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Research Article
Biosynthesized Silver Nanoparticles (AgNPs) by the Two-spotted Spider Mite *Tetranychus urticae* Against the Cotton Leafworm (*Spodoptera littoralis*)

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Abstract

Background and Objectives: *Tetranychus urticae* is a pest but also a resource of bio-nanoparticles which could be used as an effective biocide. The aim of this study was to biosynthesize silver nanoparticles (AgNPs) by *Tetranychus urticae* against *Spodoptera littoralis*. **Materials and Methods:** Biosynthesized silver nanoparticles (AgNPs) which coated by biogenic amines resulted from exploded mite's bodies were able to control the cotton leafworm, *Spodoptera littoralis* under laboratory conditions. **Results:** Bio AgNPs caused mortality with 91.24 and 83.77% in case of treated 2nd and 4th larval instars in comparison with control 10.39 and 7.02%, for the same previous arrangement. While, malformation was appeared clearly in the metamorphosis stage to pupal stage after alive treated larvae. Subsequently, oxidative stress was increased and indicating that reactive oxygen scavengers were reduced drastically in interaction effectively with induced treatments. **Conclusion:** Current research is a novel trend to control pest by another pest which could present bio AgNPs as a biocide and also cause malformation.

Key words: AgNPs, biosynthesized, biocide, oxidative stress, *Spodoptera*, *Tetranychus*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cotton leaf worm, *S. littoralis* is a serious lepidopteran pest. Larvae are able to cause a considerable damage to cotton plants as well as more than 29 hosts from other crops and vegetables[1]. Biological imbalance was caused by intensive use of insecticides in agricultural fields[2] and then new eco-friendly formulations became the target[3-5].

Nanotechnology, a promising field of research opens up in the present decade a wide exhibit of chances and is relied upon to give significant driving forces to specialized advancements in an assortment of modern segments later on. These days, nanotechnology has been grasped in the realm of pesticides, control wide spectrum of pests[6] and can possibly change current agribusiness control. Various gatherings of nanopesticide defeat like insecticides, fungicides, herbicides but with reduced environmental footprint in comparison with conventional pesticides[7,8]. Nano-silver represents as a powerful eco-friendly alternative in pest management and an improved material for crop production. Moreover, addition of AgNPs to pesticides is able to increase toxicity to exposed pests with reduction of unexpected side effects on plants[9]. Besides, AgNPs showed antimicrobial activity against plant pathogens and helpful for plants to absorb specific nutrients from the soil. The toxic effects of NPs can be attributed to the small size and large surface area, thereby increasing chemical reactivity and penetration in the living cells[10-12].

Oxidative stress is a state of redox disequilibrium in which Reactive Oxygen Species (ROS) production overwhelms the antioxidant defense capacity of a cell; thereby, it could lead to adverse biological consequences. The ROS are generated during photo-activation of some chemicals on the particle surface, or as a consequence of the interaction between particles and cellular components[13,14]. The production of bio AgNPs by *Tetranych usurticae* provided a new bio material to control *Spodoptera littoralis* as a pest product vs. pest and it was being the main target of this paper. Consequently, toxicity, occurred malformation and oxidative stress explained the mode of action of bio AgNPs against *Spodoptera littoralis*. This study is conducted to biosynthesize silver nanoparticles (AgNPs) by the two-spotted spider mite (*Tetranychus urticae*) against the cotton leafworm (*Spodoptera littoralis*).

MATERIALS AND METHODS

Maintenance of *Tetranychus urticae* colonies: The two forms, green and red of *Tetranychus urticae* were gathered from normally cowpea (*Vignaung uiculata*) and strawberry (*Fragaria ananassa*) plants, independently, in Dakahlia Governorate, Egypt. At that point, maintenance was done on the castor bean leaf discs under laboratory conditions[15] for a half year before treatments under laboratory conditions. This study was conducted between October, 2017-October, 2019.

