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RESEARCH ARTICLE

Larvicidal Effect of Pure and Green-Synthesized Silver Nanoparticles against *Tribolium castaneum* (Herb.) and *Callosobruchus maculatus* (Fab.)

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Abstract

Silver nanoparticles seem to be an excellent alternative option to control insect's pests due to their unique characteristics. In addition, the plant mediated synthesized silver nanoparticles have important applications in the field of biology, agriculture and environmental due to their safety. The present work aims to study the activity of pure and green-synthesized silver nanoparticles (Ag NP's) against *Tribolium castaneum* and *Callosobruchus maculatus*. The biosynthesized Ag NP's by *N. olrande*r were characterized by UV-visible spectra, X-ray Diffraction (XRD), Energy dispersive spectroscopy (EDS) and Scanning electron microscope (SEM). Larvae of *T.castaneum* and *C. maculates* were exposed to different concentrations of pure and green-synthesized silver nanoparticles and *Nerium oleander* leaf extract (500, 1000, 1500 and 2000 ppm). Although the Larvicidal effect of pure silver nanoparticles against larvae of *T. castaneum* (mainly first larval instar with LC_{50} value 2.019) and *C. maculates* (with LC_{50} value 3.366) , the green-synthesized silver nanoparticles have more larvicidal effect. while Nerium extract have a less effect on all larval instars of *T.castaneum* (LC_{50} value against first instar 3.853) and larvae of *C. maculates* (LC_{50} value 4.332), The green-synthesized silver nanoparticles have more Larvicidal effect than pure silver nanoparticles and *N. oleander* leaf extract.

Keywords: *Callosobruchus maculatus*, *Tribolium castaneum*, silver nanoparticles, and *Nerium oleander*.

Introduction

Storage of grains is part of the post-harvest system through which food materials pass from field to consumer. One of the most important and essential issues with the storing process is the loss in quality and quantity of the grains caused by insects leading to damages and reduction of their dry weight and nutritional value [1]. Thus, insect's pests that infect cereals are considered one of the main problems that cause damage of 10-40% of stored food cereals around the world. Two of the most important stored product insects pests are bruchid beetle *Callosobruchus maculatus* (Fab.) which is a primary pest that infects legumes and cowpea in fields and in warehouses, and red flour beetle *Tribolium castaneum* (Herb.) which is the most common pest of wheat flour as well as pulses, dried fruit and prepared cereals such as pasta, beans, nuts and cornflakes that spreads widely and internationally as it attacks stored crops with different environmental conditions causing them a serious damage [2]–[4]. Insects pests infestation in grains and dry food products is currently controlled by the use of phosphine and methyl bromide, the two most common fumigants all over the world, as well as pyrethroids and organophosphates .the persistent use of chemicals can lead to serious problems associated with human and environmental health [3]. The carcinogenic potential of the chemical pesticides and its toxic residues on food after application, besides insects continuous resistance to pesticides, are basic problems of this approach in addition, the most commonly used fumigant methyl bromide has been completely phased out due to its effect as ozone depletion compound [5]. As a result of this, there is an urgent need to find alternatives for the purpose of insects pests management that are eco-friendly, safer and inexpensive. Nanotechnology has become one of the most novel new approaches for pest management in the recent years particularly nanoparticles [6]. Nanoparticles, central dogma of nanotechnology, which are materials of 10 – 100 nm in size, show completely new properties such as morphology, size and distribution of the particles [7]. Nanoparticles are of great importance in biological and agricultural applications including protection of crops, enhancement of plants growth, detection of plant pathogens, enhancing the ability of plants to absorb nutrients as well as environmental applications including removal of wastes disinfection of water devices for trace concentration detection and in food industry [8], [9].

Nano materials show new properties such as permeability, stiffness, thermal stability, crystallinity, solubility and biodegradability that are necessary for nano pesticides formulations. Thus nano materials have been developed to offer more efficient and safe pesticides and herbicides [10]. Green synthesized silver nanoparticles mostly desired because plant extracts are free from toxics and provides natural capping agents and less expensive compared to microorganism culturing and isolation processes [11], [12].

