SCIENTIFIC NOTE

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INSECTICIDE EFFECT OF SILVER AND ZINC NANOPARTICLES AGAINST *Aphis nerii* BOYER DE FONSCOLOMBE (HEMIPTERA: APHIDIDAE)

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The oleander aphid, *Aphis nerii* Boyer de Fonscolombe, is one of the common pests of ornamental plants in the families of Apocynaceae and Sclepiadaceae and distributed throughout the world, which has been responsible for the mortality of a large number of oleander (*Nerium oleander* L.) shrubs each year. In this research, the insecticidal activity of Ag nanoparticles against the *A. nerii* was investigated. Nanoparticles of Ag and Ag-Zn were synthesized through a solvothermal method, and using them, insecticidal solutions of different concentrations were prepared and tested on *A. nerii*. For comparison purposes, imidacloprid was also used as a conventional insecticide. In the experiments, the LC_{50} value for imidacloprid, Ag and Ag-Zn nanoparticles were calculated to be 0.13 μL mL^{-1}, 424.67 mg mL^{-1}, and 539.46 mg mL^{-1}, respectively. The result showed that Ag nanoparticles can be used as a valuable tool in pest management programs of *A. nerii*. Additionally, the study showed that imidacloprid at 1 μL mL^{-1} and nanoparticles at 700 mg mL^{-1} had the highest insect mortality effect.

Key words: Imidacloprid, oleander aphid, *Nerium oleander*, solvothermal method.

Mortality of oleander shrubs (*Nerium oleander* L.) has been rapidly increasing recently. The oleander aphid, *Aphis nerii* Boyer de Fonscolombe (Hem.: Aphididae) causes oleanders wilt and produce large amounts of honeydew, which is a harmful and destructive to the plant. *Aphis nerii* is one of the common pest of several important ornamental plants in the families of Apocynaceae and Sclepiadaceae and principal insect pest on oleander shrubs (Ester *et al*., 2003). This aphid is cosmopolitan, being found in tropical to warm temperature regions throughout the world. The host range of the oleander aphid includes several genera of Asclepiadaceae (*Gomphocarpus*, *Asclepias*, and *Calotropis*) and Apocynaceae (*Nerium* and *Vinca*). Insecticides still remain a very important component among the strategies for effective control of the pest. Since integrated pest management (IPM) gained acceptance as a preferred approach of pest control, many efforts have been initiated to protect natural or introduced enemies by choosing selective pesticides (Croft, 1990; Lucas *et al*., 2004); however, using chemical control has proved insufficient for controlling populations of *A. nerii*; this is because even with the large scale use of insecticides, *A. nerii* is still widely spread in tropical regions around the world with ever increasing populations. Additionally, long term use of insecticides has risk of developing resistance in *A. nerii*. Targeted nanoparticles often exhibit novel characteristics such as extraordinary strength, more chemical reactivity, and possessing a high electrical conductivity. Thus, nanotechnology has become one of the most promising new approaches for pest control in the recent years (Bhattacharyya *et al*., 2010). Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental cleanup problems (Chinnamuthu and Murugesa Boopathi, 2009). Silver has been used in many applications in pure free metal or in compound form because it possesses antimicrobial activity against pathogens, yet it is nontoxic to humans (Yeo *et al*., 2003; Elchiguerra *et al*., 2005). Zinc nanoparticles have antimicrobial activity and can be used as fungicide (Seven *et al*., 2004; Levin *et al*., 2007). The authors of these studies have reported that the oxide nanoparticles such as ZnO and Ag have a toxic effect on bacteria, fungi, and biological cells; furthermore, various toxicity mechanisms of Ag have been reported. Examples of such mechanisms include generation of reactive oxygen species, oxidative stress, membrane disruption, protein unfolding, and/or inflammation (Bragg and Rannie, 1974; Feng *et al*., 2000; Samuel and Guggenbichler, 2004; Elchiguerra *et al*., 2005; Reddy *et al*., 2007; Meng *et al*., 2009; Donaldson *et al*., 2009).

