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Effect of *Melaleuca viminalis* Essential Oil and Biosynthesized Silver Nanoparticles on Mortality of *Sitophilus oryzae*

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ABSTRACT

Background: Insects Bio-control specially botanicals have received more attention nowadays. Essential oils are extracted from plants as a natural eco-friendly substance and as an insecticidal property. Moreover, it can be used for synthesis of nano particles pesticides. The current study aimed to use the essential oil of *Melaleuca viminalis* leaves as pesticides, synthesis of silver nano particles and verify their effectiveness on mortality of rice weevil under laboratory conditions.

Methods: The essential oil extracted from leaves of *M. viminalis* was used at concentrations (10, 20, 30 µL L^{-1} air) and silver nanoparticles (2000, 4000, 6000 mg L^{-1}) at three exposure periods (24, 48, 72 hours) to assess performance against rice weevil larvae and adults.

Result: The mortality rate of adults and larvae increased with increasing concentrations of both insecticides. For larvae after 72 hours was reached to 100% and 93.3% at 6000 mg L^{-1} and 30 µL L^{-1} air for silver nanoparticles and *Melaleuca* oil, respectively. Adults mortality after 72 hours, was 100% and 90% at 6000 mg L^{-1} and 20 µL L^{-1} air for silver nanoparticles and *Melaleuca* oil, respectively. It was demonstrated from the results that the treatment of silver nanoparticles with low concentration was more effective in mortality rate than *Melaleuca* oil. Therefore, it can be concluded that the use of nanotechnology for pest control is more economical due to lower lethal doses.

Key words: *Melaleuca viminalis,* Nanoparticles, Rice weevil, *Sitophilus oryzae*, Volatile oils.

INTROUCTION

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), a cosmopolitan pest of stored grains, infests all cereal grains. It reproduces rapidly and the larvae and adults of this weevil voraciously gnaw through the grain. The larval feeding process causes the grain to become husk-like, causing significant damage to agriculture (Baker *et al*., 1999). Seeds and grains become unacceptable due to the insect's secretion of uric acid, which makes the nutritional value of the grains poor (Trematerra *et al*., 1999; Al-Hayali and Fadel, 2024a, 2024b).

Continuous, repeated and long-term use of chemical pesticides leads to the development of resistance to the pesticide's effect, in addition to harmful negative effects such as toxic residues on grains, environmental pollution and their impact on non-target organisms, not to mention high costs (Arthur, 1996; Cui, 2021). Among these pesticides is phosphine gas, despite its good characteristics as a fumigation insecticide (Porca *et al*., 2003), it has been found that many insects that attack grains are resistant to its effects, including *S. oryzae* . Therefore, there is a need to avoid using chemical fumigants that pollute the environment (Rajendran and Sriranjini, 2008).

Therefore, it is necessary to develop new insecticide formulations (Giunti *et al*., 2019; Al-Hayali *et al*., 2024; Al-Hayali *et al*., 2025a).To reduce environmental pollution resulting from the frequent use of chemical pesticides, it is necessary to use safe alternatives from plant origin, such as extracts of essential oils to test their effect against stored product insects (Al-Hayali *et al*., 2025b, 2025c). The main components of essential oils have also been used to manufacture nano-pesticides and evaluate their efficiency and effectiveness of the effect at low doses. To preserve them, they are coated with micro- and nano-structures to maintain their biological properties and enhance their physical and chemical stability (Hashim *et al*., 2018; Benelli, 2020).

As a result of the decrease in nanoparticle size and the consequent increase in surface area, which allows the natural elements to interact better with the compounds encapsulated in Target site (Pavoni *et al*., 2019). By overcoming the physicochemical instability and solubility issues of essential oils (Turek and Stintzing, 2013) this strategy can enhance the applicability of essential oils as

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natural insecticides (Heydari *et al*., 2020). Therefore, the nature is a rich source of plants, which are the source of essential oils and thus alternative pest management solutions for a wide range of pests (Ghosh *et al*., 2013; Al-Hayali and AL-Zuhairi, 2024; Al-Jayid *et al*., 2025).