Formation of silk balls: At that point but with adopted modification[16], single discs of castor bean plants were put separately in cages (30×30×20 cm) under institutionalized conditions (26°C, 33% RH). About 500 adult females of each morph were collected by fine brush and permitted to develop on each disc. The pinnacle of the wooden stick (5 cm high, 3 mm diameter) and a square piece of graph paper (2×2 mm) was fixed on the stick to scale the surface of the ball. It was watched each hour to recognize the silk ball's arrangement at the development time. The ball was shaped by 500 mites for all time amassed over the wooden stick which buried in 2 cm thickness layer of salt. When the first mites landed on the stick, the number of others moving to the highest point of the stick was tallied over a time of 10 min, two times a day (at 8 and 14 h) until the expulsion of the ball at about day 18. Silk ball formed and then harvested occurred at 14 h. To gather the ball, the wooden stick was painstakingly expelled from the plant and from there on the ball was gathered from the stick end with tweezers.

Biosynthesis of silver nanoparticles (AgNPs) and detection of biogenic coating: Biological silver nanoparticles (AgNPs) were synthesized by reaction with sodium chloride was done for the first time and provided easier trend to do so. About 0.001 mg $AgNO_3$ was spread on the admitted plants just once for by 500 adults of *T. urticae* and the exposure was for 3 h. Then, addition of 1 mg of NaCl as a stabilizer and as a reduction agent was occurred. Released AgNPs were attached with the silk fibers of *T. urticae* away of salt particles even in the outer or inner layers of fiber balls. Characterization of resulted AgNPs was d one by Scanning Electron Microscopy (SEM) and imaged beside images of mite body with released AgNPs as presented in Plate 1 and 2.

Monoamine oxidase (MAO-A) interaction to detect coating with biogenic amines of AgNPs: MAO-A is a Flavin Adenine Dinucleotide (FAD) containing enzyme which is tightly anchored to the mitochondrial outer membrane and responsible for the reaction at Eq. 1:

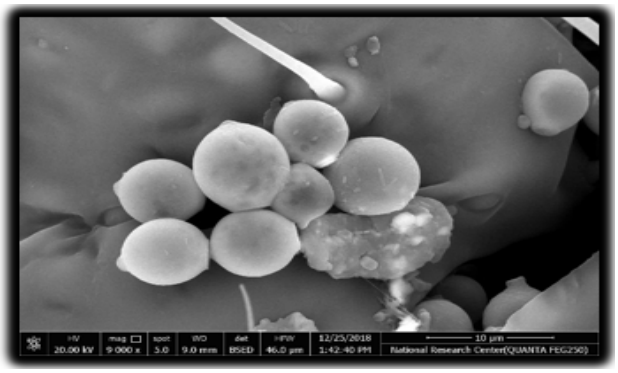

Plate 1: Silver nanoparticles (100 nm) coated by biogenic amines to produce full capsules

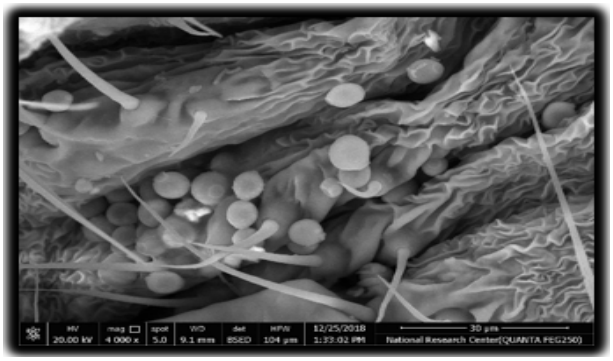

Plate 2: Spherical silver nanoparticles (100 nm) coated by biogenic amines from exploited bodies of *T. urticae*

$$R-\overset{H_2}{C}-NH_2+O_2+H_2O\xrightarrow[\text{oxidase}]{\text{Amine}}R-CHO+NH_2+H_2O_2 \quad (1)$$

Equation 1 represents the reaction of biogenic amines with mono amine oxidase (MAO).

