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Research Article

# The efficiency of ordinary and nano extract of Silverleaf Nightshade (*Solanum elaeagnifolium* Cav.) seed in controlling the Mealybug (*Phenacoccus solenopsis* Tinsley)

**Worood A. Gorge[1*], Mohammed F. Abood[1], Khalid W. Ibade[2]**

[1] College Education for Pure Science, University of Anbar, Anbar, Iraq.
[2] College of Agriculture, University of Anbar, Anbar, Iraq.
[*]Email: woroodadeil@gmail.com

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## Abstract

The excessive use of chemical insecticides has led to the emergence of many environmental problems, which was reflected in the reconsideration of returning to the use of alternative methods, including the use of plant extracts. The study was conducted in 2023 to test the efficiency of the Ordinary and nano extract of silverleaf nightshade (*Solanum eleaegnifolium* Cav.) seeds in the mortality percentage of nymph stage and adults of the mealybug (*Phenacoccus solenopsis* Tinsley) in the laboratory after 1, 3 and 7 days of treatment. Results showed that the highest percentage of cumulative mortality of the first nymph stage reached 100% after seven days when treated with alcoholic extract of silverleaf nightshade seeds at the concentration of 5% and 100% for all concentrations used of the nano alcoholic extract. The mortality percentage the third nymph stage was 77.5% at the alcoholic extract concentration of 5% and 87.5% for the nano alcoholic extract.

The mortality percentage of adults caused by the alcoholic and nano alcoholic extract in the same concentration was 84.5 and 87.1%, respectively.

**Keywords**: Cone-Beam Computed Tomography, Dental Radiography, Electronic Apex Locator, Tooth Apex

## Introduction

Mealybug (*Phenacoccus solenopsis* Tinsley) is one of the most common types of mealybugs. It spreads widely all over the world and causes severe damage to economic, horticultural, and ornamental importance plants, including cotton, tobacco, mango, papaya, citrus, cashew, cassava, jasmine, and hibiscus (Zala et al., 2021). Mealybugs are soft-bodied insects, and most stages of their life cycle are covered with white mealy wax; therefore, insecticides are ineffective against them. About 5,000 species have been recorded on 246 plant families worldwide, and approximately 70% of mealybugs are considered invasive pests (Millar et al., 2002). Mealybugs affect plants by absorbing the sap and secreting large amounts of sugar and water as a substance rich in sugar and carbohydrates known as honeydew, attracting ants and causing secondary damage, including the growth of black mold or what is called *Aspergillus niger*, which leads to reducing photosynthesis, plant weakness, yellowing, and leaf fall (Fang et al., 2020). Biological control is a safe and efficient method of reducing harmful pests in agriculture (Riseh et al., 2022). Plant extracts can be used as a part of this method to prevent the accumulation of harmful toxins and metals from the polluted ecosystem (Yaashikaa et al., 2022). Plant extracts were widely used as plant insecticides before developing synthetic insecticides. They occur naturally in plants as secondary compounds and act as repellents (Stankovic et al., 2020). Silverleaf nightshade (*Solanum elaeagnifolium* cav) is a harmful weed and represents a severe threat to agriculture (Karmezi et al., 2022). It is classified as a harmful and poisonous silver plant for both livestock and humans, threatening agricultural productivity and biodiversity around the world, as it competes with crops, causes damage to livestock, acts as a host for insects and plant diseases, and causes hallucinations, paralysis, and death through damaging the intestinal and nervous system systems (Sastry et al., 2019). Using nano-sized particles has led to enhancing crop productivity in agriculture and protecting plants from pests (Alfadul et al., 2017), which is due to the high ratio of their surface to their ultra-small size. The biological method is considered the best way to synthesize nanomaterials, and this is consistent with the advantage of renewable, cost-effective, and biodegradable substance sources in contrast to chemical and physical methods that are unsafe for the environment, expensive and require complex conditions when working (Iravani et al., 2014). Given the dramatic losses in plants caused by mealybug, studies related to the infestation and its control have expanded over the years to find new methods and means to reduce its activity and desire to reduce the use of chemical insecticides for control as a result of their harmful effects and the pollution they cause to the environment as well as the humans in the short and long term as the final consumer of these crops in addition to the emergence of resistance to pests against manufactured chemical pesticides (Oliveira et al., 2002). Modern study trends have focused on this domain, including the idea of searching for effective natural plant compounds, including the seeds silverleaf nightshade herb, which is widely spread in the Iraqi environment and contains effective secondary compounds that have an effect on important in combating mealybugs *P. solenopsis*.