Recently many plants have been used in the process of synthesizing silver nanoparticles as reducing agents particularly *Avicennia marina*, *Sargassum muticum*, green tea (*Camellia sinensis*), leguminous shrub (*Sesbania drummondii*), various leaf broth, Aloe vera plant extract, Neem (*Azadirachta indica*) and lemongrass leaves extract. In present study, *Nerium oleander* leaf extract used as effective reducing agent for the synthesis of non-toxic, clean and environmentally acceptable silver nanoparticles [11]–[13].

Materials and Methods

Materials

Silver nanoparticles of (20 – 30) nm particle size (Fig.1) were purchased from SkySpring Nanomaterials Company, Houston, USA. Identified *N. oleander* leaves were collected from public gardens around the city of Diwaniyah, Al-Qadisiyah, Iraq. Silver nitrate was obtained from the department of biology, college of science. Non- infected seeds of cowpea and infestation-free wheat flour were purchased from local market and used. Identified stock culture of *C. maculatus* and *T. castaneum* were obtained from different domestic storehouses.

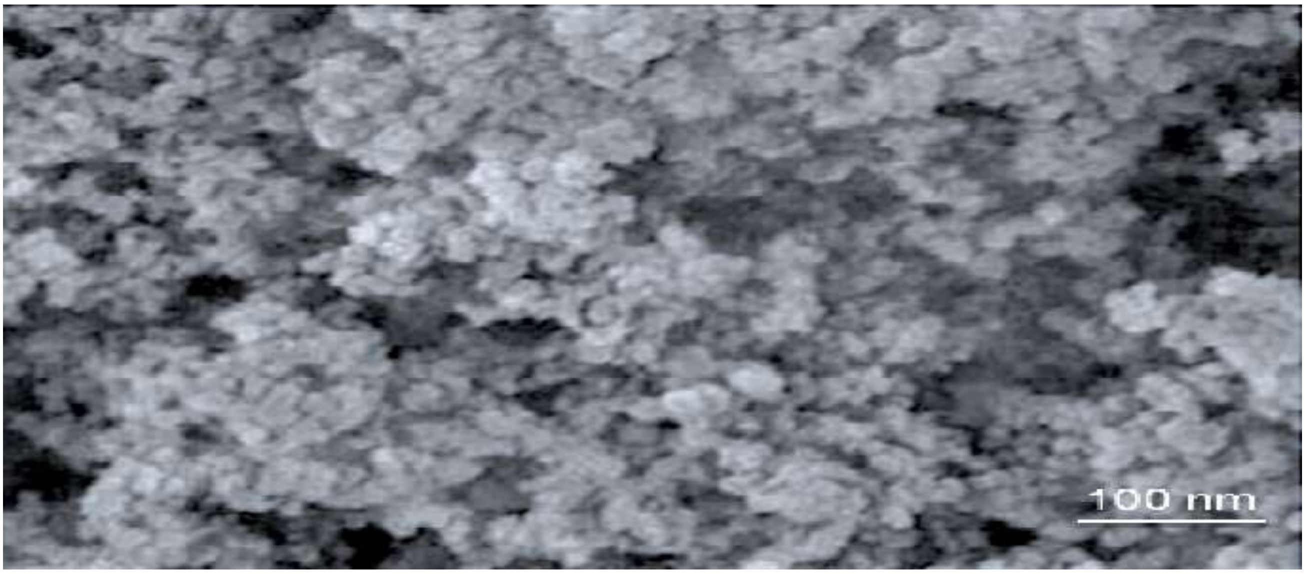

Fig1: SEM image of pure Ag NPs (http://ssnano.com/inc/sdetail/silver_nanoparticle/362)

Insects Rearing

Newly emerged adult pairs of *C. maculatus* were introduced into glass containers (covered with muslin cloth) with 100 g cowpea seeds, and newly emerged adult pairs of *T. castaneum* were introduced into glass containers (covered with muslin cloth) with 100 g wheat flour, incubated at 28 °C and 70% relative humidity for the obtainment of larvae for bioassay.

