INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

THE EFFECT OF SILVER NANOPARTICLES ON SECOND LARVAL INSTAR OF *TROGODERMA GRANARIUM* EVERTS (INSECTA: COLEOPTERA: DERMESTIDAE)

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ABSTRACT
This study was performed to characterize the impact of silver nanoparticles AgNP biosynthesis by *Metarhizium anisopliae* Sorokin with concentrations 250, 500, 1000,2000,3000,4000 ppm in second larval instar of *Trogoderma granarium*. The results of this study demonstrated the following: the most killing rate of treated hatchlings was 96.6% at concentration 4000 ppm; observation of demelanization adults from the larvae that were treated with AgNP, the AgNP impact on the quantity of eggs that were generated from adults and hatching percentages. There was an emergence of abnormal adults with increasing concentrations.

KEYWORDS: silver nanoparticles, *Trogoderma granarium* larvae, biocontrol.

INTRODUCTION

The khapra insect, *T. granarium* Everts, is one of the world's most dangerous pests. It has been depicted as one of the 100 worst species around the world (Lowe *et al*. 2000). These pests are hard to control because of the insect's capacity to live for a long time without food and it can depend on food with low humidity content. These insects crawlies tend to slither into minor breaks and hole and stay there for long time, making them moderately tolerant to many surface insecticides and fumigants (Szito, 2006). The hatching larvae about is 1.6 to 1.8 mm long, the greater part of this length comprises of a tail made up of hairs on the last abdominal section. Larvae are consistently yellowish white, with the exception of head and body hairs which are brown. As the larvae increase in size, their body changes in color to a reddish brown or golden brown, more body hairs create, and the tail turns out to be relatively shorter. Mature larvae are around 6 mm long and 1.5 mm wide (Buss and Fasulo, 2006). The specialists are searching for procedure to control the insects without dirtying the earth. Along these lines, there was a move to synthesis process, which happens to be generally of biological nature relying on the Nano biotechnology. Lately, the green approach of nanoparticles combination by biological elements has taken an increasing interest over different other physic-substance techniques. Scientists state that nanotechnology will change pest management sooner rather than later and scientists have done much research on the dangerous impact of nanoparticles on pests (Wan *et al*., 2005). The favorite choice of the nanotechnologist is fungi because they offer wide advantages over plants, bacteria, actinomycetes yeast, and other physic-synthetic properties. They require simple supplement, and they are easy to be prepared, have a high capacity to bind, in addition to intracellular metal take-up capacities. One of these fungi is the entomopathogenic organism *M. anisopliae* that can infect many species from more than 50 families of insects (Aker and Abacı, 2016). The most important points are that they have a high insect specificity compared with chemical pesticides, low toxicity to different living beings and low ecological effect (Vahabi *et al.*, 2011).

MATERIALS & METHODS

Insect culture

The culture consists of 5g of yeast and 500g grain of wheat *Triticum aestivum* which is cleaned of impurities, and were put in a frozen medium for 72 hours with a degree of -20^{o}C to be sure that they are free from any other pests.

The male and female were transferred to 1-liter Plastic jars (10 cm in diameters) for a rearing to four generations before being used in the experiment. They were covered with Aorquenza cloth held in the place by a rubber band, which did not only allow proper ventilation but also precluded the entry or exit of insects. The culture was incubated at 27±2 °C and 70±10% relative humidity (Egwurube *et al*., 2010).

Fungal isolate

Isolates of *M. anisopliae* were obtained and diagnosed by Dr. Hussam Elldeen A. Mohammad/ College of Agriculture / Baghdad University. Conidial suspensions were prepared by growing fungi on Potato Dextrose Agar (PDA) in Petri dishes with 10cm diameter and were incubated under specific conditions at 27°C for two weeks for complete sporulation. Fungal conidia were collected by

scraping away conidial layer using sterilized scalpel. A mixture of conidia was harvested by adding sterile distilled water to the Petri dishes and agitating with sterilized glass rod. (Lord, 2007).

