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The Evaluation of Silver Nanoparticles Prepared by Oily *Eucalyptus Camaldulensis* on Some Aspects of *Sitophilus Oryzae*

Sarah Ibrahim Mahmood[1], Wijdan Hasan Ibrahim[2], Aseel Riyadh Luaibi[3]

[1]Biology Department, College of science, Mustansiriyah Univesity, Iraq, Baghdad

[2]Al-Rusafa Second Directorate of Education, Ministry of Education, Iraq, Baghdad

[3]Al_Karkh first Directorate of Education, Ministry of Education, Iraq, Baghdad

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KEYWORDS

Eucalyptus camaldulensis, Sitophilus oryzae

ABSTRACT:

The present study aims to evaluate the efficacy of silver nanoparticles prepared from oil extracts of th *Eucalyptus camaldulensis* plant as an alternative to using chemical pesticides against *Sitophilus oryza* which is one of the most dangerous stored pests that cause significant economic losses. The extract wa employed at concentrations of 30, 20, and 10µg/ml. The formation of silver nanoparticles (EOEc AgNPs) by *Eucalyptus camaldulensis* was investigated using UV spectroscopy. The following step were covered in the current study: adult at age of 24 hours, 2nd larval, and eggs. The extract EOEsc AgNPs was shown to be effective against *Sitophilus oryzae.* The largest percentage of eggs th mortality resulted in was 80.4 % at 30µg/ml and 10g/ml, the percentage drops to 66.2 percent. Whi there was no evidence of egg mortality in the control treatment. In addition, the maximum death ra for 2nd larvae were 75.4 percent at 30µg/ml and fell to 56.6 percent at 10µg/ml, whereas no mortalit was seen in the control treatment. In the control adults, the greatest killing percentage was 60.2 percer at 30µg/ml and 44.3 percent at 10µg/ml, but no mortality was observed in the control test.

Introduction

The rice weevil is considered one of the most dangerous pests of stored materials that are observed all over the world, especially in tropical and subtropical regions, and attacks grass grain crops in storage and field and prefers rice, in addition to infecting healthy grains, making them vulnerable to attack other insects that are unable to attack healthy grains [1]. Also, this pest is amongst the considerable across-the-board and malignant primary pests of stored grains such as rice, maize, wheat, dried beans, nuts, and other products emanating from them [2]. Controlling these insects is challenging because the immature stages grow inside cereal, which impedes the valid detection of invasions and the efficacy of managing measures, resulting in general harm to stored grains [3]. To reduce the effects of this insect, several means have been used, the most important of which are chemical pesticides, and it is considered one of the most used methods in combating it [4]. However, chemical control of warehouse pests, in general, has become undesirable due to the danger resulting from residual pesticides or chemicals used, as well as their economic cost and consumer fear of contaminating their food and products with the toxins of these substances [5]. An important role plant secondary metabolites played as alternatives to conventional pesticides, the researchers were interested to find methods more effective, Nanotechnology, which has been one of the best and most used in many different modern fields, has proven its effectiveness in controlling many pests [6]. green Synthesis of nanoparticles by using plant extracts presents sundry advantages for the reason that plants are eco-friendly and managed without effort [7]. Moreover, it presents significant efficiency, lower toxicity, and cost-effectiveness. active compounds in plants, such as flavonoids, glycosides, alkaloids, terpenes, and phenols, play an important role in the green Synthesizing of nanoparticles [8]. In view of the importance of the insect as one of the most important pests widespread in warehouses in the world and Iraq in particular, and due to the high economic damage, it speaks of stored materials and feed stores, the aim of the research is to Impact study silver nanoparticles prepared by oily *Eucalyptus Camaldulensis* on some aspects of *Sitophilus oryzae.*

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Materials and Methods

Insect population of *S. oryzae*

Insects were obtained from a sample of infected rice grains and were raised inside plastic containers of dimensions (20 X 15) cm they covered the plastic container's nozzles with muslin not to escape the insect and to allow ventilation They are placed in the incubator at 28 ± 2 C and 20% ± 2 relative humidity [9]. The rice grains were placed after being frozen at -18°C (for a period of two weeks to ensure that no further insect infestation was possible).

Preparation of oily Leaf Extracts

The leaves of *E. camaldulensis* were gathered and dried from trees established on the grounds of Mustansiriya University(Iraq). According to the technique of [10]. the oil extract was obtained by water distillation using the Clevenger equipment for essential oil extraction. To get the volatile oil, the contents were distilled for 3 hours. The essential oil was gathered in a tube, sealed tightly, and stored in the refrigerator at 4-6 degrees Celsius until needed [11].

