgNPs.

for *Culex quinquefasciatus*, which collected from different aquatic environments. After applicated of (Kokhs, hypothesis) it were isolated, purified and growth on Potato Dextrose Agar (PDA). $AgNo_3$ was converted into a nanoparticle by *Beauveria bassiana* where took 5 mm diameter tablets from the fungal colonies that grew on the food medium (PDA). The fungal colonies added to the liquid medium Potato Sucrose (PS) in the glass containers of capacity of 1000 ml in sterile medium (PS) in steam pressure at 121°C and pressure of 15 pounds/ inch for 20 minutes added (Omoxicillin) (2 g/L) and Tetracyclin. In order to prevent the growth of undesirable microorganisms, move the flasks slightly and then incubate in the cooled incubator at 25±2°C for 21 days with daily shaking for flasks. The biomass harvested after 21 days of incubation at the above mentioned conditions by filtration using the Bochner funnel and by the filtration paper (Watman No. 1). The biomass of the fungus was washed by distilled water three times, then washed with non ionic water three times also for the purpose of disposing of the media residue and took 20 g of biomass and transferred to 750 ml glass flask containing 500 ml of non ionic water, put the beaker in an electric incubator at 25±2°Cfor 120 hours. For obtaining on the solution of fungal biomass filtered and filtered by filtration paper Watman No. 1 (ALShammari, 2015). The biomass extract of the fungus put in a glass flask and left in the incubator at a temperature of 25±2°C then took 100 ml of it and add to 1000 ml glass flask containing 900ml of silver nitrate solution ($AgNO_3$). The solution transferred to the dark electric incubator at 25±2°C for 120 hours with the daily shake. The solution was monitored during this period to observe the chromatic changes that indicate the occurrence of the biodegradation process and the composition of nanoparticles (Bb-AgNPs) (Shape 1). Samples sent to the University of Technology for testing UV, scanning electron microscopy (SEM) and FTIR to make sure it converted to nanoscale (Banu and Balasubramanian, 2014). The steps of interaction can be expressed as follows :

Silver nitrate solution + (Enzyme + protein) / reducing agents = Silver nanoparticles

Three concentrationes of the fungal nanoparticles (Bb-AgNPs) (1000, 2000 and 3000ppm) were tested on 20 larvae of the fourth instar for mosquitoes *C. quinquefasciatus*. They were put in a volume receptacles 250 ml containing 150 ml of swamp water with 1.5 g of rat larvae. In order to feed the larvae, the experiment was performed at three replicates per concentration with treatment of control and the results were recorded after 24, 48 and 72 hours of treatment (Ishii and Ohba, 1993). The percentage of larvae was corrected according to equation of Schneider and Abbott (Abbott, 1925). In other side, *Beauveria bassiana* used to prepare three non-nano-fungal concentrations (3×10^4, 3×10^5 and 3×10^6 spore/ml) by the Haemocytomete (Gottel and Ingilis, 1997). They tested on fourth instar larvae of *C. quinquefasciatus* mosquitoes and in the same steps as previous experiment. The tests are designed according to CRD design.

Results and Discussion

Larvicidal effect of Bb-AgNPs on fourth instar for *C. quinquefasciatus* larvae

Table 1 indicate the effect of concentration factor in the percentage of mortality where reached rate of mortality 47.2% by the concentration 3000 ppm its was highest rate of mortality in fourth instar larvae of *C. quinquefasciatus* with a cumulative of death 90.0% while mortality rate reached 30.5% by the concentration 1000 ppm its was lowest rate of mortality and the cumulative loss rate was 68.3% with significant differences in the proportions between the concentrations. Time periods were infiuence on the rate of mortality

Table 1 : Larvicidal effect of Bb-AgNPs on the Forth instar for *C. quinquefasciatus* larvae.