MAO-A potencies were determined in the released homogenates as coatings of AgNPs through each interaction. The rate of the MAO catalyzed oxidation of Kynuramine was measured[17]. Kynuramine is non-fluorescent until undergoing MAO-catalyzed oxidative deamination and subsequent ring closure to yield 4-hydroxyquinoline, a fluorescent metabolite. The concentrations of the MAO-generated 4-hydroxyquinoline in the incubation mixtures was determined by comparing the fluorescence emitted by the samples to that of known amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer and contained kynuramine as substrate, MAO-A (0.0075 mg mL^{-1}) and various concentrations of the test inhibitor (treatment). The reactions were carried out for 20 min at 37°C and were terminated with the addition of 200 µL of NaOH (2 N). After the addition of distilled water (1200 µL) to each reaction, the reactions were centrifuged for 10 min at 16000×g. To determine the concentrations of the MAO generated 4-hydroxyquinoline in the reactions, the fluorescence of the supernatant at an excitation wavelength of 310 nm and an emission wavelength of 400 nm were measured[18]. Mono amine oxidases (MAO) was used to detect the presence of biogenic amines in the coatings of AgNPs produced by green and red morphs of *T. urticae*. With lower MAO activity, the more accumulation of biogenic amines specifically with the presence of AgNPs. The specific activity of MAO was higher in case of both morphs released the AgNPs than control. Affected MAO activity with 2.9 and 1.5 mOD min^{-1} mg^{-1} proteins in comparison with control (5.1 and 3.7 mOD min^{-1} mg^{-1} proteins) in green and red forms of *T. urticae*, respectively.

Maintenance of *Spodoptera littoralis* colony: *Spodoptera littoralis* was cultured on leaves of the castor oil plant (*Ricinus communis* L.) in Plant Protection Research Institute, Agriculture Research Center, Mansoura Branch, Egypt. Larvae were kept at 25±1°C, 70% RH and 16L:8D of photoperiod. The second instar larval stage of the insect was used in the insecticidal bioassay.

Toxicity of biosynthesized AgNPs against larval instars of *Spodoptera littoralis*: Emulsion was composed of collected 0.01 g of AgNPs with addition of required emulsifiers. Then bioassay was done by leaf discs of castor oil plant which were prepared and dipped in the prepared formulation with its concentrations. Three replicates for each treatment with 90 larvae/replicate. Mortality results were taken after 24 h of the exposure.

Morphological changes of treated *Spodoptera littoralis*: Both 2nd and 4th larval instars were treated with bio AgNPs for 24 h and then alive larvae were reared on cleaned castor oil leaves till metamorphosis to pupal stage, the deadline for all of them for death. Changes in their bodies were photographed and discussed.

Antioxidant of enzyme activities in treated larvae by AgNPs: APX activity was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM cm^{-1}). The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzyme activity was expressed as unit's min mg^{-1} protein[19].

Statistical analysis: The statistical software SPSS for Windows 16.0 was used to perform t-test. Values of $p<0.05$ and $p<0.001$ were considered as statistically significant values. Gained data through perceptions were not in every case typically dispersed. As long as, both parametric and non-parametric tests were utilized in this research. Linear regression was used to define the relation between treatments and the non-linear squares was utilized in non-linear relationships.

RESULTS

Certain effects of nanoparticles on *S. littoralis*: Results showed that used bio AgNPs at 0.01 g quantity caused mortality and malformation in Fig. 1. Mortality recorded 91.24 and 83.77% in case of treated 2nd and 4th larval instars in comparison with control (10.39 and 7.02%) for the same previous arrangement.

Malformation was noticed with treatments by AgNPs against 2nd larval instar (4.47%) while AgNPs malformed *S. littoralis* 4th larval instar by 15.94%. All malformed larvae in all treatments died and counted in mortality (%) and also showed as separately ratios.