## Materials methods

The experiment was conducted at the laboratories of the Biology Department - College of Education for Pure Sciences / University of Anbar in 2023. Mealybug was raised on sprouts of squash and potatoes grown under laboratory conditions. It was incubated at 30 ± 2°C and a relative humidity of 60-80%. The insect growth was monitored, and the first and third nymph stages and adults were isolated. The insect was identified morphologically at the Natural History Museum / Baghdad and molecularly at the Wahj Al DNA Center, Baghdad - Iraq, with PCR to study the insect by recognizing the nucleotide sequences of the double-stranded DNA, and comparing them with the International Gene Bank. Samples of silverleaf nightshade plants were collected from Al-Qaim, west of Anbar Governorate, and identified morphologically by the Desert Studies Center/Anbar University Herbarium. The plants were rinsed with running water and dried under air conditions, and the seeds were collected, ground, and placed in polyethylene bags in a refrigerator until use.

**Preparation of plant extract from the silverleaf nightshade (*S. elaeagnifolium*) seeds**

The aqueous extract of eggplant seeds was prepared by taking 3, 4, and 5 g of the ground seeds and mixing them with 100 ml of deionized water, heating at a temperature of 60 °C and stirring for an hour, then filtering the solution with a piece of cloth and taking the filtrate for using it in the laboratory experiment. The alcoholic extract was prepared with a Soxhlet extractor using absolute ethanol as a solvent. The extract was collected and dried in an incubator at 60°C (Harborne, 1998). The nano alcoholic extract was prepared according to the procedure described by (Abdullah & Mohammed, 2021) through the reaction process of magnesium nitrate dissolved in water and its transformation into its ionic state, as the phytochemicals decompose at temperatures to produce oxygen. The oxygen binds to the magnesium to form the Mgo nanomaterials. A sample of this extract was subjected to atomic force microscopy (AFM) to determine crystalline size.

**Effect of silverleaf nightshade (*S. elaeagnifolium*) seed extract on mealybug stages.**

Leaves of ornamental plants were collected and examined under a microscope to ensure that they were free of any infection. Sterile nine cm Petri dishes containing sterile filter papers were used in this experiment. In each dish, one of these plants' leaves was placed, in addition to cotton soaked in water, and ten insects of each stage (the first and third nymphs and adults) were released with four replicates for each mentioned stage. Information was recorded on each dish regarding the type of extract, its concentration, the stage, and the replicate number. Each treatment was sprayed with 1 ml of the three prepared extracts (aqueous, alcoholic, and nano) under laboratory growth conditions separately according to the type and concentration of the extract. The dishes were placed inside the incubator at a temperature of ±302°C and a humidity of 60-80%, and data involving the number of insects killed were recorded daily for seven days from the treatment, then corrected using the Orell and Schneider equation as follows,

$$\text{Corrected mortality percentage } (\%) = \frac{\text{mortality percentage at treatment} - \text{mortality percentage at control}}{100 - \text{mortality percentage at control}} \text{x}100$$

In this experiment, the chemical insecticide Goldti was used at the recommended concentration (1 ml/L water) as a positive control sample, while water was used as a negative control sample. The experiment was analyzed according to a completely randomized design (C.R.D.), based on the Least Significant Difference (L.S.D.) at the significance level of P≤0.05.

