IHJPAS. 37 (1) 2024
Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407

Effect of Alcoholic Phenol and Nanocapsules Extract of Grape Seed (*Vitis Vinifera*) on Egg Hatching and Adult Death of Southern Cowpea Beetles

[1]Rusul A.S. Mall* **[2]Sahar A. Kathier**

[1,2]Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq

***Corresponding Author:** rusal.abdulsatar1202a@csw.uobaghdad.edu.iq

Received 13 February 2023, Received 11 April 2023, Accepted 18 April 2023, Published in 20 January 2024

doi.org/10.30526/37.1.3283

Abstract

Pulses are the second-most significant economic crop that has been attacked by many storage pests. One of these pests is the cowpea beetle, *Callosobruchus maculatus (Fabricius*, 1775). This study aims to study the effect of phenol grape seed *Vitis vinifera* extract (1000, 1500, 2000, 2500, and 3500 mg/L) on eggs and adults. The results showed that the alcoholic extract of the grape seed *Vitis vinifera* recorded for egg hatching was (80, 56.67, 53.33, 40.00, and 10.00%), respectively. Results in the use of nanocapsules at concentrations of 100 and 500 mg/L showed the egg hatching ratio was (53.33, and 16.67%), respectively. While the results of alcoholic extracts of the grape seed *Vitis vinifera* recorded for adult mortality were (20.00, 70.00, 73.33, 76.67, and 96.67%), respectively after 72 hours of treatment. The results showed that the nanocapsules at concentrations of 100 and 500 mg/L had an adult mortality rate of (26.67, and 93.33%), respectively after 72 hours of treatment. The adult mortality rate increases by increasing the concentrations of both alcoholic extract and nanocapsules.

Keywords: Phenol, Nanocapsules, *Vitis vinifera*, *Callosobruchus maculatus*.

1. Introduction

The cowpea *Vigna unguiculata (L.)* is an important legume that provides dietary protein and animal fodder, in addition to its importance in soil fertility. It is cultivated in many tropical and subtropical regions in Africa, Asia and America. There are approximately 600 species of beetles that cause damage to Cereals in warehouses [1]

Callosobruchus maculatus (Fabricius, 1775), also known as the cowpea weevil [2] is one of the most important storage pests that harm cowpeas and other legumes. The negative economic effects caused by this insect are associated with the larvae penetrating and feeding inside the grain, resulting in significant weight loss. Reducing the nutritional value of the seeds and their germination potential, *Callosobruchus maculatus* (Fabricius, 1775) often multiplies massively,

and within a few months can make entire stores unfit for future consumption or cultivation. Both larvae and adults are more lethal to crop plants [3]. Plant extracts are used in insect control. An alternative to synthetic insecticides is botanical insecticides. These botanical insecticides, consisting of ground dried botanical materials, crude plant extracts, or chemicals isolated from plants and used for insect pest management in general, botanical secondary metabolites, such as alkaloids, are non-protein amino acids, steroids, phenols, flavonoids, glycosides, glucosinolates, quinones, tannins, and terpenoids are responsible for protective actions against insects[4،5] .Grapes *Vitis vinifera* is of great importance as the roots, stems, leaves, fruits, seeds and peel contain various types of phytochemicals, phenolic compounds, flavonoids and proanthocyanidins. Grapes contain nutrients and elements such as minerals, proteins, carbohydrates, fats, fiber, vitamin C and sugar in addition to biologically active substances [6]. The amount of soluble phenolic compounds found in grapes is not evenly distributed, as 70% of the total soluble phenolic compounds are found in the seeds, 28-35% are in the peel and the remaining about 10% are found in the pulp. [7] Nanotechnology is used in the control of insect pests as one of the alternatives to overcoming problems related to the use of traditional pesticides [8]. Nanocapsules represent a technology that preserves the concentration and effectiveness of plant extracts from external influences, because it does not dissolve automatically due to environmental factors, and are characterized by a short period in their impact on the target organism, due to their small size, as the size of the nanocapsules ranges from 100-1000 nanometers [9].

2. Materials and Methods

2.1. *Callosobruchus maculatus* (Fabricius, 1775) Culture

Insects were collected from infected cowpea seeds from the local markets in Alwa Jamila in Baghdad, then placed in glass containers with the nozzle covered with a piece of tulle and tied with rubber, and then placed in the incubator at 29 ± 2˚C, relative humidity 70±5%. Eggs were treated at 24 hours of age, and hatching was followed up after 5 days. New adults were treated, and mortality levels were calculated after 24 hours.