Synthesis and Characterization of Ag Nanoparticles

Nerium oleander leaves have been washed and left to dry at room temperature for 4 days and then sliced into pieces. 10 gram of the leaves have been weighed and placed in a flask with 100 ml distilled water and then boiled for about 5 minutes, filtrated by filter paper and left to chill [21]. 7 ml of the extract were added to an Erlenmeyer flask containing 100 ml of 1mM Silver nitrate. The reaction was performed at room temperature and darkness. A sample of the mixture has been analyzed by UV-Visible spectra (Fig.), XRD, EDS and SEM (Fig.) to characterize the formed silver nanoparticles.

Bioassay

20 larvae were placed in a petri dish (3 replicates for each concentration) and sprayed with Ag NPs mixture prepared by Nerium leaf extract, pure Ag NPs solution and Nerium water-leaf extract separately then incubated at 28 °C and 70% RH for one day. Mortality percentages were estimated after 24 hours.

Statistical Analysis

The data have been analyzed with SPSS 21 software to estimate means and standard deviation of the data and were significant at p value less than 0.05. Probit analysis has been performed to calculate LC_{50} values.

Results

Characterization of Green-synthesized Silver nanoparticles

Transformation of the color occurred after addition of plant extract to silver nitrate solution from colorless to brown which gives us primal evidence of the formation of silver nanoparticles due to the reduction of Ag ions by reduction agents in the plant extract (Fig. 2).

Fig. 2: Silver nitrate before (a) and after (b) addition of plant extract,(c) plant extract The mixture characterized by UV-VIS spectra within 24 hours, showed absorbance peak at 490 nm (Fig.3) indicated the presence of silver nanoparticles of spherical particle shape[22].

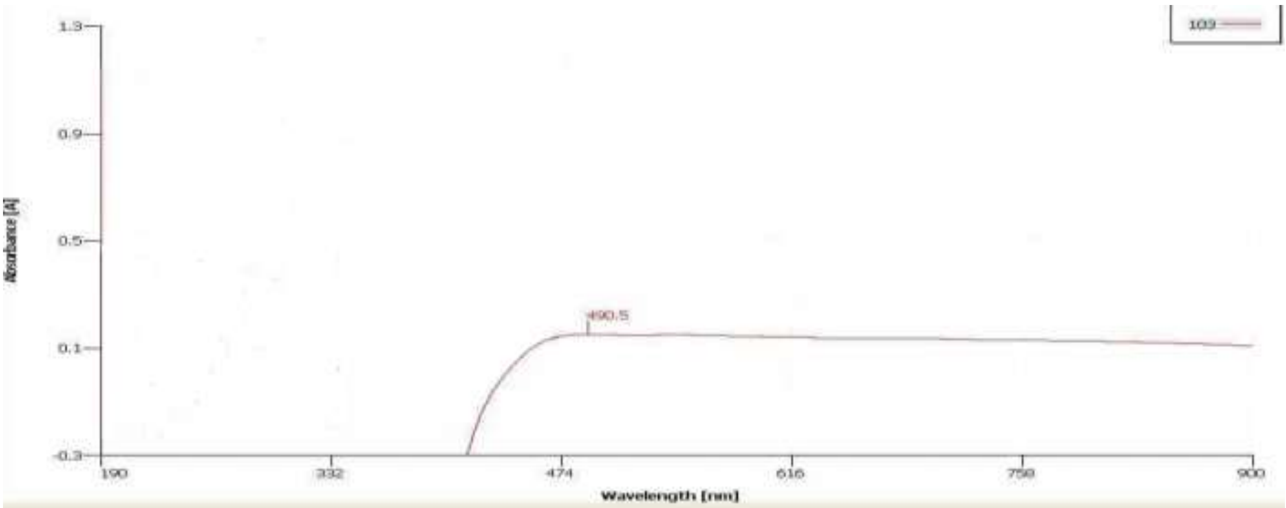

Fig.3: UV-VIS spectra of green synthesized silver nanoparticles

By applying Scherrer equation on the XRD pattern, the particle sizes have been calculated from multiple peaks and the mean size of these peaks was 52 nm, confirming the nanoparticles formed (Fig.4). These facts obtained from UV and XRD analyses, were confirmed by EDX and SEM (Fig. 5).