Although there have been numerous studies on the toxicity effects of nanoparticles on bacteria, fungi, and animal pathogens (Bragg and Rannie, 1974; Feng *et al*., 2000; Samuel and Guggenbichler, 2004; Elchiguerra *et al*., 2005; Reddy *et al*., 2007) little research has carried out to investigate the toxicity effect of nanoparticles on insects. In this work, we studied the mortality effect of Ag and

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Ag-Zn nanoparticles, synthesized using a solvothermal method, in comparison with imidacloprid, on *A. nerii*.

MATERIALS AND METHODS

Synthesis of Ag-Zn and Ag nanoparticles

A 2 mmol solution of $Zn(AC)_2{\cdot}2H_2O$ in 20 mL EtOH/H_2O solvent was added to a 2 mmol solution of $AgNO_3$ in 20 mL EtOH/H_2O solvent. Sodium hydroxide solution (10 M, 10 mL) was added to the above mixture at room temperature under stirring. After 60 min stirring the mixture was transferred into Teflon-lined stainless steel autoclaves, sealed, and maintained at 155 °C for 12 h. Subsequently, the reactor was cooled down to -15 °C temperature immediately. The resulting gray solid products were centrifuged, washed with distilled water and ethanol to remove the ions possibly remaining in the final products, and finally dried at 50 °C in air.

Sodium hydroxide solution (4 M, 10 mL) was added to a solution of $AgNO_3$ (2 mmol) in EtOH/H_2O solvent (25 mL). To investigate the role of surfactants on the size and morphology of nanoparticles, 0.5 g of polyethylene glycol (PEG) was used in the reactions with optimized conditions. The obtained mixtures were sonicated for 150 min with ultrasound powers followed by centrifugation and separation of the solid and liquid phases.

Insect and bioassay

A colony of the test insect, *A. nerii*, was established in the insectary from field collected, disease-free aphids and reared under constant temperature of 26 ± 2 °C, 65 ± 5% RH with a photoperiod 14:10 h. Aphids were reared in plastic box (20 × 30 cm) on fresh leaves of oleander.

Bioassay for effects of the Ag and Ag-Zn nanoparticles and imidacloprid ([*E*]-1-[6-chloro-3-pyridylmethyl]-*N*-nitroimidazolidin-2-ylideneamine) on 1-d old first instar nymphs were determined by dipping infested leaves in different concentration 300, 371, 458, 566, and 700 mg mL^{-1} for nanoparticles, 0.08, 0.14, 0.25, 0.5, and 0.8 $\mu L\ mL^{-1}$ for imidacloprid. The experiments carried out with three replicates while each of them consisted of 15 nymphs. Distilled water was used as control. Treated nymphs were maintained in a climate chamber and reared on fresh leaves. Insect mortality was counted after 24 h.

Statistical analysis

The morphology and size of Ag and Ag-Zn samples were characterized by scanning electron microscope (SEM) (Philips XL 30, Tehran, Iran) with gold coating. The mortality data was analyzed with SPSS 16 software followed by one-way ANOVA and Duncan's multiple range tests to compare effects among treatments. The results were expressed as means (± SE) of untransformed data and considered significantly different at $P < 0.05$. Probit analysis was used for estimation of the LC_{50} by the Probit Analysis-MSChart 2009 software (Chi, 2009).

RESULTS

Structural study of nanoparticles and contact toxicity bioassay

The morphology, structure and size of the samples were investigated by Scanning Electron Microscopy (SEM). Figures 1a and 1b indicate that the original morphology of the Ag and Ag-Zn nanoparticles are approximately spherical with the diameter varying from 46 to 41 nm, respectively.