Cumulative percentage of mortality (%)	Percentage of mortality (%)	Mortality of *C. quinquefasciatus* (%)			Bb-AgNPs (PPM)	No.
		After 72 h	After48 h	After 24 h		
68.3	30.5	48.9	25.9	16.7	1000	1
78.3	37.6	59.7	33.2	20.0	2000	2
90.0	47.8	79.2	42.6	21.7	3000	3
		62.6	33.9	19.4	Rate	
Intefere		Concentrations		Times		
1.08		6.40		6.40	L.S.D.	

PH = 8.51 Ec = 3.4 Turb. = 107

Table 2 : Larvicidal effect of *B.bassiana* against the Forth instar for *C. quinquefasciatus* larvae.

Cumulative percentage of mortality (%)	Percentage of mortality (%)	Mortality of *C. quinquefasciatus* (%)			*B. bassiana* Spore ml.	No.
		After 72 h	After48 h	After 24 h		
50.0	19.1	40.1	10.7	6.6	$10^4 \times 3$	1
40.0	14.6	33.5	7.0	3.3	$10^5 \times 3$	2
33.3	11.8	28.6	5.1	1.6	$10^6 \times 3$	3
		34.1	7.6	3.8	Rate	
Intefere		Concentrations		Times		
6.00		3.46		3.46	L.S.D.	

PH = 8.51 Ec = 3.4 Turb. = 107

where reached highest rate of mortality (62.6%) after 72 hours of experiment while the mortality rate reached 19.4% after 24 hours of the experiment, the statistical analysis showed significant differences between the treatment periods. The concentration 3000 ppm recorded highest percentage of mortality its reached 79.2% after 72 hours, while the concentration of 1000 ppm was the lowest rate of mortality 16.7% after 24 hours. Significant differences were observed between the concentrations and the time periods. In study of Salunkhe *et al*. (2011) obtained the AgNPs by *Cochliobolus lunatus*, it tested on the fourth instar larvae of *A. aegypt* and *C. quinquefasciatus* by three concentrations (1,250, 2500, 5000 ppm), they gave results of 74-81% during 96 hours. $AgNO_3$ which converted to nanoparticles by seaweed gave a clear effect against *C. quinquefasciatus* larvae during one hour of experimentation and with concentrations ranging from 2000 to 10,000 ppm.

Larvicidal effect of *B. bassiana* against the Forth instar for *C. quinquefasciatus* larvae

Table 2 showed that the concentrated solution (3×10^4 spore / ml) gave the highest mortality rate in the fourth instar of *C. quinquefasciatus* mosquitoes, which were placed in quagmire where it reached 19.1% with a cumulative kill rate of 50.0%, while the concentration (3×10^6 spore / ml) gave mortality rate 11.8% where, it was the lowest of mortality rate with significant differences between the concentrations.

The same table showed that the time difference infiuence on the increase mortality rates where reached highest rate of mortality 34.1% after 72 hours of experiment while the mortality rate reached 3.8% after 24 hours of the experiment. The statistical analysis showed significant differences between the treatment periods. The concentration (3×10^4 spore/mL) recorded highest percentage of mortality its reached 40.1% after 72 hours, while the concentration (3×10^6 spore/mL) was the lowest rate of mortality 1.6% after 24 hours after treatment. Significant differences were observed between the concentrations and the time periods. The organic and inorganic particles and the suspended materials in the water of the swamp are important in determining the efficiency of the fungal fungicide in targeting the larvae and its effect on the kill of *Culex quinquefasciatus* mosquitoes where conidia congregates fungus or toxic fungal molecules around organic and inorganic suspended as a result of not reaching the target larvae (Al-Hamdanee, 2014). Note that the increase in the concentration of the biocide was evident in the effect

on the rate of homicide (Ali, 2007) showed a positive relationship between concentrations of pathogen and the mortality rate of larvae *Culex* spp. In comparison with the results of tables 1 and 2 observed that Bb-AgNPs have achieved high mortality rates.

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