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Magnesium Oxide Nanoparticles Synthesized from *Solanum elaeagnifolium* and their Effect Against Mealybugs *Phenacoccus solenopsis* Tinsley

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The excessive use of chemical insecticides and the emergence of accompanying environmental problems has led to a return to environmentally friendly plant extracts through nanotechnology and the production of particles loaded on many metals to control insects. Accordingly, the study was conducted in 2023 to investigate the efficiency of the nano- extract of the seeds and leaves of the nightshade *Solanum elaeagnifolium* Cav loaded on magnesium, producing MgO nanoparticles and the regular alcoholic seed extract with a concentration of 5% on the relative efficiency of killing the first and third nymph instars as well as the adults of the mealybug *Phenacoccus solenopsis* Tinsley, grown on moss rose seedlings. The results showed that the highest relative efficiency in killing the insects at the first instar was 100% three days after treatment with nano seed extract, 84% at the third instar, and 75% for mealybug adults after seven days of treatment. In contrast, nano extract of nightshade leaves recorded a relative mortality efficiency of 91.6, 47.4 and 54.3% for the first and third nymph instars and the adults, respectively, and 97.1, 55.0 and 58.7% for the regular alcoholic seed extract after seven days of treatment.
Keywords: Alcoholic extract, nightshade, nanotechnology, nanoextract, mealybug.

INTRODUCTION

Mealybug, *Phenacoccus solenopsis*, is a harmful pest that invades and infects crops, vegetables, and ornamental plants and is widely spread worldwide (Caliscan, 2019). Mealybugs transmit viral diseases when they feed on plants by piercing and sucking plant sap, thus facilitating the infection of secondary diseases and reducing the nutritional and aesthetic value of the plants they infect (Culik and Gullan, 2005). This insect secretes honeydew on leaves and other plant parts, constituting a medium for the growth of fungi, including gray mold fungi, which reduces the efficiency of photosynthesis (Saeed *et al*., 2007). The indiscriminate use of agricultural chemical pesticides, most commonly used for pest control, can cause environmental problems and human health risks. The need for environmentally friendly insecticides, including plant extracts, has emerged as a safer natural alternative than synthetic ones (Damalas *et al*., 2020). Therefore, modern study directions have focused on this field, including the idea of searching for effective natural plant compounds, including the seeds and leaves of the silverleaf nightshade herb *Solanum elaeagnifolium* Cav, which is widely spread in the Iraqi environment, because it contains effective secondary compounds having an essential effect in controlling pests. Silverleaf nightshade is classified as a harmful plant poisonous for both livestock and humans, as it threatens agricultural productivity and biodiversity around the world, competes with crops, causes damage to livestock, and acts as a host for insects and plant diseases. This plant is rich in solanidine, a toxic substance belonging to the group of glycoalkaloids. Some studies state that the seeds and leaves of silverleaf nightshade have insecticidal and repellent properties against many crop pests (Hamouda *et al*., 2015). The HPLC analysis showed that the fruit and leaf extracts of the silverleaf nightshade herb were rich in the main substances: Luteolin, Quercus, Gallic acid, and Neringin (Bouslamti *et al*., 2022). Nanotechnology is one of the latest sciences that has caused a breakthrough in many fields by improving the properties of matter and giving it unique, more effective properties by controlling the arrangement of its atoms (Muto *et al*., 2020). Nanomaterials have distinctive and unique properties, including a large surface area relative to their size, which may enhance their effect on living organisms. Recent studies have tended to the use of plant extracts and chemicals relying on nanotechnology by producing particles loaded with many metals, including silver (Ag), and oxides of some metals such as zinc oxide (ZnO), magnesium oxide (MgO), and iron oxide (Fe3O4) for use in

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pest control (Alloosh 2020). One of this method's advantages is that it is environmentally friendly and does not require energy (Ribeiro *et al.*, 2020). It is expected that by the year 2026, the global production of metallic nanoparticles will reach 10,000 tons and total sales will reach 50 billion dollars (Ovais *et al.* 2018). Therefore, the study aimed to test the nano-extract of the seeds and leaves of the silverleaf nightshade loaded with magnesium and the producing MgO nanoparticles and the regular alcoholic extract of the seeds and testing their efficiency in controlling mealybugs on moss rose plants under the field conditions.

MATERIALS AND METHODS

Raising the mealybug *Phenacoccus Solenopsis*: The experiment was conducted in a garden on August 15, 2023. The mealybug was raised on some plants, such as ornamental plants, potatoes, and tomatoes, as August is one of the months in which the insect grows abundantly due to the appropriate conditions of temperature and humidity since the temperature was recorded at 40-44 °C and the humidity was 60 -70. All stages of insect growth were prepared, including eggs, nymphs, and adults, to use in the field experiment.