## Results and discussion

### Morphological identification of mealybug (*phenacoccus solenopsis*)

The insect was morphologically identified at the Natural History Museum / University of Baghdad, recognizing its scientific name as *phenacoccus solenopsis* (figure 1), which is a type of harmful economic pest that affects vegetables, cotton, and ornamental plants.

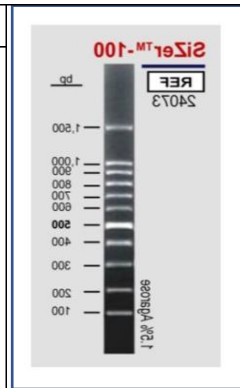
**Figure 1.** Adults of the mealybug *Phenacoccus solenopsis*

### Molecular identification of the mealybug

Results of comparison between the nucleotide sequences of the DNA strand amplified with the primer 28S and the sequences reserved in the World Gen Bank showed that the genetic region matches 100% with the gene of the mealybug *Phenacoccus solenopsis*. Table 2 refers to the electrophoresis of the PCR results using the insect-specific primer. The figure demonstrates a single band for the insect with a molecular size of 500 base pairs.

**Table 2.** Molecular identification of the mealybug

| Phenacoccus solenopsis | |
|---|---|

| No. | Sequence ID with compare | Source | |
|---|---|---|---|
| 1 | ID: KJ461274.1 | Phenacoccus solenopsis 28S ribosomal RNA gene, |  |

**Nano formula of the silverleaf nightshade seeds**

The result of examining the silverleaf nightshade seeds extract with an atomic force microscope (AFM) showed that the size of the atoms is 76.5 nm, which is within the nanoscale limits (Figure 2), showing a three-dimensional image of nano-magnesium nitrate prepared using the silverleaf nightshade extract with the homogeneous distribution of the nano substance and the spherical shape of molecules.