Morphological effect: The 4th larval instar of *S. littoralis* was affected highly by the acute exposure to bio AgNPs more than 2nd stage. It was appeared specifically in the metamorphosis to pupal stage when exposed larvae at certain as showed in Plate 3a-c. Even exposed 4th stage larvae colors were turned to dark grayish and all noticed malformed larvae (Plate 3d) and intermediate larvae were being in apoptosis which their bodies liquefied and melanized as usual when infected with baculoviruses which mainly occurred in case of affected by other nanoparticles.

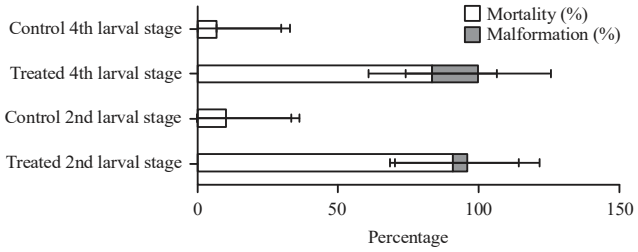

Fig. 1: Effect of bio AgNPs produced by *Tetranychus urticae* against 2nd and 4th larval instars of *Spodoptera littoralis*

Plate 3(a-d): Malformation caused after treatments with bio AgNPs produced by *Tetranychus urticae*, (a) Intermediate pupa resulted of treating 2nd larval instar of *S. littoralis* compared with control, (b) Intermediate pupa resulted of treating 2nd and 4th larval instars compared with control, (c) Small malformed pupa and (d) 4th larval instar treated with AgNPs which is malformed dead larva

Table 1: Reactive oxygen scavengers (ROS) ratio during nano-metals treatments with control comparison

	Reactive Oxygen Scavengers (ROS)			
Spodoptera littoralis larval instars	Superoxide dismutase (SOD)	*Decreased ratio (%)	Ascorbate peroxidase (APX)	*Decreased ratio (%)
Bio AgNPs produced by *T. urticae*				
Treated 2nd larval stage	7.68±0.10[a]	34.10	10.22±1.741[b]	34.75
Treated 4th larval stage	9.15±1.61[a]	24.03	13.25±2.09[b]	29.24
Control 2nd larval stage	22.53±3.78[c]		29.41±4.78[c]	
Control 4th larval stage	38.07±4.21[c]		45.31±8.04[c]	

Values are expressed as the Mean±SE, same letters have the same significant degree, (a, b and c are high, moderate and least significance respectively), *Decreased ratio (%): ROS ratio of the tested strain/ROS ratio of the control strain×100

The integuments were exploded and body contents were out later then bio AgNPs coated with biogenic amines would be new resource again to injure other larvae to control them biologically.

Reactive oxygen scavengers (ROS): Reactive Oxygen Scavengers (ROS) in treatments were significantly lower decrease than control ($p<0.05$). Table 1 showed that superoxide dismutase (SOD) in control were higher than treatments. Decreased ratio percentages of SOD than control recorded 34.1 and 24.03% for treated 2nd larval stages. In the same arrangement, APX ratio decreased in treatments than control with 34.75 and 29.24%. Paired samples test showed high significant differences in case of APX of 2nd larval stage with $t = 2.558^{**}$ and it was $t = 2.619^{*}$ in case of SOD. Furthermore, paired samples test showed high significant differences in case of APX of 2nd larval stage with $t = 2.705^{**}$ and it was $t = 2.91^{*}$ in case of SOD. Moreover, nonparametric correlations showed high significance for treatments with bio AgNPs as appeared through Kendall's Tau_b correlation coefficient = 0.816** and Spearman's rho = 0.894**.

DISCUSSION

New bio nanomaterial, AgNPs produced by *Tetranychus urticae* is starting its way to control agricultural pests with specific advantages more than any other bio resource of AgNPs. Silver ions with certain formulation, characterization and effect of their characteristics are predicted to be extended even against plant diseases or pests such as *Helicoverpa armigera* and fourth instar larvae of *Anopheles subpictus* and *Culexquinque fasciatus*[20-23]. Besides, addition of NaCl was a cofactor for mites to produce nanoparticles with their silk which noticed through this research and could be a revolution of production of bio nanomaterials with their unique mode of action.