Preparation alcoholic extract of seeds of *S. elaeagnifolium*: The extraction was prepared in the laboratories of the Biology Department - College of Education for Pure Sciences of University of Anbar in 2023 by taking 100 g of powdered seeds and leaves of the nightshade and placing each of them in a 1000 ml glass beaker, then adding 500 ml of absolute ethanol to it. The flask was lid with a stopper and shaken with an electric shaker. Next, the extract was filtered using a piece of cloth. After that, the final filtrate was collected and concentrated using a rotary vacuum evaporator at a temperature of 40 °C to get rid of the solvent, obtaining a thick liquid, and then this process was repeated several times to get an appropriate amount of the extract. Finally, the extract was placed in a sealed glass bottle and kept in the freezer until use (Harborne, 1984).

***Converting the extract of nightshade seeds and leaves into the nano formula*:** The nano extract was prepared according to the procedure described by Osama and Mohemmed (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 give oxygen, which binds to the magnesium, forming the MgO nanomaterials. A sample of this extract was taken for an atomic force microscope (AFM) to determine crystal size.

***Field experiment*:** Seedlings of the moss rose plant, *Portulaca grandiflora*, at the same age planted in plastic pots were prepared. They were examined to confirm they were free of pest infestation. The previously bred mealybug at three stages (eggs, first nymph instar, third nymph instar, and adults) were released onto the seedlings to establish themselves on the plant. After leaving them for two weeks to grow, the reading before treatment was recorded, as a branch well infested with insects was selected, and the number of various stages on it was counted. Then, the infested target branch was surrounded by a transparent rectangular plastic cage perforated on all sides dimensioned 8*6*3 cm (Figure 1). The treatments, including an alcoholic extract of nightshade seeds, nano extract of seed and leaves loaded with magnesium, producing MgO particles at a concentration of 5% after one day of reading, were applied, in addition to the control treatment (water and the commercial insecticide Goldti, Lambda-cyhalothrin 5% EC) at a concentration of 1 ml-1. The experiment included five treatments for each insect stage with three replicates. The treatments were distributed according to the completely randomized design (CRD). The mortality percentage of each stage was recorded after 1, 3, and 7 days of treatment. The relative efficiency was calculated according to the equation:

$$\text{Relative efficiency} = \frac{\text{number of indivisuals after spray}}{\text{number of indivisuals before spray}} \times \frac{\text{number of indivisuals at the control before spray}}{\text{number of indivisuals at the control after spray}} \times 100$$

Figure 1. Application of alcoholic and nano extracts of seeds and leaves to insects.

RESULTS AND DISCUSSION

***Nano-formula of wild nightshade seed extract*:** Examining the silver-leaf nightshade seed extract with an atomic force microscope (AFM) showed the size of the atoms to be 76.5 nm, which is within acceptable nanoscale limits. Figure 2 demonstrates a three-dimensional image of magnesium oxide (MgO) nanoparticles prepared by the wild nightshade extract, exhibiting the nanomaterial's homogeneous distribution and the spherical shape of the particles.

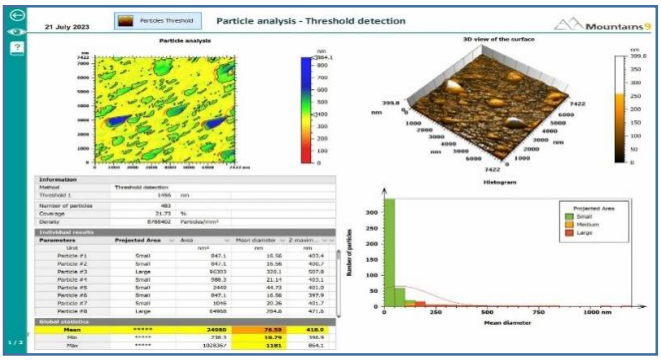

Figure 2. The topography and distribution of granular accumulation of MgO nanoparticles.