2.2. Collect *Vitis Vinifera* grape seeds and prepare the phenolic extract

Grape seeds were collected from the local markets in Baghdad, then washed and left to dry with stirring from time to time to prevent rotting, then finely ground to be used for phenol extraction. Phenols were prepared according to the method described in [10]. 50 g of seed powder was taken, then 500 ml of petroleum ether solvent was added to it at a ratio of 10:1 (solvent: plant) and placed in a Soxhlet device at a temperature of 60 °C for a period of 4-6 hours. The seeds were left to dry, after which ethanol was added at a concentration of 99% and filtered, then the filtrate was taken and concentrated, then hydrochloric acid (HCL) was added to it at a concentration of pH 2.0, then ethyl acetate was added at a rate of 10 ml with stirring, after which it was filtered and the solvent was left to evaporate, and we obtained phenol compounds and left to dry and then freeze until use. Detection of phenols according to the method of [11].

2.2.1. Ferric chloride reagent:

Drops of ferric chloride reagent were added to the plant extract and a bluish-green color appeared indicating a positive detection.

2.2.2. Lead acetate reagent:

Several drops of lead acetate reagent were added to the plant extract, and a white-yellow precipitate appeared, indicating a positive detection. The stock of phenols was prepared by dissolving 1 gm of phenol in 2 ml of DMSO (Di Methyl Sulfoxide) and then adding the volume to 100 ml of distilled water to make the required concentrations (1000, 2000, 1500, 2500, and 3500 ppm). According to the law, C1V1 = C2V2.

2.2.3. Preparation of phenol nanocapsules

It was prepared according to the method of [12], using Melt-dispersions. Technique, where 50 g of polyethylene glycol (PEG 6000) was placed in a glass beaker with a capacity of 100 ml, and it was gradually melted on a device (a Magnetic Stirrer with a hot plate) at a temperature of 65 °C; After that, 1 gm of the phenol extract, which was previously prepared, was added after it was dissolved with a little absolute ethanol alcohol, and it was mixed with PEG gently using a glass stick (stirring rod) to ensure equal distribution of the mixture, then it was mixed by the magnetic stirrer for a period of 60 minutes, and then the mixture was poured. In sterile glass Petri dishes and leave to cool, then put in the refrigerator for 15 minutes, then grind using the electric grinder, and fill in clean and sterile glass bottles, with the addition of small bags of calcium chloride to prevent moisture absorption, and keep the bottles at a temperature of 25°C. The required concentrations (100, 250, and 500 ppm) were prepared for the nanocapsules, according to the law C1V1=C2V2.

2.3. Characterization of nano-composite

Three methods were used to diagnose nanocapsules to ensure that they carried their properties through infrared spectrum spectroscopy (FTIR, Fourier transfer infrared), measuring granular-size DLS, and examining the nanocapsules through the FE-SEM scanner electronic microscope**.**

2.3.1. Fourier Transform Infrared Spectroscopy (FT-IR)

This is a tool for determining what types of functional groups are present in vehicles. The wavelength of light absorbed is a feature of the visible chemical bond in the annotated spectrum. Infrared interpretation of the absorption spectrum of chemical bonds in a molecule can be determined through the use of the PerkinElmer Spectrum Two N FT-NIR..

2.3.2. Nanoparticulate size and distribution

Particle size was measured with a convenient size analyzer (Horiba Scientific Nanopartica SZ-100). The droplet size was Analyzed. using the Dynamic Light Scattering (DLS) technique. Particle size was measured at an ambient temperature of 25.1ºC.

2.3.3. Scanning electron microscope (SEM)

Nanoparticle morphology was analyzed using (TESCAN MIRA3 FRANÇAIS).

2.4. Statistical analysis

The statistical analysis was the Relative Standard Deviation (RSD) for Microsoft Excel and R 4.0.4. R Core Team (2020). R: A language and environment for statistical computing.