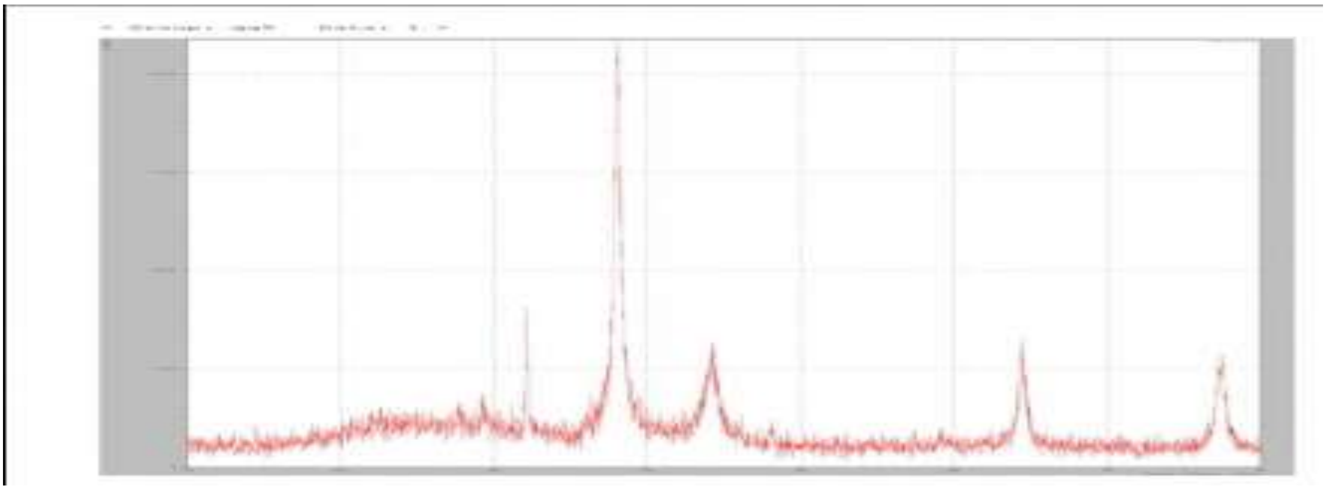

Fig 4: XRD analysis of bio synthesized silver nanoparticles

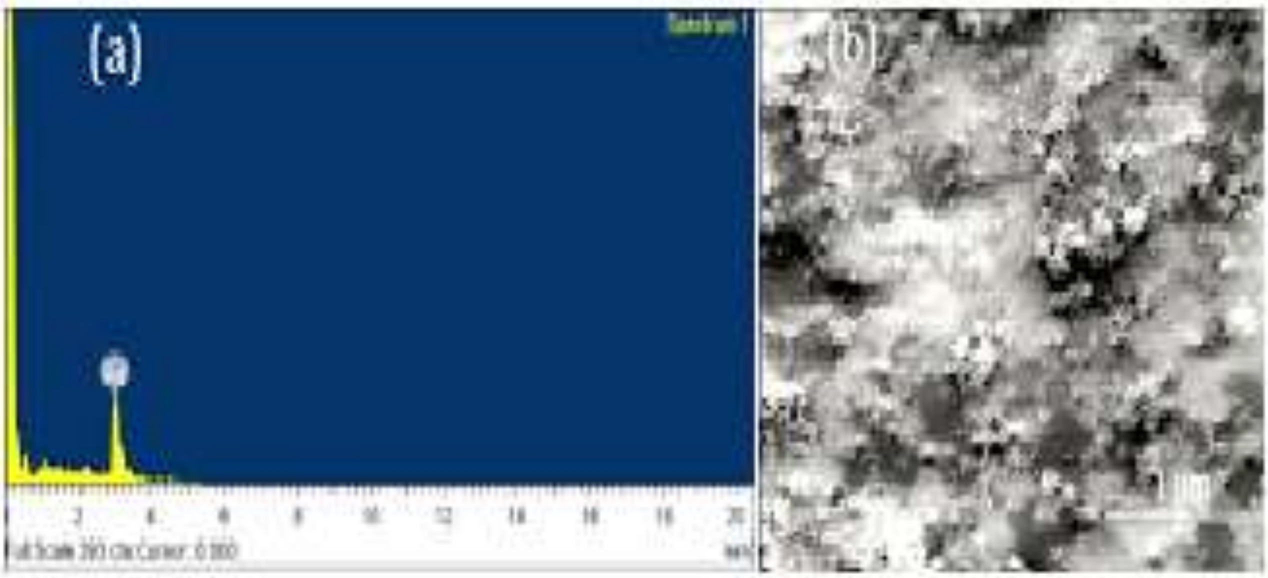

Fig.4: EDX (a) , and SEM image (b) of Green-synthesized silver nanoparticles

Larvicidal effect of Pure, Green-synthesized Silver Nanoparticles and N. oleander Extract on Callosobruchus Maculatus

Larvae of *C. maculatus* significantly effect by pure and green-synthesized silver nanoparticles in compared with control ($p<0.05$) as in Table (1). Also results showed that high concentrations of silver nanoparticles (1500 and 2000) ppm had more effect on larvae. The LC_{50} value of Green-synthesized silver nanoparticles (2.992) showed that they had the strongest lethal effect on larvae of *C. maculatus*, compared to pure silver nanoparticles (LC_{50} value 3.366) and *N. oleander* extract (LC_{50} value 4.332) which has the weakest effect.

Table 1: Means of mortality percentages of larvae of Callosobruchus maculatus

Con. PPM	Green synthesized Ag NPs	Pure Ag NPs	*N. oleander* extract
500	35 *	20	10
1000	40 *	20	15
1500	55 *	40 *	20
2000	85 **	50*	20
control	0	0	0

*= Significant association (p <0.05) in compared with control, **= Significant association (p<0.001) in compared with control.

Table 2: LC_{50} (ppm) value of pure Ag NPs, Green-synthesized Ag NPs and N. oleander leaf extract against Callosobruchus maculatus

Material	LC_{50}	Slop	Limits	Chi square	Sig.
Pure Ag NPs	3.366	1.492±0.685	3.157 – 6.080	1.199	0.549
Green synthesized Ag NPs	2.992	1.978±0.658	2.761 – 3.155	17.584	0.000