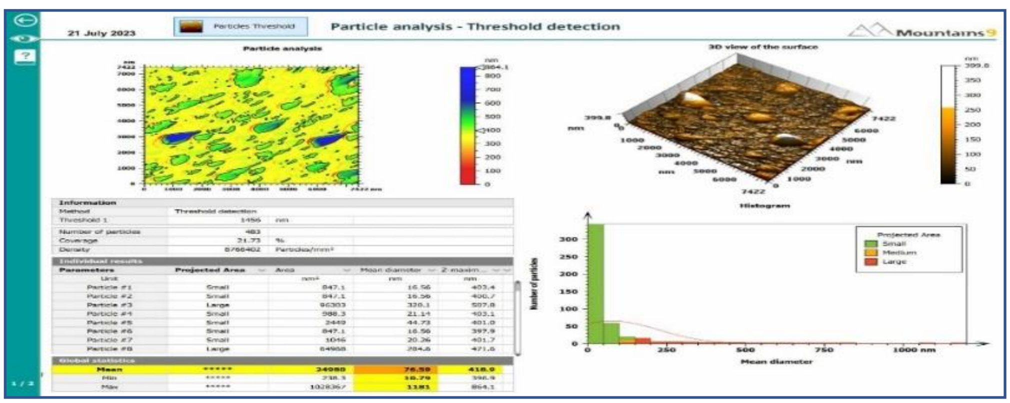



**Figure 2.** The distribution of granular accumulation of magnesium nano particles.

**Effect of the silverleaf nightshade (*S. elaeagnifolium*) seed extract on the first nymph stage of mealybug**

Results in Table 2 illustrated that the aqueous extract significantly decreased the cumulative mortality percentage of the first nymph stage, achieving a mortality percentage ranging from 16 to 69% after seven days of treatment. The highest mortality percentage was 100% obtained from all concentrations prepared in the nano-alcoholic extract, while 52-100% in the alcoholic extract. In the same table, we notice that the chemical insecticide achieved mortality by 100% within a day after treatment. These results are consistent with the results reached by (Zala et al., 2021) when treating mealybugs with extracts of *Azadirachta indica*, *Allium sativum*, and *Datura stramonium*, which caused an evident effect on the first nymph stage in a great deal which is due to the lack of the wax layer small size of the nymphs at this stage. Likewise, the high mortality percentage at the first nymph stage may be due to the toxic compounds that are secondary nutritional metabolites of the silverleaf nightshade herb. These substances affect and interfere with some of the physiological activities necessary for the growth and survival of insects, as some plant compounds act in killing epithelial cells lining the middle gastrointestinal tract of an insect that feeds on these compounds, or these toxic compounds affect the nervous tissue of the insect, causing paralysis and then failure to continue growing (Bowers, 1984). This is consistent with what he mentioned the (Lengai et al., 2020) the death of insects may be due to the action of some plant extracts in killing the cells of the insect feeding on these compounds, which leads to paralysis and subsequent death, or that these toxic compounds have anti-feeding and repellent properties, stimulate moulting abnormalities, hinder egg laying, and disrupt the endocrine system.

**Table 2.** Effect of the extract of the silverleaf nightshade seeds on the mortality percentage of the mealybug first nymph stage

| **silverleaf nightshade seed extract** | | **Mortality percentage of the mealybug first nymph stage (%)** | | |
|---|---|---|---|---|
| | Concentration | 1 day | 3 days | 7days |
| Aqueous extract | 3g | 22.5 | 30.0 | 36.0 |
| | 4g | 32.5 | 32.5 | 36.0 |
| | 5g | 45.0 | 67.5 | 69.3 |
| Alcoholic extract | 3g | 75.0 | 77.0 | 94.4 |
| | 4g | 72.5 | 75.0 | 94.4 |
| | 5g | 92.5 | 95.0 | 100 |

| | | | | |
|---|---|---|---|---|
| Nano extract | 3g | 100 | 100 | 100 |
| | 4g | 100 | 100 | 100 |
| | 5g | 100 | 100 | 100 |
| Goldti Insecticide | 1ml | 100 | 100 | 100 |
| Control (water) | 1ml | 0.0 | 0.0 | 2.5 |
| L.S.D. P<0.05 | | 11.7 | 12.1 | 8.8 |

**Effect of the extract of the silverleaf nightshade (*S. elaeagnifolium*) seeds on the mortality percentage of the mealybug third nymph stage**

Results in Table 3 refer to a positive relationship between the mortality of the third nymph stage treated with the alcoholic and nano extract of the silverleaf nightshade herb and increasing the concentration used in the treatment. The mortality percentage for the three concentrations of both the nano- and alcoholic extracts of the seeds was significantly high. The nano-extract achieved the highest mortality percentage, ranging between 42.5 and 87.5% seven days after treatment. In comparison, it ranged between 32.5 and 77.5% in the alcoholic extract, while the aqueous extract showed a noticeable decrease in the mortality percentage of the third nymph, reaching 22.5-37.5%. The positive relationship between the mortality percentage and the concentration of the plant extract used in the current study treatment is similar to the relationship found by (Jbilou et al., 2006), who referred to an increase in the cumulative mortality percentage with an increase in the concentration, as it was found that the cumulative mortality percentage in the larvae of the red rusty flour beetle reached 58% after ten days of treatment with the alcoholic extract of *Piganium harmalla*, while it was just 14% after two days of treatment. The efficiency of the regular and nano alcoholic extract may have affected the level of some enzymes and components in the insect's body, which affected its death. This is consistent with what he mentioned the (Madasamy et al., 2023) the effect of plant extracts, including neem, as an aqueous extract loaded on silver nanoparticles, can reduce the aqueous enzymes present in the insect, including amylase, protease, invertase, and glycosidase. It also leads to a decrease in the level of carbohydrates, fats, and proteins by 10%, which weakens the insect's ability to feed and thus destroys it .