Likewise, the apoptosis of hemocytes happened with bio AgNPs in the exposed *S. littolaris*, it was significantly induced at high zinc concentration (1000 mg kg^{-1}) in the insect diet and the apoptosis rate was 63.63%, which was remarkably higher than that at control and lower concentrations (50-500 mg kg^{-1}). That action can be explained depending on the toxic actions of metals which cause oxidative damage to DNA, proteins and lipids[24]. Nanometals able to be accumulated and penetrated more than normal molecules.

Consequently, the mode of action can be mentioned in specific points as a result of the interaction of free radicals with DNA which may occur in different ways, including modification of sugar moieties of DNA and production of apurinic/apyrimidinic[25]. Furthermore, reactive oxygen species (ROS) can also cause damage to all components of a cell[26]. ROS-induced DNA damage is the main cause of p53 activation in the DNA damage response which explains the apoptosis clearly. Furthermore, the morphological changes revealed that multiple numbers of proteins bind to a single or multiple NP molecules. That concept was detected through the interaction between α-lactalbumin protein and nanoparticles by tryptophan fluorescence and circular dichroism spectroscopic techniques[27]. The tryptophan fluorescence measurement of the protein revealed the fact that the protein undergoes a havoc structural change while interacting with NP. The tryptophan fluorescence quenching revealed that tryptophan residues are possibly in the binding site. The disruption of the cell membrane and mitochondrial membrane leads to the production of additional ROS[28,29].

Further, loss of the biological functions of proteins may be due to oxidative modification that leading to the production of carbonyl groups (=C=O). These groups are stable and specific and their appearance causes permanent changes in the structures of the proteins[30,31]. Circular dichroism spectroscopic measurement confirmed the change of secondary structure of the protein in the presence of NP which could also explained the apoptosis that occurred in DNA bands in this research.

Although the protein may retain most of its native structure after adsorption on the NP surface, in some cases the thermodynamic stability of the protein was decreased, making the protein more sensitive to chemical denaturants such as urea[32]. Thus, while the exact mechanisms of the antibacterial action had not yet been clearly understood, it had been suggested that the rule of Reactive Oxygen Species (ROS) generated on the surface of the particles, zinc ion release, membrane dysfunction and nanoparticles internalization were the main cause of cell swelling[33]. It was detected that the same occurred in case of silver ion released and all led to the accumulation of biogenic amines which were targeted[34,35] by Ag^{+}. They recently proved that biogenic amines affected mosquito fertility. Even egg melanisation was regulated by adrenergic signaling, whose disruption caused premature melanisation specifically through the action of tyramine. The strong cumulative negative effect was on mosquito locomotion and survival. Dopaminergic and serotonergic antagonists such as amitriptyline and citalopram recapitulated this effect. Biogenic amines have a wide variety of functions in both the central and peripheral nervous systems of insects. They can act as neurotransmitters, neuromodulators and even circulating neurohormones.

It's recommended to expand use of new nanomaterials which is being pest vs. pest in biological control system in order to let it be done automatically with more required studies on other components in the environment.

CONCLUSION

Production of bio AgNPs coated with biogenic amines by *Tetranychus urticae* provided a renewable resource of bio nanoparticles to control agricultural pests as *Spodoptera littolaris*. Auxiliary compound, NaCl activated more production of AgNPs till a specified point in which mites were exploded and AgNPs released. Consequently, AgNPs caused mortality and malformation of exposed *S. littoralis*. Bio progression of NPs upon mites presented new alternative tools to control the population of pests by certain modes of action.

SIGNIFICANCE STATEMENT

This study showed novel and cheap method to produce bio AgNPs coated with biogenic amines upon pest vs. pest. Thereby, *T. urticae* is able to produce silk filaments also which are helpful to spread the effect of resulted AgNPs, even when their bodies exploded. This research will help expanding production of bio nanoparticles and self-control of wide spectra of insects and mites biologically under field conditions soon.

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