The effect of Silver nanoparticles on Tetranychus urticae

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ABSTRACT: The two-spotted spider mite, Tetranychus urticae Koch is one of the most important pests of agricultural systems. One of the major obstacles to the control of T. urticae is its ability to rapidly develop resistance to many important acaricides, even after only a few applications. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. In this study results from bioassay condition by leaf dipping and leaf spray methods showed significant points so that mortality effect of silver nanoparticles on adult mites using leaf dipping method in concentrations of 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm showed different effects on mites which is based on increase in concentrations at different time interval.

Key words: nano particles, Tetranychus Urtica, nano silver

INTRODUCTION

The two-spotted spider mite, Tetranychus urticae Koch is one of the most important pests of agricultural systems, (Helle and Sabelis, 1985). The mite has been reported to attack about 1200 species of plants, (ZhangZ, 2003) of which more than 150 are economically important (Gavanji et al. 2012 ;Jeppson LR et al.,1975). One of the major obstacles to the control of T. urticae is its ability to rapidly develop resistance to many important acaricides, even after only a few applications (Stumpf et al., 2001). Spider mites have evolved resistance to more than 80 acaricides to date, and resistance has been reported from more than 60 countries. The economic threat posed by these mites is constantly increasing because of the development of pesticide resistance and the resurgence of mite populations (Cranham JE and Helle W,1985). Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (M. A. Albrecht,et al,2006). Nanotechnology is a field that is burgeoning day by day making an impact in all dimensions of human life. (R. Vaidyananathh et al.,2009) The word “nano” is used to indicate one billionth of a meter or 10-9. (N. Taniguchi, 1974) Among many natural antibiotic compounds, silver or silver ions have long been used in many areas due to their strong antimicrobial activity against pathogenic microbes such as bacteria (Gavanji et al. 2013; T.Y.Beveridge and Murray R.G.E.1980; Golab Z. 1981; Brierley J.A.1990), yeast (M. A. Albrecht,2006), fungi (N.Frilis and Myers-Keith. P. 1986; H.Niu.,et al.1993) algae (T.Sakaguchi et al.,1979; D.W.Darnall et al.,1986) and non-toxicity to humans (Elchiguerra et al., 2005; Yeo et al., 2003). It may be used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides (Park, H.-J.,et al 2006). Until now, limited research provided some evidence of the applicability of silver for controlling plant diseases (Park, H.-J.,et al 2006). Silver ions are very reactive, which are known to cause the inhibition of microbial respiration and metabolism as well as physical damage (Gavanji et al. 2013; Bragg and Rannie, 1974; Thurman and Gerba,1989).
MATERIAL AND METHODS

**preparation of Silver nanoparticles**

In this study, the solution containing Ag nanoparticles with commercial name, NONO24460, was produced by Mobin chemical spadana Company. The concentration of Ag-NPs in this compound was 4000 ppm and it was in form of colloidal suspension. This compound keeps its stability in cultural medium. The size of this Ag-NPs was between 18 to 34 nm and Zeta potential of silver nanoparticles was -33.5 that showed the average stability of this compound (Figure 1). All the applied concentrations have obtained by diluting different amount of the Ag-NPs solution with appropriate amount of distilled water.

![Characterization of AgNPs in test media by using TEM](image)

**Source of mites**

In the first step Tetranychus urticae collected from bean plants in laboratory of research center of Isfahan plant medicine. Then 100 bean plants free of pests obtained and in order to increase the number of mites, the number of 50 adult male and female mite in controlled conditions including temperature of 25 ±1°C, relative humidity equal to 70±5 and 16:8 L: D photoperiod transferred on 20 bean plant and by increasing the number of mites, the number of pots increased. Also to prepare fresh leaves, 100 bean plants free from pests were kept in another greenhouse. The experiments were based on leaf spray method.

**Plants and mites**

At first the bean plants (Phaseolus vulgaris) were cultivated in the greenhouse of agricultural research center of Isfahan. After that a T.urticae population was collected from bean plants in the plant protection laboratory of Khorasgan University. Then in order to increase the number of mites, 100 bean plants free of pests were selected and 50 adult male and female mites were transferred on 20 bean plants in controlled conditions including temperature of 25 ±1°C, relative humidity equal to 70±5 and 16:8 L: D photoperiod and by increasing the number of mites, the number of pots also increased. Also to prepare fresh leaves, 100 bean plants free from pests were kept in another greenhouse. The experiments were based on leaf spray method. T.urticae reared on bean plants at 25±4°C and 60±4% RH under a 16-h light regime without any exposure to pesticides.
**Leaf spray method**

In this experiment new bean leaves with long petioles picked over and laid on wet cotton in Petri dishes and covered with aluminum foil. Then five mites were transferred on leaves and solutions of Silver nanoparticles at 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm concentrations were sprayed on the leaves. Weight of liquid that sprayed on leaves was between 1 mg/cm$^2$ and 5 mg/cm$^2$. The effect of nano particles on mites was checked at 24, 48 and 72 hours interval. For counting the number of alive or dead mites, a hand magnifier with magnification of 10X was used.