In bioassay of contact toxicity the ANOVA showed a significant difference at 1% level between the effect of imidacloprid ($F_{5,12} = 139.08$, $P = 0.00$), Ag nanoparticles ($F_{5,12} = 29.3$, $P = 0.00$) and Ag-Zn nanoparticles ($F_{5,12} = 59.5$, $P = 0.00$) on *A. nerii*. In the all tested pesticides, mortality of first nymphal instar increased by increasing in the pesticide concentrations. In other words, mortality of *A. nerii* increased as a function of the pesticide concentrations (Tables 1, 2 and 3).

The highest mortality of imidacloprid (94.44%),

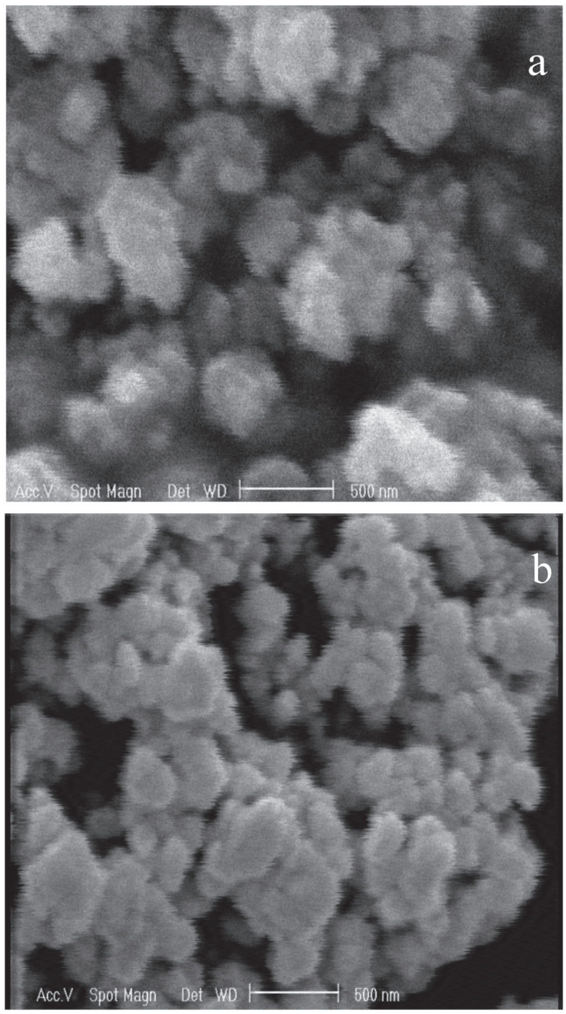

Figure 1. Scanning electron microscopy images of synthesized Ag (a) and Ag-Zn (b) nanoparticles.

Table 1. Mortality of *Aphis nerii* treated with imidacloprid.

Concentration	Mortality (mean ± SE)
μL mL^{-1}	
1.00	11.00 ± 0.57a
0.56	9.00 ± 0.57b
0.31	7.33 ± 0.88c
0.17	4.66 ± 0.66c
0.10	2.33 ± 0.33d
0.00	0.00 ± 0.00e

The same letter in column indicates lack of significant difference at 5% level; SE: Standard error.

Table 2. Mortality of *Aphis nerii* treated with Ag nanoparticles.

Concentration	Mortality (mean ± SE)
mg mL^{-1}	
700	7.00 ± 0.57a
566	5.66 ± 0.66a
458	5.33 ± 0.66a
371	2.33 ± 0.88b
300	1.33 ± 0.33bc
0.000	0.00 ± 0.00c

The same letter in column indicates lack of significant difference at 5% level; SE: Standard error.

Table 3. Mortality of *Aphis nerii* treated with Ag-Zn nanoparticles.

Concentration	Mortality (mean ± SE)
mg mL^{-1}	
700	8.00 ± 0.57a
566	6.33 ± 0.66ab
458	5.00 ± 0.57b
371	3.00 ± 0.57c
300	2.00 ± 0.57c
0.00	0.00 ± 0.00d

The same letter in column indicates lack of significant difference at 5% level; SE: Standard error.