Researchers have turned to finding alternative methods to traditional chemical pesticides in order to protect crops from pests by introducing nano-technology to improve the stability of biopesticides and overcome their delivery problems. Lade and Gogle, (2019). Various metals, such as silver, are used to manufacture nanoparticles to combat pests. AgNPs have attracted attention due to their unique magnetic, mechanical and optical properties (Chaloupka *et al*., 2010). Overall, nanotechnology appears to increase the stability and efficacy of natural plant products against pests and reduce their costs (Mishra *et al*., 2017). Due to their small size, large surface area, eco-friendly behaviors and low toxicity, nano-pesticides have many advantages over bio-pesticides (Sahayaraj, 2014; Wang *et al*., 2021). The overall objective is to formulate pesticides with eco-friendly method. The using essential oil of *Melaleuca viminalis* leaves as pesticides, synthesis of silver nano-particles and verify their effectiveness against *S. oryzae* were the specific objectives.

MATERIALS AND METHODS

The study was carried out in the plant protection laboratory of the Directorate of Agriculture, Diyiala, Iraq, in 2025 and *S. oryzae* was bred in 800 ml glass jars at 30-32°C and 60-70% R.H. in an incubator.

The healthy leaves of the *M.viminalis* plants were collected for the purpose of extraction of essential oils required for present study.After washing them in sterile water, they were exposed to ventilation at room temperature for 120 hours to dry. Then grinded using an electric grinder and after obtaining the powdered plant leaves, they were placed in bags and laminated (Mohemed and Abbas, 2017).

Extraction of essential oils

To obtain vegetable oils, 100 grams of leaf powder of *M. viminalis* were transferred to a 1000 ml beaker, then the volume was made upto 1000 ml by adding water. Oil samples were obtained using a Clevenger device through a steam distillation process for 3 hours for each extraction cycle and were separated. The oils were extracted using a separating funnel. The amount of extracted oils from *M. viminalis* was 2% v/w (Mathlouthi *et al*., 2018). Then stored at 4°C in tight, dark containers.

Preparation of AgNPs

Volatile oils extracted from the leaves of the *M. viminalis* were used to prepare silver nanoparticles, as they work to reduce, stabilization and encapsulation of $AgNO_3$. Preparation steps were carried out according to (Lalitha *et al*., 2013). Prepared the standard aqueous solution by adding 8.4935 g of $AgNO_3$ to a 1000 ml glass beaker with a concentration of 50 mmol. and supplementing this volume by adding deionized water. This aqueous solution was heated for 10 minutes at 60°C with continousstiring, then cooled. To be ready to prepare silver nanoparticles. Added 1 ml of the volatile oil of *M. viminalis* to 15 ml of an aqueous solution of $AgNO_3$ in a beaker and placed it in a water bath at 100°C with continuous stirring while boiling for one minute, quickly reducing the $AgNO_3$ ions. It was confirmed the formation of AgNPs by seeing the colour changes from pale yellow to reddish brown.due to biological reduction process (Manju *et al*., 2014). The solution was then placed in opaque and tightly closable 50 ml glass container and stored at refrigerater with 4-5°C temperarture.

Determination of structural characteristics nanoparticles

The structural characteristics of the nanoparticles were determined in terms of shape and size by examination of 24 h air dried droplets silver nanoparticle solution placed on a glass slide under microscope (HITACH-4300 Scanning Electronic microscope). This was done in the laboratories of the Nano and Advanced Materials Research Center at the University of Technology in Iraq.

Details of treatment imposition done in the present investigation Fumigation treatment

Laboratory experiments were conducted in January 2025 using the essential oil of *M. viminalis* at three concentrations of 10, 20 and 30 µL L^{-1} air to know the effect on 7 days aged larvae and 48h aged adults. The Volatile oils were added according to the required concentrations using a micropipette by making discs with a diameter of 5 cm from (Whitman) No. (1) type filter paper, which are provided with the three concentrations of the mentioned oils under study.