Synthesis of Silver Nanoparticles

Silver nanoparticle synthesis was done using *E. camaldulensis* leaf oil extract with a Pre-prepared silver nitrate solution. A flask containing 80 ml of AgNO3 (1 mM) was filled with 20 ml of plant extract, the pH of the solution was measured, and the combination was then warmed at 60 ºC for 25 minutes. The success of the biosynthesis process has been proven by seeing the reaction mixture's color change shown in Figure 1 from light yellow to dark brown [12].

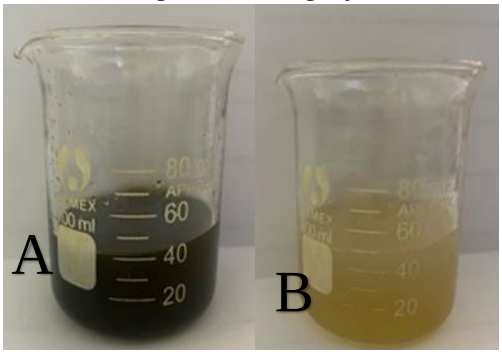

Fig. 1. A: extract of *Eucalyptus Camaldulensis* B: after the adding of AgNO3

Characterize silver nanoparticles biosynthesized by Eucalyptus Camaldulensis

UV-Visible Spectroscopy

The spectrum investigation verified the production of EOEc-AgNPs.A dark brown solution with a 2 mM concentration was diluted with deionized water in a ratio of 1:2. A UV-visible spectrophotometer with a wavelength of 190-1100 nm and a speed of 1000 nm per minute was used to record the UV spectra of biosynthesized silver nanoparticles [13].

Function = SPECTRUM
Start wavelength = 190.0 nm
Stop wavelength = 1100.0 nm
Low value = 0.0000 ABS
High value = 3.0000 ABS
Scan speed = 1000 (nm/min)
Accessory = Single cell

The Fourier Transform Infrared (FTIR) Spectroscopy

The analysis was conducted to indicate the functional group in the biosynthesized of AgNPs. To make the final pellet, dried AgNPs were combined with KBr. The finished pellet was then processed using FTIR spectroscopy in the 4000 to 500 cm- [14].

Effect of Oily Extracts on 24 hour old Eggs of *S. oryzae*

The experiment used 30 eggs divided into three replicates, with treatment consisting of spraying the extract with three concentrations (30, 20, and 10µg/ml) plus a control test, after which the eggs were placed in a petri dish and moved to the same incubator under the same conditions; mortality ratio and hatching period were recorded for each insect.

Effect of Oily Extracts of leaves of *Eucalyptus camaldulensis* on 2nd Instars' larvae of S. oryzae

The second instar larvae were collected after hatching the egg and following instars until reaching the second larva, using 30 larvae for each insect, placed in a Petri dish, treated by spraying the extract with three concentrations (30, 20, and 10µg/ml) in addition to the control test, and moved into an incubator under the same conditions; mortality ratio, larval stage period, and mortality ratio were recorded for each insect.

Effect of Oily Extracts of leaves of Eucalyptus camaldulensis on 24 hour adults of S. oryzae

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In this treatment, adults (24 h) were collected by flowing up pupa to grow thing to adults, put in Petri dish, 30 adults divided into three replicates for each insect, treatment by direct spraying the extract with three concentrations (30, 20, and 10µg/ml) in addition to controlling test, killing ratio were recorded for each insect.

Results:

UV-Visible Spectroscopy

After noticing the color change of the colloidal solution from light yellow to dark brown, the formatted silver nanoparticles created utilizing the oily extract of *E. camaldulensis* were detected by UV-Visible analysis at 190 to 1100 nm. As illustrated in Figure 2 the results indicate the synthesis of silver nanoparticles.