N .oleander extract	4.332	0.776±0.783	1.735 – 6.339	0.049	0.976

Effect of Pure, Green-synthesized Silver Nanoparticles and N. oleander Extract on Tribolium Castaneum

Tables (3) ,(4) and (5) showed that larvae of *T. castaneum* (especially first and second instars) Significantly affected by pure and green-synthesized silver nanoparticles in compared with control ($p < 0.05$). Also results showed that high concentration of silver nanoparticles (1500 and 200) ppm were more effective, while nerium extract had the least activity as larvicidal . Table(6) showed that the pure silver nanoparticles had the strongest effect on first and second instars than the green-synthesized silver NPs, while the third and fourth instars were affected by the green-synthesized Ag NPs more than the pure.

Table 3: Means of mortality percentages of pure silver nanoparticles on larvae of Tribolium castaneum

Con. PPM	1st instar	2nd instar	3rd instar	4th instar
500	80**	70*	60*	50
1000	85**	75*	70*	50
1500	90**	8**	75*	70*
2000	95**	90**	90**	85**
Control	0	0	0	0

*= Significant association ($p < 0.05$) in compared with control, **= Significant association ($p<0.001$) in compared with control.

Table 4: Means of mortality percentages of Green-synthesized silver nanoparticles on larvae of Tribolium castaneum

Con. PPM	1st instar	2nd instar	3rd instar	4th instar
500	80**	70	70*	65
1000	90**	85**	80**	75*
1500	90**	90**	85**	85**
2000	100**	95**	90**	90**
Control	0	0	0	0

*= Significant association ($p < 0.05$) in compared with control, **= Significant association ($p<0.001$) in compared with control.