***Field experiment*:** After conducting the experiment procedure by spraying the nano extract loaded with magnesium, producing MgO particles for the seeds and leaves, and the regular alcoholic extract of the wild nightshade on the seedlings of the moss rose plant and monitoring them for seven days, the results were recorded and statistically analyzed. The figures below refer to the mortality percentage occurring in the different insect stages of the mealybug, including eggs, nymphs, and adults. Figure 3 denotes the unhatched egg percentage, as the regular alcoholic extract of the seeds recorded the highest relative efficiency of 96.9%, which did not differ significantly from the other treatments of nightshade extract. The nano extract treatment of the leaves, the nano extract of the seeds, and the chemical pesticide Goldti recorded a relative efficiency, reaching 96.8%, 96.8%, and 100%, respectively. They all differed significantly from the water spray treatment, which recorded a relative efficiency of 5.6%. The cause of the egg mortality may be due to the entry of the toxic compounds present in the nightshade herb in regular and nano forms into the egg, causing rapid mortality of the embryo in the egg through its direct cytotoxic action due to its penetrance through the chorion of the egg, leading to the failure of the embryonic development process; or, it may be due to its effect on the embryo muscle tissue, causing damage to the effectiveness of the egg's outer shell, which causes the embryo to lose its ability to hatch (Nisar *et al*., 2021). The results of the effect of the alcoholic extract of nightshade herb loaded with MgO particles as egg-fatal substances are consistent with findings obtained by Madhiyazhagan *et al*. (2015) that the aqueous extract as a nano-silver form from *Sargassum muticum* algae played a role in killing the eggs of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*.

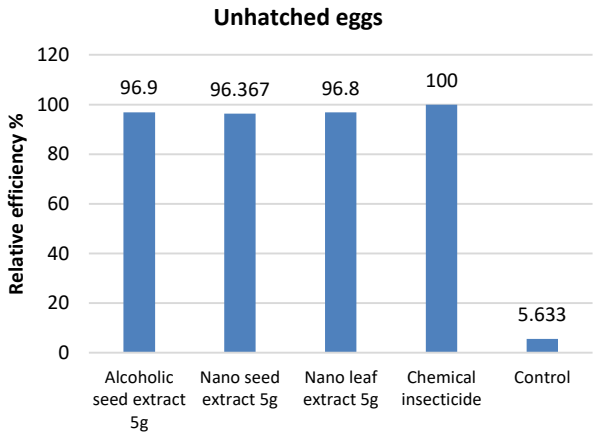

Figure 3. Effect of nightshade extract on the percentage of mealybug unhatched eggs.

Figure 4 illustrates the effect of treatments on the first nymphal instar of the mealybug. It reveals that the nano extract of the seeds recorded a relative mortality efficiency of 100% three days later, and the regular alcoholic extract recorded a relative mortality efficiency of 57.1% three days later. In comparison, the nano extract of the leaves recorded a relative efficiency of 90.4%. This result is considered good as the propagation of the mealybug can be controlled by consistently targeting the first instar nymphs, thus blocking the completion of the rest of the instars, which are difficult to control due to their protective waxy layer. This result is consistent with the results reached by Zala *et al*. (2021) when treating mealybugs with extracts of *Azadirachta indica*, *Allium sativum*, and *Datura stramonium*. These extracts dramatically caused an apparent effect on the first nymphal instar because the nymphs in this stage lack the wax layer and are also small in size. Also, the reason for the high mortal percentage of the insect at the first nymph instar may be due to the presence of toxins that are secondary metabolites of the nightshade herb, which affect and interfere with some of the physiological activities necessary for the insects' growth and survival, which is consistent with the findings of Lengai *et al*. (2020) who reported that the insects' mortality affected by some plant extracts might be due to the feeding on these compounds killing the cells of insects, which leads to paralysis and subsequent death, or that these toxic compounds have anti-feeding and repellent properties, stimulate ecdysis abnormalities, hinder egg-laying, and disrupt the endocrine system.

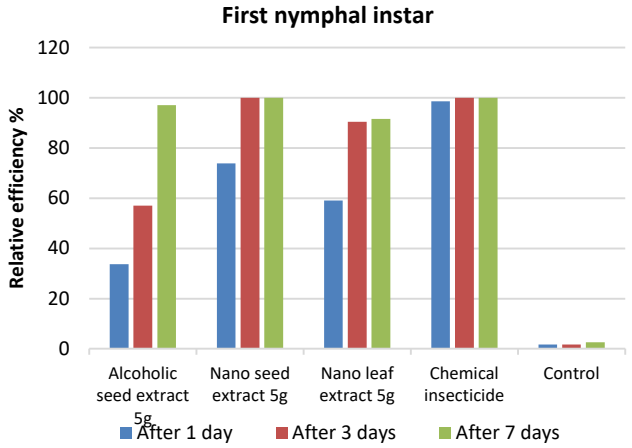

Figure 4. Relative efficiency of nightshade extract on the insect's first nymphal instar.

