The Application of the Silver Nanoparticles in Control of Arthropoda under Laboratory Conditions

Ali Sharifian [1], Asgar Jafarzadeh [2], Sina Azodian [3], Ahmad Zabetian [1], Ahmad Riahi [4]

1-Department of Plant Protection, College of Agriculture, University of Zabol, Zabol, Iran.
2-Department of Food Sciences and Technology, College of Agriculture, University of Zabol, Zabol, Iran
3-Institute of Agriculture, University of Zabol, Zabol, Iran
4- Department of Biology, Payam Noor University, Tehran, Iran

Abstract
The two-spotted spider mite (*Tetranychus urticae* Koch.) is one of the most harmful phytophagous pests, dangerous not only due to its fast development cycle and high fertility, but also due to its ability to rapidly develop resistance to active substances of acaricides. In this work acaricidal relative toxicity of silver nanoparticles (AgNPs) was evaluated against adults of mite. Silver nanoparticles synthesized by chemical reduction method and six concentrations (190, 285, 356, 441, 551 and 685 mg ml^{-1}) by leaf dipping method were tested under laboratory conditions. Result showed that, the silver nanoparticles were highly effective against this mite causing more 96.04% mortality in highest concentration indicating the effectiveness of AgNP to control this pest. In the experiments, the LC_{50} and LC_{90} value for Ag nanoparticles were calculated 363.10 mg $mL^{-1}$and 629.26 mg mL^{-1}, respectively. The result also showed that AgNPs can be used as a valuable tool in pest management programs of *T. urticae.*

Key words: Arthropoda, acaricide, silver nanoparticles

Introduction

The two-spotted spider mite, *Tetranychus urticae* (Koch, 1836) (Acarina: Tetranychidae), is considered to be one of the most serious plant-feeding mite that infests a wide range of hosts worldwide (Zhang, 2003) and affects crops by direct feeding, thereby reducing photosynthetic leaf area and causing leaf abscission in severe infestations (Gorman *et al*., 1975). The control of this species is challenging due to the quick development of resistance to many miticides within a short period of time and leading to the appearance of resistance in mite populations. (Flamini, 2006). It is therefore important using other approaches with a different mode of action to achieve the sustainable management of *T. urticae* (Van Leeuwen *et al*., 2015). Nanotechnology is one of the fastest growing field in agricultural with novel tools for pest and disease management (Tarafdar et al. 2013). Silver has been used in many applications because its compounds possess strong inhibitory and antimicrobial activities against bacteria, fungi, and virus yet it is nontoxic to humans (Sahayaraj and Rajesh, 2011); however little research has carried out to investigate the toxicity effect of silver nanoparticles on mites. In this work, we studied the mortality effect of Ag nanoparticles, synthesized by chemical reduction method on T. *urticae.*

Materials and Methods Synthesis of Silver Nanoparticles

The silver nanoparticles synthesized in *Department of Chemistry*, Faculty of *Science*, University of Zabol, Zabol, Iran. Silver nitrate ($AgNO_3$) used as the source for silver nanoparticles. A volume of 30 mL of the distilled water poured into an Erlenmeyer flask containing dry ice. 67 mL of solution borohydride ($NaBH_4$) 2mM added to it. $AgNO_3$ dripped into the stirring $NaBH_4$ solution at approximately 1 drop per second. $AgNO_3$ added to turn darker yellow, then gray as the nanoparticles aggregate. Erlenmeyer flask was removed from the ice and the solution of trisodium citrate (2 mM, 20 mL) added into flask on shaker (150 rpm). The final solution divided into 1.5 mL micro tubes and centrifuged at 14,000g for 15 minutes. To remove contaminants and excess material, sediments was washed three times with distilled water. The final sediment was dispersed in 10 mL of distilled water. The concentration of recovered silver in the solution was equal to 0.2 mM.

Rearing of mites

Stock population of *T. urticae* was collected from naturally infested plants in greenhouse and transferred to a climate controlled room (26°C and 50-60% RH, 16:8 [L:D] in a laboratory at Institute of Agricultural Research, University of Zabol (Iran). The population of *T. urticae* was reared on leaves of bean (*Phaseolus vulgaris*) placed on moistened cotton in Petri dishes (Overmeer, 1985). Only young adult (24 h old) females were used in the experiments.

Bioassays

In order to determine the effects of Ag nanoparticles, experiments carried out in a Completely Randomized Design with three replications and each of them consisted of 15 virgin young adult female *T. urticae*. The leaf-dip bioassay method was used in different concentrations 190, 285, 356, 441, 551 and 685 mg ml^{-1}. Leaves immersed into silver nanoparticle solution for 5 second and allowed to dry in the air and were placed on wet cotton into plastic petri dishes and then adult mites were transferred on leaves. Control *leaves* were *dipped* in distilled water. Treated adults were kept at same condition. The number of dead mites were counted after 24 hours

Statistical analysis

Adult mortality counts were made after 24 h of exposure and corrected using Abbott formula in the control treatment (Abbott, 1925). The data were analyzed by using a two-way ANOVA and compared with Duncan's multiple range tests to compare effects among treatment (SPSS, 2007).Mean percent larvalmortalitydataweresubjectedtoanalysisofvarianceandcomparedwithDuncan'smultiplerange tests to determine any differences between plant species and within species and concentration (SPSS, 2007). Data were subjected to probit regression analysis (Finney 1971). The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and the 95% confidence intervals of LC_{50} (upper confidence limit) and (lower confidence limit) were calculated. Results with $p<0.05$ were considered to be statistically significant.