Table 5: Means of mortality percentages of N. oleander extract on larvae of Tribolium castaneum

Con. PPM	1st instar	2nd instar	3rd instar	4th instar
500	20	15	15	10
1000	25	20	20	10
1500	30	20	25	15
2000	35	25	25	15
Control	0 ±0.0	0±0.0	0±0.0	0±0.0

Table 6: LC_{50} (ppm) value of pure Ag NPs, Green-synthesized Ag NPs and *N. oleander* leaf extract against *Tribolium castaneum* larvae

Material	Instar	LC_{50}	Slop	Limits	Chi square	Sig.
Pure Ag NPs	1st	2.019	1.161 ± 0.795	1.012 – 3.145	11.352	0.010
	2nd	2.315	1.196±0.709	1.442 – 3.316	0.352	0.893
	3rd	2.566	1.394±0.677	-8.167 – 2.783	0.911	0.634
	4th	2.803	1.551±0.651	1.694 – 2.991	2.161	0.340
Green synthesized Ag NPs	1st	2.227	1.691±0.881	-1.667 – 3.731	13.560	0.004
	2nd	2.404	1.746±0.775	-1.459 – 2.709	0.059	0.971
	3rd	2.273	1.192±0.716	1.131 – 2.881	0.044	0.978

	4th	2.462	1.443±0.704	-9.202 – 2.772	0.171	0.918
***N.oleander* extract**	1st	3.853	0.750±0.681	1.451 – 6.839	0.032	0.984
	2nd	4.609	0.542±0.725	2.447 – 8.993	0.063	0.969
	3rd	4.298	0.643±0.720	3.129 – 7.441	0.037	0.982
	4th	5.474	0.476±0.817	3.212 – 9.774	16.144	0.001

Discussion

The data in present study clearly indicate that silver nanoparticles (especially green synthesized Ag NPs) could provide excellent larval control of *Tribolium castaneum* and *Callosobruchus maculates*. The exact mechanisms of larvicidal effect of Ag NPs are yet not known. But it can be assumed that the Ag NPs penetrate through the larval membrane which leads to the death of the delicate larvae [14]. Current research agreement with study of Jayaseelan *et al*., 2011 who showed that larvicidal activity of synthesized silver nanoparticles using an aqueous leaf extract of *Tinospora cordifolia* showed maximum mortality against the head louse *Pediculus humanus* and fourth instar larvae of *Anopheles subpictus* and *Culex-quinque fasciatus* [15].

This study reported that Ag NPs, like almost all nanoparticles, are potentially toxic beyond a certain concentration because the survival of the organism is compromised due to scores of pathophysiological abnormalities past that concentration [16], [17]. However, the degree of Larvicidal effect of green-synthesized silver nanoparticles on insects differ according to type of used nanoparticles, bio-chemical way of nanoparticles production and sensitivity or larval stage of insects [18].

Bhuvaneswari *et al*., 2016 found that green synthesized AgNPs using the leaf extract of B. kewensis offered an excellent larvicidal activity. Green route of Ag NPs synthesis shows to be an eco-friendly, time consuming, cost-effective, and alternative to chemical methods of NPs production would be an appropriate for extending the biological route for large-scale production of the bio-larvicidal product [19] whereas Naik *et al.,* (2011) showed moderate larvicidal effects of the synthesized Ag NPs of leaf of *Pongamia pinnata* for mosquito control and found that plant extracts was found to be toxic to larvae at LC50 = 0.25 ppm and LC90 =1 ppm [20]. Rawani *et al.* 2013 also reported the larvicidal activities of the synthesized Ag NPs from fresh leaves, dry leaves and green berries of *S. nigrum* against larvae of *Culex quinquefasciatus* and *Anopheles stephensi* [14].

Conclusion

Nanostructure of silver that synthesized by the green chemistry approach by the use of the aqueous leaf extract of *Nerium oleander* have been showed an excellent larvicidal activity against *Tribolium castaneum* and *Callosobruchus maculates* compared to the extract alone. Present research move toward, avoiding the use of hazardous, toxic insecticidal chemicals.

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