Results of Figure 5 showed that the nano extract of nightshade seeds caused a noticeable increase in the relative efficiency of killing the insects at the cumulative third nymph instar, achieving an efficiency of 84.0% after seven days of treatment, while it was 55% at the regular alcoholic extract, while the nano extract of nightshade leaves gave a relative efficiency of 47.4%. In the same figure, we observe that the chemical insecticide gave a relative efficiency of 96% after three days of treatment. The efficiency of the regular and nano extract may have affected the level of some enzymes and components in the insect's body, resulting in its death. This result is consistent with what was stated by Madasamy *et al*. (2023) that the effect of plant extracts, including neem aquatic extract, loaded on silver nanoparticles, could reduce the water enzymes present in insects, including amylase, protease, invertase, and glycosidase, it also decreased the level of carbohydrates, fats, and proteins by 10%, which weakens the insect's ability to feed and thus causes its mortality.

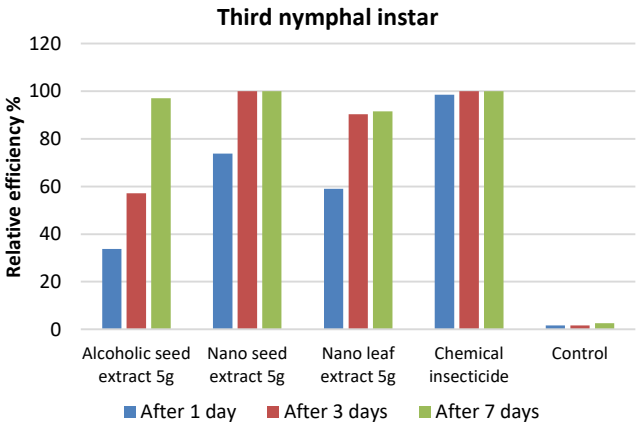

Figure 5. Relative efficiency of nightshade extract on the third nymphal instar.

Figure 6 shows the effect of the treatments on mealybug adults, indicating that the nano extract of the seeds recorded a relative mortality efficiency reaching 75% three days after treatment. The regular alcoholic extract recorded a relative mortality efficiency of 41.1% after three days, while the nano extract of the leaves recorded a relative efficiency of 38.5%. The efficiency of the nano extract has been proven to fatal insect adults in shorter periods, and this may, in turn, be reflected in the economic and environmental returns. We notice from the same figure that the chemical insecticide gave a relative efficiency of 96.4% three days after treatment. Results showed that adults were less sensitive than nymphs when treated with the herb extract. This result is consistent with those obtained by El-Sayed Ibrahim *et al*. (2023) at a laboratory and field study, where essential vegetable oils were extracted from the seeds of different plants and tested to ensure their efficiency as repellent insecticides against adults and second nymph instar of the mealybug on the cotton crop. According to the results of this study, the nano extract gave more efficient results in killing mealybug at different instars than the regular alcoholic extract beginning from the third day after treatment, and this is consistent with what was reported that the nanoparticles increased the mortality rate at the first, second, and third nymphal instars as well as the adults of the mealybug, compared with non-nanoparticles, as the nanoparticles penetrating the insect membranes and binding to proteins containing sulfur or phosphorus, as is the case in nanoparticles, thus leading to the destruction of organelles and enzymes (Rai *et al*., 2009).

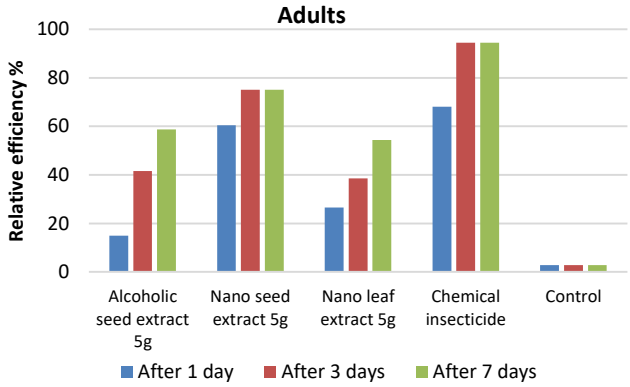

Figure 6. Relative mortal efficiency of nightshade extract on the mealybug adults.

***Conclusion*:** The decision to reconsider the use of plant extracts is a correct one, which was demonstrated through conducting a study investigating the efficiency of the ordinary and nano extract of nightshade *Solanum eleaegnifolium* Cav loaded on magnesium and producing of MgO particles on the relative mortality efficiency of killing the mealybug *Phenacoccus solenopsis* Tinsley at the various stages in the field after days of treatment.

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***Ethical statement*:** the study is in vitro study mainly and conducted according to laboratory ethical protocols.

***Availability of data and material*:** Data are available when requested by journal.

***Consent to participate*:** All authors of present manuscript are participated in present research-work.

***Informed consent*:** N/A

***Consent for publication*:** All authors of present manuscript are agreed and confirmed draft for publication of present work.

***SDGs addressed*:** Zero Hunger, Good Health and Well-Being, Responsible Consumption and Production.

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