Results and Discussion Structural study of nanoparticles

Particle sizes and shapes were analyzed using Scanning Electron Microscope (SEM). Figure 1, indicates that the original morphology of silver nanoparticles is approximately spherical with the diameter varying between 10 to 30 nm.

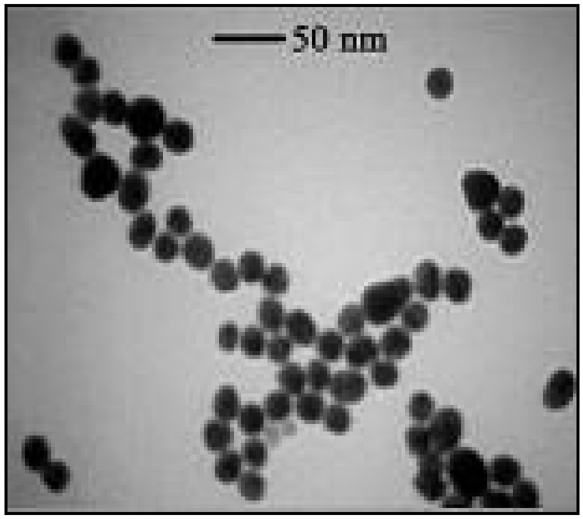

Fig. 1. The SEM images of spherical silver nanoparticles and its particle size distributions.

Efficacy of AgNPs against *T. urticae*

In order to *calculate the* appropriate *concentration of silver nanoparticles* in solution, preliminary experiments were carried out on *T. urticae* to determining appropriate range of applied concentrations. The results obtained for highest AgNPs concentration was presented in table 1.

Table 1. Corrected mortality rate (%) of *T. urticae* treated with AgNPs

Nanoparticles	Concentration	Mortality (%)
AgNPs	685 (mg mL^{-1})	96.04%

The different log concentrations (190, 285, 356, 441, 551 and 685 mg mL^{-1}) of AgNPs were tested against adults of *T. urticae* and were found to be highly susceptible to the AgNPs after 24 h of exposure. Data illustrated in Table (2), revealed that the percentage of accumulative mortality of *T. urticae* adults increased gradually with the increased of concentration. The two concentrations of Ag nanoparticles 190 and 285 $mg\ mL^{-1}$, showed mortality 14.16 and 19.87 %, respectively. The other concentrations 356, 441, 551 and 685 $mg\ mL^{-1}$ recorded with significant differences 61.73, 70.55, 91.87 and 96.04 %, respectively compared with control that recorded 4.87 mortality percentages Fig. 1)

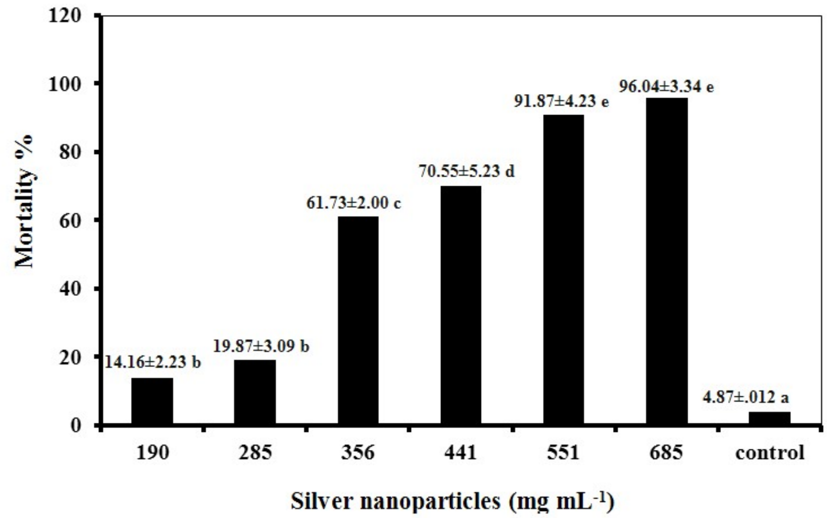

Fig.1. Mortality percentage of *Tetranychus urticae* adults treated with silver anoparticles after 24 hours. Means followed by the same letter are not significantly different at the 5%

The lethal concentrations ($LD_{50/90}$) values for Ag nanoparticles on adults of *T. urticae* was calculated 24 h after treatment. LC_{50} and LC_{90} values of silver nanoparticles were 363.10 mg mL^{-1} and 629.26 mg mL^{-1}, respectively (Table 2). Results indicated that LC_{50} decreased with increasing in NP concentrations.

Table 4- LC_{50} (mg mL^{-1}) value of silver nanoparticles against *Tetranychus urticae* after 24 hours

Nanoparticles	**Slop± SE**	**LC_{50}(mg mL_{-1}) (Confidence limits 95%)**	**LC_{90}(mg mL_{-1}) (Confidence limits 95%)**	**(χ^2)**
AgNPs	5.36± 0.75	363.10 (327.44×393.63)	629.26 (561.50×754.40)	0 .17

Mortality effect of silver nano particles on adult mites using leaf-dipping method in tested concentrations showed different effects on mites which is based on increase in concentrations. Obtained results showed that the nanoparticles have significant toxicity on two spotted mites and can be used as a new tool in pest management programs of mites. However, environmental effects of using AgNPs as acaricide are subject for further studies.

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