**Leaf dipping method**

In this method the rose leaves disinfected from any pests were cut freshly with their long tail before experiment. Each tail was placed in wet cotton and covered with aluminum cover. Then the leaves were individually immersed in concentrations 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm of silver nanoparticle for 5 seconds with gentle agitation. Discs were glued individually to plastic Petri dish and then adult mites were transferred on leaves. Then all Petri dishes were kept at same condition. The effect of silver nanoparticles on mites was recorded at 7 periods of 24, 48, 72, 96, 120, 144 and 168 hour.

**Preparing leaves to culture the mites from Phaselus vulgaris**

To test the effect of nanoparticles in greenhouse, bean plant (Phaselus vulgaris) had been used. In this step, the bean seeds were disinfected and then they were cultivated in plastic jardinière with 17.5 cm height in 3 to 3.5 cm depth at 25±1 °C and 45 to 50% RH. This step had been completely done at Research Center of Agriculture and Natural Resources of Isfahan.

**Bioassay test**

Direct toxicity to adults (LC$_{50}$ values) was determined using the leaf-spray method. Each leaf was placed on wet cotton in Petri dish and 5 adult female mites were transferred to leaf disc to which treatments were sprayed by a hand sprayer. Mortality was recorded after 24 h. Each concentration of nano particles was tested with 5 replicates and water as control. The Petri dishes were stored in a cabinet at 25±4 °C, 60±4% RH, 16 L: 8D photoperiod.

**Greenhouse test**

For greenhouse test, the T.urticae colonies were transferred on Phaselus vulgarises leaves. The cultivation conditions were as follow: temperature 25±1 °C, relative humidity 70% and 16:8 L: D photoperiod. In the next stage according to the results that obtained from laboratory stage, the concentrations (0, 25, 50, 100, 200, 500, 1000, 2000 and 3000 ppm) of Silver nanoparticles solution were selected. For counting the number of mites, 3 leaves from any bean plant at 24, 72, 120, 168 and 216 h interval were picked and the counting was performed with a hand magnifier with 10X magnification.

**RESULT AND DISCUSSION**

**Nymph and adult mite mortality in bioassay condition using silver nano particle**

Obtained results from bioassay condition using leaf dipping and leaf spray methods showed considerable points so that mortality effect of silver nano particles on adult mites using leaf dipping method in concentrations of 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm showed different effects on mites which is based on increase in concentrations. So the highest mortality effect is related to the times of 48, 72, 96, 120 and 144h using concentrations 500, 1000, 2000 and 3000 ppm and the lowest mortality effect occurred in control group in concentrations of 2.5, 5 and 10 ppm. Also using leaf spray method at different mite life stages showed that the highest mortality effect happened at the times of 24, 48, 72, 96, 120, 144 and 168 h using 100, 200, 500, 1000, 2000 and 3000 ppm of silver nano particles. Comparative effect of these two methods showed that of spray method had a better controlling effect on mites so that it can be concluded that silver nano particles are more effective when they are put on the surface rather than penetrating way. Results from leaf dipping method on Nymph mites showed the highest mortality effect using concentrations 500, 1000, 2000 and 3000 ppm of silver nano particles and like the leaf spray method, concentrations 2.5, 5 and 10 ppm showed the lowest mortality effect which is referred to control group. The effect of silver nano particles in these two methods showed that in leaf dipping method the highest mortality effect is related to concentrations of 500, 1000, 2000 and 3000 ppm of silver nano particles on Nymph mites at 96, 120 and 144h. The effect of silver nano particles in leaf spray method at the times
of 24, 48, 72, 96 and 120h on Nymph mites showed the highest mortality effect in concentrations 100, 200, 500, 1000, 2000 and 3000 ppm of silver nano particles in which concentrations 500, 1000, 2000 and 3000 ppm had been shown to have a meaningful effect compared with other treatments (table 1).