**Table 3.** Effect of the extract of the silverleaf nightshade seeds on the mortality percentage of the mealybug at the third nymph stage

| **Silverleaf nightshade seed extract** | | **Mortality percentage of the mealybug (%) third nymph stage** | | |
|---|---|---|---|---|
| | Concentration | 1 day | 3 days | 7days |
| Aqueous extract | 3g | 12.5 | 12.5 | 22.5 |
| | 4g | 17.5 | 25.0 | 25.0 |
| | 5g | 27.5 | 27.5 | 37.5 |
| Alcoholic extract | 3g | 17.5 | 17.5 | 32.5 |
| | 4g | 27.5 | 32.5 | 50.0 |
| | 5g | 40.0 | 42.5 | 77.5 |
| Nano extract | 3g | 42.5 | 42.5 | 42.5 |
| | 4g | 52.5 | 62.5 | 62.5 |
| | 5g | 85.0 | 87.5 | 87.5 |
| Goldti Insecticide | 1 ml | 95.0 | 100 | 100 |
| Control (water) | 1ml | 0.0 | 0.0 | 0.0 |
| L.S.D. P<0.05 | | 12.8 | 10.7 | 11.1 |

**Effect of the extract of the silverleaf nightshade (*S. elaeagnifolium*) seeds on the mortality percentage of the adult stage of mealybug**

Results in Table 4 showed that the aqueous extract of silverleaf nightshade seeds gave a noticeable decrease in the cumulative mortality percentage of the first nymph, ranging between 22.5 and 37.5% after seven days of treatment for the three concentrations, while the mortality percentage ranged between 32.5 and 77.5% in the alcoholic extract, while the nano extract achieved the highest mortality percentage, ranging between 42.5 and 87.5%. From the results of this study, we notice that the nano-alcoholic extract gave more efficient results in killing the mealybug than the alcoholic extract beginning from the third day after treatment, which is consistent with what was reported that nanoparticles caused an increase in the mortality percentage at the first, second, and third nymph and adults stages of the mealybug compared to the non-nanoparticles due to penetration of the nanoparticles into the insect's membranes and binding to proteins containing sulfur or phosphorus, as is the case in DNA, and this led to the destruction of organelles and enzymes. (Mariappan et al., 2022; Rai et al., 2009).

**Table 4.** Effect of the extract of the silverleaf nightshade seeds on the mortality percentage of the adult mealybug

| **Silverleaf nightshade seed extract** | | **Mortality percentage of the mealybug (%) adult** | | |
|---|---|---|---|---|
| | **Concentration** | **1 day** | **3 days** | **7days** |
| Aqueous extract | 3g | 2.8 | 12.5 | 23.9 |
| | 4g | 20.0 | 27.5 | 24.8 |
| | 5g | 27.5 | 37.5 | 40.9 |
| Alcoholic extract | 3g | 32.5 | 40.0 | 46.1 |
| | 4g | 30.0 | 42.5 | 64.0 |
| | 5g | 37.5 | 45.0 | 84.5 |
| Nano extract | 3g | 40.0 | 50.0 | 51.2 |
| | 4g | 40.0 | 70.0 | 71.7 |
| | 5g | 37.5 | 85.0 | 87.1 |
| Goldti Insecticide | 1 ml | 92.5 | 100 | 100 |
| Control (water) | 1ml | 0.0 | 0.0 | 2.5 |
| L.S.D. P<0.05 | | 12.9 | 13.0 | 12.8 |

## Conclusion

Returning to using plant extracts is a wise decision that can be conducted by testing the efficiency of the Ordinary and nano extract of silverleaf nightshade seed on the mortality percentage of mealybug (*Phenacoccus solenopsis*) nymphs and adults grown in the laboratory after days of treatment. It has been proven that the efficiency of the alcoholic extract in increasing the mortality percentage of the insect at different stages, and the nano alcoholic extract showed the same efficiency in killing insects at lower concentrations, which may be, in turn, reflected positively in the environment and economic returns.

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