The discs are then transferred to the bottom of 1 litre glass containersand released 10 adult individuals, then closed tightly and placed in 30-32°C and 60-70% RH. In the same way, the larvae are treated, after which data are taken on mortality rates after 24, 48 and 72 hours of exposure. The death rates were corrected using the Abbott equation.

Effect of AgNPs solution treatment on larvae and adults *S. oryzae*

The following concentrations (2000, 4000, 6000) mg L^{-1} of silver nitrate nanoparticles were prepared using deionized water. Prepared using extract of Volatile oils (Manju *et al*., 2014). Larvae and adults were treated after transferring 10 individuals separately in plastic Petri dish (dia. 9 cm and a height of 1.5 cm). Then each petridish was sprayed with 1 ml AgNPs solution of above mentioned each concentrations using 50 ml sprayer at a height of 20 cm. There were three replications were maintained for each concentrations and were compared with a petridish sprayed with 1 ml deionized water as a untreated check After spray these dishes containing solution and insects were rolled and air dried for 20 mins to dry moisture present in petridish. Then the dishes were transferred to the incubator at 30±2°C. R.H. is 65±5%. mortality of treated insects were recorded after 12, 24 and 48 hours. The death rates were corrected by Abbott equation (Abbott, 1925).

Statistical analysis

Using a completely randomized design (CRD) and comparing the differences between the averages of the coefficients by Duncan test at the probability level P<0.5, using the SAS statistical program.

RESULTS AND DISCUSSION

Optical and spectroscopic properties of AgNPs

The absorption value of AgNPs was determined by a UV spectrophotometer at a wavelength of 197.08 nm. Different absorption values were recorded, which confirms the difference in the size and shape of the nanoparticles. This difference in values indicates that the essential oils are coated with silver nitrate, with the angle of encapsulation varying according to the different components of the oils and the formation of a new material that carries the Nano scale properties of the silver particles formed.

Size and shape of AgNPs

The shape and size of AgNPs manufactured using *M. viminalis* essential oils were determined using a scanning electron microscope (SEM), an average of 222.29 nanometers and an oval to spherical shape for the nanoparticles. It became clear that the spherical shape was the most stable and that the nanoparticles had good properties. They were formed by the interaction of a large number of biomolecules in the solution. Determining the chemical and biological properties of nanoparticles is related to their shape and size.

Effect of silver nanoparticle concentrations and exposure period on larvae mortality

The data presented in Table 1 indicate of that treating the larvae with extracts of *M. viminalis* essential oil or with silver nanoparticles led to an increase in the mortality achieved progressively with increasing the levels of concentrations used gave the highest mortality rate of 90% at 6000 mg L^{-1} under the influence of silver nanoparticles prepared from *M. viminalis* oils, compared to the fumigation with essential oil, which gave the highest mortality rate of 88.8%. At 30 µL L^{-1} air, which indicates a significant superiority in favor of silver nanoparticles. While the time factor had a significant impact on increasing mortality as exposure periods increased. In terms of the interaction between fumigation with *M. viminalis* oil and silver nanoparticles with exposure periods, it increased the mortality rate, reaching 100% at 4000 and 6000 mg L^{-1} after 72 hours. Which indicates that the treatment with silver nanoparticles was significantly superior to the fumigation treatment with *M. viminalis* oil, which amounted to 100% at 20 µL L^{-1} of air after 72 hours. Nanopesticides have many advantages over essential oils. As a result of their small size and large surface area, high solubility, low toxicity, fast delivery time to plants and eco-friendly behaviors (Wang *et al*., 2021; Arab *et al*., 2022).

Effect of silver nanoparticle concentrations and exposure period on adult mortality

The data presented in Table 2 indicate the significant effect of the treatments used against insect adults, as the highest mortality reached 86.6% at 6000 mg L^{-1} for the AgNPs

Table 1: The effect of treatments concentrations and exposure period on larvae.