Ag nanoparticles (80.55%) and Ag-Zn nanoparticles (72.22%) was observed at the highest concentration of each pesticide (i.e., 1 μL mL^{-1}, 700 mg mL^{-1}, and 700 mg mL^{-1}, respectively). Statistical analysis between maximum concentration of pesticides ($F_{3,8}$ = 153.94, P = 0.00) showed that all pesticides significantly decreased density of *A. nerii* compared with the control (Table 4). Furthermore, Table 4 compares the insect mortality effect of nanoparticles with imidacloprid. As shown in the table, the mortality effect of insecticides based on Ag and Ag-Zn based nanoparticles is less than (but comparable with) the mortality effect of imidacloprid.

The LC_{50} values for imidacloprid, Ag nanoparticles, and Ag-Zn nanoparticles after 24 h were estimated to be 0.13 μL mL^{-1}, 424.67 mg mL^{-1} and 539.46 mg mL^{-1}, respectively (Table 5). The LC_{50} value comparison using the LC_{50} ratio (0.0003) and their lower and upper 95% confidence limits (0.0002-0.0004) showed that there was significant difference between LC_{50} value for imidacloprid (0.13 μL mL^{-1}) and this value for Ag nanoparticles (424.67

Table 4. Effect of the highest concentration of the test insecticides against first instar nymphs of *Aphis nerii*.

Pesticide	Concentration	Mortality (mean ± SE)
Imidacloprid, μL mL^{-1}	1.00	11.33 ± 0.66a
Ag nanoparticles, mg mL^{-1}	300	9.66 ± 0.33b
Ag-Zn nanoparticles, mg mL^{-1}	300	8.66 ± 0.33b
Control	0.00	0.00 ± 0.00c

The similar letter in column indicates the lack of significant difference at 5% level; SE: Standard error.

Table 5. Evaluation of imidacloprid, Ag and Ag-Zn nanoparticles against first instar nymph of *Aphis nerii*.

Pesticide	Slop (± SE)	LC_{50}	Limits 95%	Chi square (χ^2)
Imidacloprid	1.79 ± 0.32	0.13 μL mL^{-1}	0.09 - 0.17	0.07
Ag nanoparticles	4.31 ± 0.79	424.67 mg mL^{-1}	384.30 - 463.36	2.38
Ag-Zn nanoparticles	5.48 ± 0.96	539.46 mg mL^{-1}	502.94 - 589.32	0.90

SE: Standard error; LC_{50}: mean lethal concentration.

mg mL^{-1}). In compression between imidacloprid and Ag-Zn nanoparticles, the LC_{50} ratio (0.0002) with their lower and upper 95% confidence limits (0.00-0.09) showed that there was significant difference between the LC_{50} value for imidacloprid (0.13 mg mL^{-1}) and Ag-Zn nanoparticles (539.46 mg mL^{-1}). Also the LC_{50} value comparison using the LC_{50} ratio (0.78) and their lower and upper 95% confidence limits (0.002-298.28) showed that there was no significant difference between LC_{50} value for Ag nanoparticles (424.67 mg mL^{-1}) and this value for Ag-Zn nanoparticles (539.46 mg mL^{-1}). For the all pesticides the statistic t ratio was > 1.96, the g factor was < 0.5 and the heterogeneity factor was < 1.

DISCUSSION

The oleander aphid management has traditionally depended on the use of synthetic insecticides. Long term application of pesticides, for controlling oleander aphid may cause in developing resistance in this pest to pesticides. In the recent years, nanoparticles have received much attention for controlling pathogens in agriculture (Guan *et al*., 2008; Sang Woo *et al*., 2009; Eleka *et al*., 2010). In the present study, bioassay was followed for assessing the insecticidal activities of Ag and Ag-Zn nanoparticles on the *A. nerii*. Results showed that these nanoparticles could be an effective pest control approach for *A. nerii*. Our study showed that the insect mortality increased significantly with increase in pesticide concentrations. Although insect mortality as a result of using nanoparticles was slightly less than imidacloprid (which is consistent with other researcher's reports such as Guan *et al*., 2008; Samih *et al*., 2011; Rouhani *et al*., 2011), one advantage of using them is low risk of developing resistance in long term usage.