3. Results

3.1. FTIR characterization

The wavelength values of the infrared absorption areas are within the range of 400-4000 cm-1, where each value represents a specific functional group. **Figure 1** shows the functional groupings of phenol nanocapsules of *Vitis vinifera* grape seed extract are hydroxyl, amine, amide, aldehydes, nitrates, ketones, and alkanes. It is observed that there are peaks representing the absorption of infrared radiation in the regions within the wavelengths between 1109.14 and 3645.91cm-1.

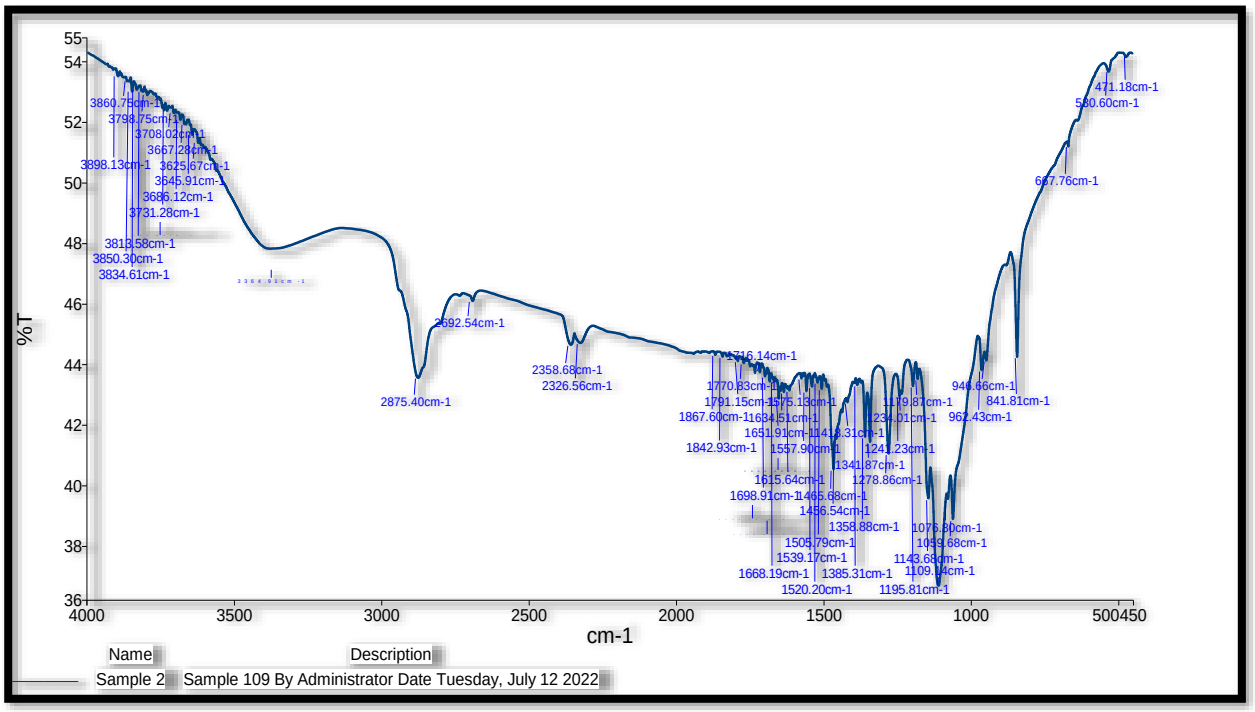

Figure 1. FT-IR of phenol nanocapsule

3.2. Dynamic Light Scattering (DLS)

The mean particle diameter and particle size distribution of the phenol nanocapsules were measured using dynamic light scattering techniques (**Figure 2**), The granular size of the phenol nanoscapsules measured using the DLS device is 252.6 nm.