Concentrations of treatments	% Mortality			Effect-treatments
	72 hours	48 hours	24 hours	
*Essential oil 10 µL L^{-1} air	60.0^{fg}	76.6^{c-f}	83.3^{a-e}	73.3^{C}
Essential oil 20 µL L^{-1} air	56.6^{g}	93.3^{abc}	100^{a}	83.3^{AB}
Essential oil 30 µL L^{-1} air	80.0^{b-e}	93.3^{abc}	93.3^{abc}	88.8^{AB}
**AgNPs 2000 mg L^{-1}	56.6^{g}	86.67^{a-d}	96.67^{ab}	80.0^{BC}
AgNPs 4000 mg L^{-1}	66.6^{e-g}	93.3^{abc}	100^{a}	86.6^{AB}
AgNPs 6000 mg L^{-1}	70.0^{d-g}	100^{a}	100^{a}	90.0^{A}
Effect of periods	65.0^{B}	90.5^{A}	95.5^{A}	

Numbers with similar letters mean that there are no significant differences. (p<0.05).

*Essential oil = *M. viminalis* essential oil: **AgNPs =Silver nanoparticles.

Table 2: The effect of treatments concentrations and exposure period on adult.

Concentrations of treatments	% Mortality			Effect-treatments
	72 hours	48 hours	24 hours	
Essential oil 10 µL L^{-1} air	53.3^{f}	70.0^{d-f}	76.6^{b-e}	66.6^{C}
Essential oil 20 µL L^{-1} air	53.3^{f}	83.3^{a-d}	90.0^{a-c}	75.5^{B}
Essential oil 30 µL L^{-1} air	73.3^{c-e}	90.0^{a-c}	83.3^{a-d}	82.2^{AB}
AgNPs 2000 mg L^{-1}	53.3^{f}	90.0^{a-c}	96.6^{a}	80.0^{AB}
AgNPs 4000 mg L^{-1}	63.3^{ef}	90.0^{a-c}	96.6^{a}	83.3^{AB}
AgNPs 6000 mg L^{-1}	66.6^{d-f}	93.3^{ab}	100^{a}	86.6^{A}
Effect of periods	60.0^{B}	80.1^{A}	90.5^{A}	

treatment, significantly superior to the fumigation treatment with essential oils, which reached 82.2% at 30 µL L^{-1} air. While the interaction between the fumigation treatment and silver nanoparticles with exposure periods had a significant effect in terms of the achieved killing rates, as it reached 100% at 6000 mg L^{-1} for the AgNPs, which was significantly superior to the fumigation treatment with essential oils, which recorded 83.3% at 30 µL L^{-1} air over a period of time the third exposure of 72 hours. Physiologically, nanopesticides affect insects in several ways, whether repellent, lethal, or anti-feeding and their mechanism of action on insects is through influencing their nervous systems by inhibiting octopamine acetylcholine receptors. As a result of disruption of the octopamine receptor, it causes the nervous system to stop working in insects. GABA receptor is also an excellent choice receptor for inducing toxicity in insects by nano-pesticides (Mossa, 2016).

Through comparison with previous studies, our study agreed with what was found by Hazafa *et al*. (2022) regarding the effect of AgNPs on the insect showed that the mortality reached 100% at 50% after 72 hours. When using nanopesticides of *Azadirachta indica* and *Chrysanthemum Coronarium* extracts.

In another study, the effectiveness of nanopesticides based on *Moringa oleifera* extract against stored product insects was found to be 83 and 92.48% dead after a period of 72 hours. Compared to the killing rate of *M. oleifera* extract of 79.30 and 81.15% against *Rhyzopertha dominica* and *T. castaneum*, respectively (Iqbal *et al*., 2024).

CONCLUSION

It concluded from the present study that the essential oil of *M. viminalis* can be used as a bio-insecticide for *S. oryzae*. The sliver nanoparticles was successfully synthesis using the essential oils of *M. viminalis.* The essential oil and silver nanoparticles were effective in bringing the mortality of the larvae and adult insects and silver nanoparticles was better than essential oil of *M. viminalis.*

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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