It has been suggested that nanometer-sized Ag particles possess different physical and chemical properties from their macroscale counterparts that affect their interaction with biological structures and physiological processes (Nel *et al*., 2003; Sang Woo *et al*., 2009). Samih *et al*. (2011) compared the efficacy of amitraz with ZnO and $ZnOAl_2O_3$ nanoparticles, against pistaccio psyllid *Agonoscena pistaciae* Burckhardt and Lauterer and found that the mortality effect of amitraz was greater than these with nanoparticles. Rouhani *et al*. (2011) reported that $ZnO\text{-}TiO_2$-Ag nanoparticles have insecticidal activity on *Frankliniella occidentalis* Pergande and showed the most mortality effect pertained to 28%ZnO-70%TiO_2-2%Ag (LD_{50} = 195.27 mg L^{-1}). Guan *et al*. (2008) showed that toxicity of the imidacloprid (IMI) increased when coated with nanoparticles; they reported toxicity of the 50% nano-SDS/Ag/TiO_2-IMI was higher in the adult stage of *Martianus dermestoides* Chevrolat (Coleoptera: Tenebrionidae) compared to the 95% IMI as indicated by the lower LC_{50} value. It is well obvious from our results that insect mortality effect of Ag nanoparticles was slightly more than that of Ag-Zn nanoparticles. Rouhani *et al*. (2011) showed that the insecticidal effect of ZnO-TiO_2-Ag increased when deal of Ag is more.

CONCLUSION

Motivated by the fact that little is known regarding the effects of Ag on pests, we evaluated the insecticidal activity of silver nanoparticles against the *Aphis nerii*. In our study, Ag and Ag-Zn nanoparticles were synthesized by solvothermal method. Our results suggest the possibility of using Ag nanoparticles to eradicate pests and can be used as valuable tools in pest management programs of *A. nerii*. While the environmental effects of using Ag and Ag-Zn nanoparticles as insecticide is subject of further study, one obvious advantage of using them as insecticides is the low risk of developing resistance by the insects in long term uses.

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Efecto insecticida de nanopartículas de plata y zinc contra *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae). El áfido de la adelfa, *Aphis nerii* Boyer de Fonscolombe, es una de las plagas más comunes de plantas ornamentales en las familias Apocynaceae y Sclepiadaceae y tiene distribución mundial, ha sido responsable de la mortalidad de un gran número de arbustos de adelfa (*Nerium oleander* L.) cada año. En este estudio se investigó la actividad insecticida de nanopartículas de Ag contra *A. nerii*. Nanopartículas de Ag y Ag-Zn fueron sintetizadas a través de un método solvotérmico, y con ellas se prepararon soluciones insecticidas de diferentes concentraciones y se probaron contra *A. nerii*. Con fines de comparación, también se usó imidacloprid como un insecticida convencional. En los experimentos, el valor LC_{50} para imidacloprid, nanopartículas de Ag y Ag-Zn se calculó como 0.13 μL mL^{-1}, 424.67 mg mL^{-1}, y 539.46 mg mL^{-1}, respectivamente. El resultado mostró que nanopartículas de Ag pueden ser usadas como una herramienta valiosa en programas de manejo de plagas de *A. nerii*. Adicionalmente, el estudio mostró que 1 μL mL^{-1} imidacloprid y 700 mg mL^{-1} de nanopartículas tuvieron el mayor efecto en mortalidad de los insectos.

Palabras clave: imidacloprid, áfido de la adelfa, *Nerium oleander*, método solvotérmico.

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