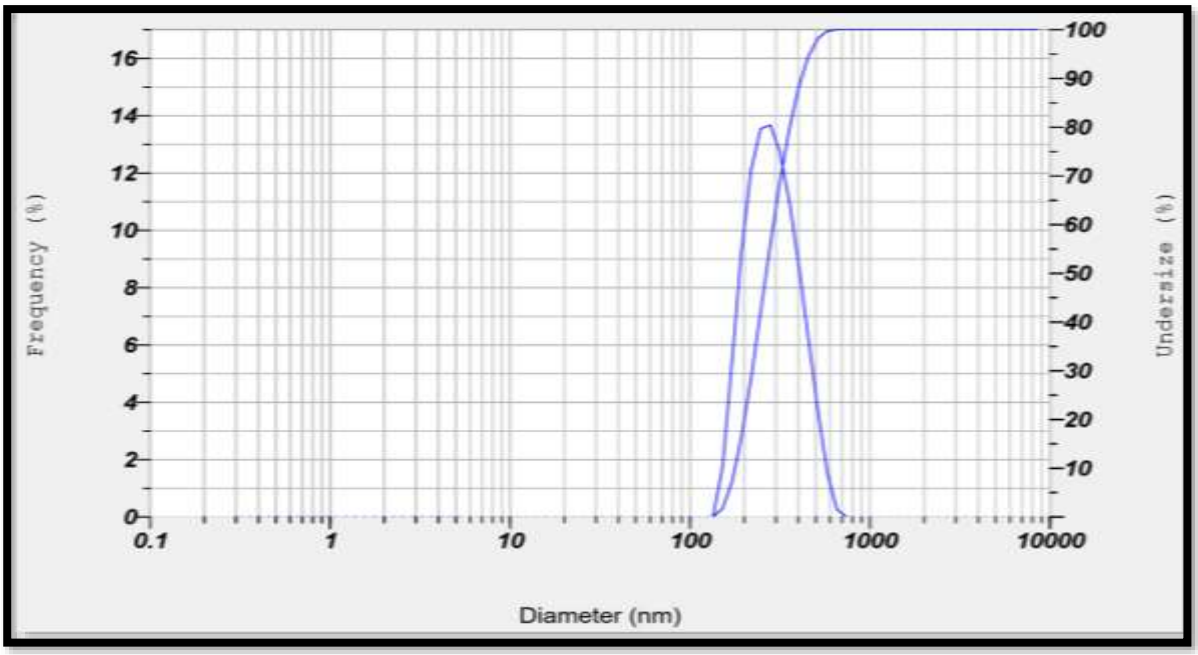

Figure 2. Phenol nanocapsules droplet size by DLS

3.3. FE-SEM Test

The scanning electron microscope is an important way to analyze the surface morphology of phenol nanocapsules shown in **Figure 3**. The electronic microscope scanning phenolic nanocapsules for grape seeds showed that they had an irregular shape with a grainy surface. The nanocapsules were 360.29 nm in diameter, while the smallest nanocapsule diameter was 103.12 nm.