Synthesis of silver nanoparticles AgNPs

For biosynthesis of AgNPs, 100 ml of Aqueous extract of fungi biomass was mixed with 900 ml AgNO3 solution within 1 liter glass jar. The prepared solutions were incubated at 25±2 °C for 120 h. to avoid any photochemical reactions during the experiment. All solutions were kept in dark (Devi *et al*., 2013).

The Second larval instar treatment

10 larvae were used for each concentration and replicate 4 times. The larvae second instar was obtained by isolating the eggs and follow-up stages of development until reaching the second larval instar. Treated by AgNPs concentrations and placed in sterile petri dishes diameter of 5 cm one larvae per dish that contains Crushed wheat grains and yeast than incubated. The control treatment was prepared by spraying the larvae with three ml of sterile distilled water and the treatment spraying with three ml of AgNPs, and then records the following data:

mortality percentage of the larvae, the average period of larval stage starting from the second instar larval, the mortality percentage of pupa, the average period of pupa stage, the proportions of the emergence of normal and abnormal adults, the average age of adults and the total number of eggs produced by adult emerging from larval treatment .

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

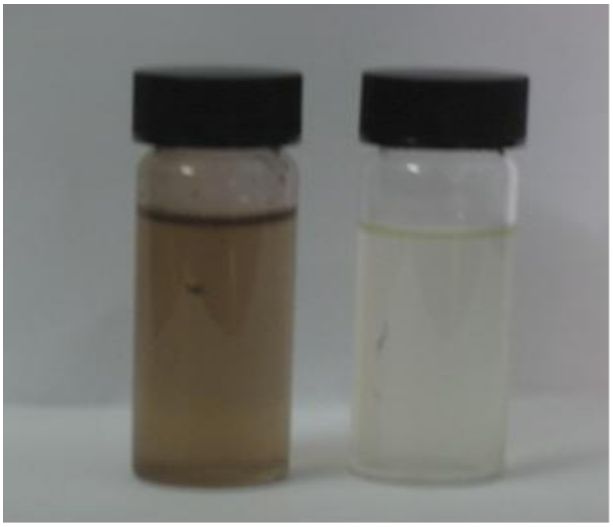
RESULTS

The second larval instar treatment

Table (1) shows that the silver nanoparticles significant effects in the second larvae instar of *T. granarium* reached the highest mortality rates 96.6% at 4000 ppm concentration compared with 250ppm concentrations and control treatment had low mortality rates 60% and 0% respectively. Differences were noticed in the average duration of larval stage for all concentrations 250, 500, 1000, 2000, 3000, 4000 ppm respectively which reached 20,24,21,21.5,22 and 20 days for all the concentrations compared with control treatment for 16 days.

The highest mortality rate of pupa stage at 2000ppm was about 3.35% compared with other treatments. The highest emergence of normal adult was 10% at 1000 ppm and become 30% at 250ppm. There was an emergence of abnormal adults with increasing concentrations. The study shows that the exposure of the second larval instar to AgNPs caused demelanization of adult's cuticle in *T. granarium.* The highest percentage of abnormal adults was 10.05% at 2000 ppm compared to 0% at control treatment. There was a decrease in the average of male age to 4 days at 4000 ppm concentration and average female age was (10,11,6,4,5) days at concentrations of 250,500,1000,2000 and 3000 ppm respectively.

The results clearly show a decrease in eggs production; 2 eggs at 2000ppm concentration compared with control treatment which consisted of significant different 60 eggs. In addition to that there were no eggs production at 3000 and 4000 ppm concentrations. AgNPs was synthesized by using a reduction of aqueous Ag+ with the culture of *M. anisopliae*. It was clearly recognized that AgNPs turned brown in the water solution.