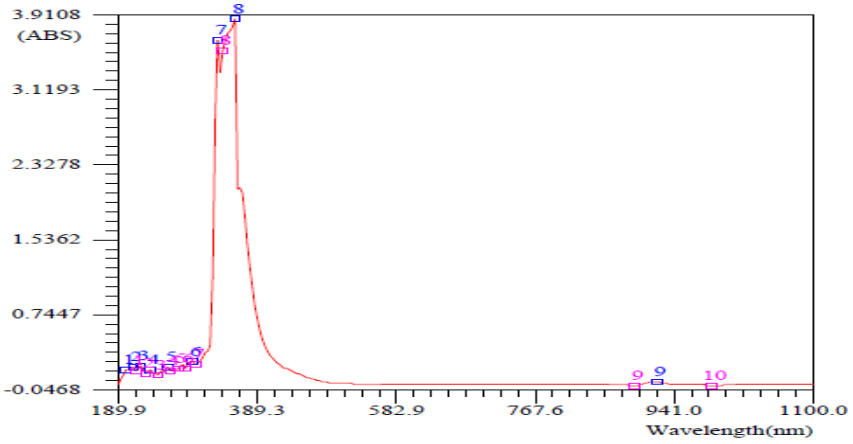

Fig. 2. UV– Visible Spectroscopy of EOEc-AgNPs performance absorbance height at various wavelengths.

Fourier Transform Infrared Spectroscopy (FTIR)

Silver nanoparticles synthesized by using an oily extract of E. camaldulensis were analyzed through FTIR to demarcate functional groups of biochemical compounds. FTIR spectrum displayed different bands in the range of infrared rays from 4000-250 cm The band at 3913.57 cm-1 suggests the binding of silver ions. The other bands are illustrated in Table 1, Fig 3. The findings suggested that numerous functional groups from E. camaldulensis biochemical compounds may work as reducing agents in the green production of AgNPs.[15].

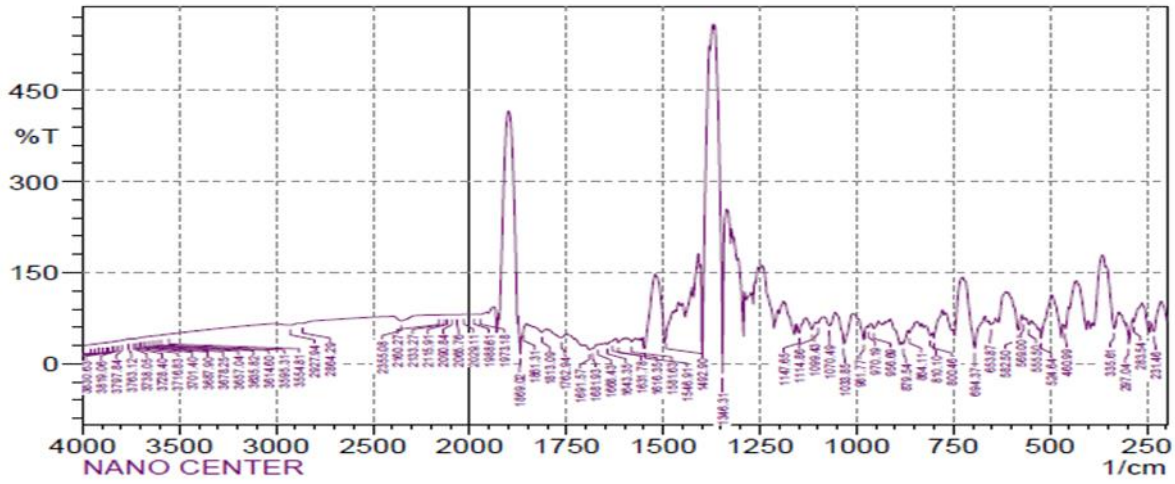

Fig. 3: FTIR spectra of EOEc-AgNPs

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Table 1.

Number	Peak Value	Intensity	Compound Class	Frequency in cm−1
1	1546.91	25.4	nitro compound	1550-1500
2	1581.63	35.5	nitro compound	1550-1500
3	1616.35	34.6	cyclic alkene	1650-1566
4	1643.35	32.3	cyclic alkene	1650-1566
5	1681.93	24.7	Imine and Oxime	1690-1640
6	1762.94	43.2	carboxylic acid	1720-1706
7	1973.18	81.2	allene	2000-1900
8	2355.08	70.7	isocyanate	2275-2250
9	2864.29	66.8	alkane	3000-2840
10	3554.81	48.8	alcohol	3700-3584
11	3635.82	45.7	alcohol	3700-3584

Effect of Oily Extracts of leaves of Eucalyptus camaldulensis on 24 hour old Eggs of S. oryzae

The results of the experiment revealed that the extract was effective in regulating *S. oryzae* eggs. Table 2 shows that the greatest death rate for eggs was 80.4 at 30 µg/ml concentration, 73.6 at 20 µg/ml concentration, and 66.2 at 10 µg/ml concentration, while no mortality rate was obtained in the control test.