<table>
<thead>
<tr>
<th>Concentration(ppm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>0</td>
<td>1.57f</td>
<td>2.60d</td>
<td>1.40f</td>
<td>2.60f</td>
</tr>
<tr>
<td>2.5</td>
<td>1.51f</td>
<td>2.68d</td>
<td>1.46f</td>
<td>2.68ef</td>
</tr>
<tr>
<td>5</td>
<td>1.63f</td>
<td>2.72d</td>
<td>1.60f</td>
<td>2.68ef</td>
</tr>
<tr>
<td>10</td>
<td>1.63f</td>
<td>2.92d</td>
<td>1.69f</td>
<td>2.92e</td>
</tr>
<tr>
<td>50</td>
<td>2.37e</td>
<td>3.24c</td>
<td>2.23e</td>
<td>2.92e</td>
</tr>
<tr>
<td>100</td>
<td>2.71de</td>
<td>3.52c</td>
<td>2.14e</td>
<td>3.60d</td>
</tr>
<tr>
<td>200</td>
<td>2.97d</td>
<td>3.92b</td>
<td>2.89d</td>
<td>3.92c</td>
</tr>
<tr>
<td>500</td>
<td>3.97c</td>
<td>4.68a</td>
<td>3.97c</td>
<td>4.76ab</td>
</tr>
<tr>
<td>1000</td>
<td>4.49b</td>
<td>4.92a</td>
<td>4.49b</td>
<td>5a</td>
</tr>
<tr>
<td>2000</td>
<td>4.83ab</td>
<td>5a</td>
<td>4.83ab</td>
<td>5a</td>
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<tr>
<td>3000</td>
<td>4.97a</td>
<td>5a</td>
<td>4.97a</td>
<td>5a</td>
</tr>
</tbody>
</table>

A: mean of mortality of adults mites using leaf dipping method.
B: mean of mortality of Nymph mites using leaf spray method.
C: mean of mortality of Nymph mites using leaf dipping method.
D: mean of mortality of Nymph mites using leaf spray method.

Calculation of adult mite mortality percentage using different concentrations of silver nano particles by leaf spray and leaf dipping methods

Results from adult mites mortality showed that at concentration 100 ppm and by the use of leaf spray method, more than 50% of mites were died and at concentrations 200, 500, 1000, 2000 and 3000 ppm mortality effect increased so that concentration 3000 ppm showed a mortality effect more than 90% (figure 2). Figure 3 shows that leaf spray method is more effective than leaf dipping method.
Calculation of Nymph mite mortality percentage using different concentrations of silver nano particles by leaf spray and leaf dipping methods

Results from Nymph mites mortality showed that at concentration 100 ppm and by the use of leaf spray method, about 50% of mites were died and at concentration 200 ppm mortality effect increased up to 50% so that concentrations 500, 1000, 2000 and 3000 ppm showed the highest mortality effect. Using leaf dipping method (3000 ppm) on Nymph mites caused 90% mortality (figure 4). Leaf dipping method using 1000, 2000 and 3000 ppm showed the highest percentage of mortality (figure 5).
The two spotted spider mite, T. urticae is one of the most important agricultural pests that annually destroy 10-15 % of agricultural products (Raworth, 1986). The two spotted spider mites is the most important specie from 1200 species of Tetranychidae family and can proliferate and propagate very quickly and also this species can be adapted to different environmental conditions. Furthermore they had a broad host range (Bollend et al., 1998). This pest is now considered as polyphagous species and reported as the first resistant pests to the pesticides (Hussey and Scopes, 1985). Until now this pest showed resistance to 80 types of pesticides and its economical restriction because of increase in its resistance to pesticides and its growth rate is growing daily (Cranham and Andhelle, 1985). As mites has an exceptional intrinsic potential for development of resistance to much acaricides, so the species of Tetranychidae become a global phenomenon (Cranham and Andhelle, 1985; Van Leeuwen et al., 2009). Today developments in Nano science caused many gains with agricultural uses. These developments from one
side lead to modernization of old agriculture and cause to produce wide range of products and from another side increased the human control over natural resources. Silver is a material with bactericidal effect, and in smaller sizes has more influence (Sharma et al., 2009). In this study results from bioassay condition by leaf dipping and leaf spray methods showed significant points so that mortality effect of silver nanoparticles on adult mites using leaf dipping method in concentrations of 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm showed different effects on mites which is based on increase in concentrations.

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REFERENCES