B A

FIGURE 1: tubes containing (A) filtrate biomass of *Metarhizium anisopliae* without $AgNo_3$ (B) filtrate biomass of *Metarhizium anisopliae* with $AgNo_3$ and production AgNPs after 120 h incubation

ISSN 2229 – 6441

TABLE 1: effect of silver nanoparticles on Second larval instar of *Trogoderma granarium* Everts

Treatments		mortality percentage of the larvae%	average duration of larval stage (day)	mortality percentage of pupa %	average duration of pupa stage (day)	proportions emergence of a normal adults %	proportions emergence of abnormal adults %	average age of adults (day)		average of eggs produce	Percentage Of Hatched Eggs %
								male	female		
	250	60	20	0	7.5	30	10	7	10	10	30
concentration	500	73.3	24	0	6	23.3	3.3	7	11	7.5	14.2
ppm	1000	80	21	0	6.5	10	10	5	6	7	14.2
	2000	86.6	21.5	3.35	5	-	10.05	4	4	2	0
	3000	90	22	0	6	-	10	4.5	5	-	-
	4000	96.6	20	0	6	-	3.4	4	-	-	-
Control		0	16	0	4	100	-	6	7	60	96.6
value LSD		*11.57	*4.86	3.35 NS	*2.05	*11.62	*3.79	*2.31	*3.76	*9.06	*10.53

NS: non significant different
*($p<0.05$) significant different

(A) (B)

FIGURE 2: female adult of *Trogoderma granarium*

(A) Showa the demelanization which appears much lighter in body color than control as a result of treatment of the second larval instar with sliver nanoparticles concentration 3000 ppm Magnification power by dissecting microscope 160X
(B) Female adults from control treatment Magnification power by dissecting microscope 160X

DISCUSSION

AgNPs was synthesized by using a reduction of aqueous Ag+ with the culture of *M. anisopliae*. It was clearly recognized that AgNPs turned brown in the water solution because of the surface Plasmon resonances (SPR) reduction and the effect of $AgNO_3$ (Wiley *et al*., 2006). The crude cell filtrate of *M. anisopliae* changed from light yellow to brown in a few hours after the addition of $AgNO_3$ solution, while no color change has been seen in the culture incubate without $AgNO_3$ (Figure 1). Thus, color changing clearly indicated the formation of AgNPs. The color intensity of the filtrate biomass with $AgNO_3$ was increased at a time. Table (1) shows that AgNPs have clearly effects on the second larval instar of *T. granarium*. The mortality rate increased with concentrations increased. This agrees with Rouhani *et al*. (2012) who studied the effect of silver nanoparticles on cowpea seed beetle, *Callosobruchus maculatus* F. were highly effective on larval with 83% mortality. This study reports that the exposure to AgNP from early development causes demelanization of adult's cuticle in *T. granarium* (Figure 2). As a result, most adults appeared to have a lack of melanin pigments due to Intracellular copper (Cu+) transport which happens through transporter proteins found on cell membrane known as bound copper transporter 1 (Ctr1) inside the cell, Cu+ is utilized via copper dependent proteins (*e.g*., tyrosinase). Presence of Ag in the extracellular environment inhibits the process of intracellular entry of Cu due to Ag and Cu competing for the same copper transporters. Copper dependent tyrosinase plays an important role in the conversion of tyrosine to dopa and dopa to dopaquinone, which is then converted to melanin pigments (Armstrong *et al*., 2013). This result agreed with Armstrong *et al*. (2013) who studied the effect of AgNP on insect pigmentation.

CONCLUSION

In summary, this study showed high susceptibility of *T. granarium* second larvae instar to certain concentrations of metallic silver NPs. We have unequivocally demonstrated that *M. anisopliae* convert silver ions into metallic silver NPs in a medium, clearly recognized by turn brown the solution. However, the successful demonstration of biosynthesis of metallic silver NPs motivates us to search for their application as biocontrol.

ACKNOWLEDGMENTS

We thank Dr. Nasr Noori (College of Agriculture, Baghdad University) for Statistical Analysis.

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