Table 2. Effect of Extracts on 24 hour old Eggs of *S. oryzae*.

Insect	Concentrations	Mortality rates %	Hatching period (days)
S. oryzae	30µg/ml	80.4 ± 4.29 a	2.4 ± 0.06 b
	20µg/ml	73.6 ± 3.07 b	3.5 ± 0.09 ab
	10µg/ml	66.2 ± 3.52 b	3.4 ± 0.09 ab
	Control	0 ± 0 c	4.5 ± 0.14 a
LSD value		6.89 *	1.268 *
* (P<0.05). Means having with the different letters in same column differed significantly			

2nd Instars' larvae of *S. oryzae.*

Table 3 revealed that the extract had a distinct effect on the larvae. At the second larval stage, the greatest death rate was 75.4 at 30 µg/ml, 58.2 at 20 µg/ml, and 56.6 at 10 µg/ml, whereas the control treatment had no mortality rate.

Table 3. Effect of Oily Extracts 2nd Instars' larvae of *S. oryzae.*

Insect	Concentrations	Mortality rates %	Larval stage period (Days)
S. oryzae	30µg/ml	75.4 ± 3.47 a	7.7 ± 0.37 c
	20µg/ml	58.2 ± 2.94 a	7.8 ± 0.34 c
	10µg/ml	56.6 ± 2.15 b	13.8 ± 0.51 b
	Control	0 ± 0 c	17.2 ± 0.74 a
LSD value		6.63 *	2.85 *
* (P<0.05). Means having with the different letters in same column differed significantly			

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Effect of Oily Extracts of leaves of *Eucalyptus camaldulensis* on 24 hour adults of *S. oryzae*

Table (4) shows that all of the concentrations tested had a noticeable effect, resulting in an increase in adult mortality. Adult death rates were 60.2, 52.7, and 44.3 at 30 g/ml, 20 g/ml, and 10 µg/ml, respectively.

Table 4. Effect of oily extracts on 24 hour adults of *S. oryzae*

Insect	Concentrations	Mortality rates %
S. oryzae	30µg/ml	60.2 ± 3.04 a
	20µg/ml	52.7 ± 2.35 b
	10µg/ml	44.3 ± 2.84 b
	Control	0 ± 0 c
LSD value		7.94 *
* (P<0.05). Means having with the different letters in same column differed significantly		

Discussion

Nanoparticles have extensive use across diverse industries medical and agriculture applications due to their distinctive and unique characteristics[16]. Multiple studies have demonstrated the increased efficiency of green synthesis of nanoparticles by plant extracts in the various biological domains [17]. Also, the researchers proved the ability of nanoparticles to hinder the biofilm formation and virulence factors produced by certain local clinical human pathogens resistant to multiple drugs [18,19]. In this study's findings demonstrated the effectiveness of silver nanoparticles made from *Eucalyptus camaldulensis* Oily Extract against *Sitophilus oryzae.* In various parts of this insect, it has attained significant death rates. The findings are consistent with [20] A study found that eucalyptus oil was effective against Anthrenus verbasci that infects museums. The results were also in agreement with [21], which revealed several of the tested essential oils had a high level of contact toxicity against T. castaneum and S. oryzae adults. Also, the results of this study are consistent with [22] which evaluated the nanoemulsions synthesized of essential oil from anise and thyme, against the adults of *Sitophilus oryzae* and the efficiency of nanoemulsions has been proven in pest control. Also, this study agrees with the [23]. where prepared silver nanoparticles of essential oil cinnamon were against *Oryzaephilus surinamensis* this study approves the effectiveness of the extract for repellency in the adult pest the mortality was 85%. It's possible that this is due to the extract's active components and their influence on *S. oryzae*, for example, phenolic chemicals such as thymol and carvacrol may be found in Oily Extracts of *Eucalyptus camaldulensis* The existence of essential chemical effectiveness can be linked to the efficacy of eucalyptus oil, Eucalyptol (cineole) is a torpedoed molecule that gives the plant its destructive action [24,25]. It could also be because the particles of silver nanoparticles have different properties than the material made from them, which is due to their nanoscale, which makes them more efficient and able to interfere with biological activity in insects, such as enzyme activity, resulting in the insect's death [26].

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