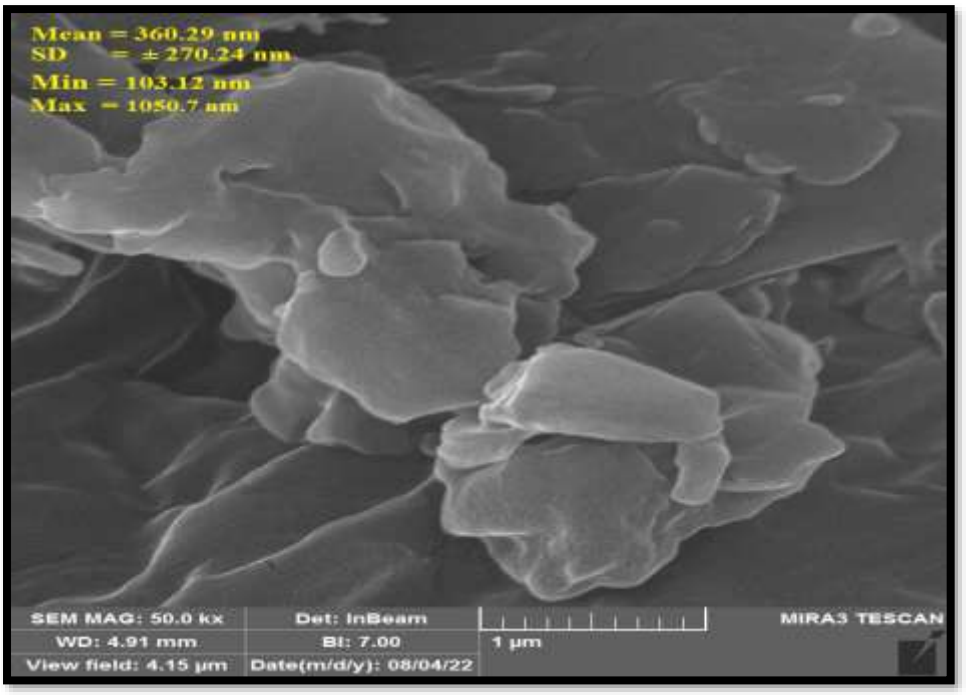

Figure 3. FE-SEM image of phenol nanocapsule

3.4. Effect of alcohol and nanocapsules extracton on egg hatch *C. maculatus*

Table 1 shows the effects of Alcohol Phenolic Extract on Grape Seeds *Vitis vinifera* in Concentrates 1000, 1500, 2000, 2500, and 3500 mg/L. The egg hatching ratio in concentration 3500 mg/L for eggs was 10%, while in concentration 1000 mg/L it showed an egg hatching rate of 80%. The results of the statistical analysis showed that there were moral differences at the probability level of 0.05.

The **Table 1** also shows the effect of phenol nanocapsules in concentrations of 100, 250, and 500 mg/L on egg hatching, where it was found at a concentration of 500 mg/L. The hatching rate was 16.67%, while the concentration of 100 mg/L showed a hatching rate of 53.33%. The statistical analysis results showed moral differences at the level of probability.

Table 1. Effect of phenol extracted and nanocapsules on egg hatch of *Callosobruchus maculatus*

Type of material	Concentration Mg/L	Mean of egg hatching%	std. Error of Mean	Substantial effect rate
Alcohol Phenol Extract	1000	80.00	0.577	48.00
	1500	56.67	0.333	
	2000	53.33	0.882	
	2500	40.00	0.577	
	3500	10.00	0.577	
	Control	100.0	0.00	
Phenol nanocapsules	100	53.33	0.577	41.11
	250	53.33	0.577	
	500	16.67	0.577	
	Control	93.33	1.155	

3.5.Effects of Alcohol and nanocapsules extracton on adult death of *C. maculatus*

Table 2 shows the effect of the phenol extract of *Vitis vinifera* in concentrations of 1000, 1500, 2000, 2500, and 3500 mg/L on the adult death rate. At the concentration of 3500 mg/L, the death rate was 96.67%, while the concentration of 1000 mg/L showed a death rate of 20.00%. Statistical analysis results showed significant differences.

Table 2 shows the effect of phenol nanocapsule extract at concentrations of 100, 250, and 500 mg/L on the adult death rate, where the adult death rate at the concentration of 500 mg/L was found to be 93.3%, while the concentration of 1000 mg/L was 26.67%. Statistical analysis results showed that there were moral differences at a probability level of 0.05.

Table 2. Effect of phenol extracted &nanocapsules on adult death of *Callosobruchus maculatus*

Type of material	Concentration Mg/L	Mean adult death%	std. Error of Mean	Substantial effect rate
Alcohol Phenol Extract	1000	20.00	0.577	68.00
	1500	70.00	0.577	
	2000	73.33	0.333	
	2500	76.67	0.333	
	3500	96.67	0.333	
	Control	03.33	0.577	
phenol nanocapsules	100	26.67	1.155	72.22
	250	70.00	0.577	
	500	93.33	1.537	
	Control	00.00	0.00	

4. Discussion

The lower incidence of hatching *Callosobruchus maculatus* may be caused by the extract preventing air from entering the egg and thus preventing the fetus from breathing [13, 14, 15, 16].

Plant extracts can penetrate the egg wall and thus prevent the embryo from developing [17, 18, 19] this study is similar to [15, 20, 21, 22], where the higher doses of saponin nanocapsules at a concentration of 500 mg/L had an effect on egg hatching in *Callosobruchus maculatus* of 06.67 %.The reason may be physical due to the treatment of the new eggs with nanocapsules of a phenol extract of grape seed, which's resulting in a shield layer that prevents gas exchange, resulting in fetal death and non-hatching of eggs. The effect of extracts on insect adults is due to direct contact as they travel between seeds, resulting in the extract adhering to the insect's body wall and causing the insect to dry and die [23, 24, 25, 26]. The search is similar [15, 27, 28] when high-dose alcoholic saponin extract is used to treat *Callosobruchus maculatus* larvae, and the case mortality rate is 76.67%. Toxic substances enter the joints, respiratory tract, and digestive tract of insects. This leads to the association of these substances with the receptors of octopamine neurotransmitters, causing convulsions and the death of insects [29,30].

5. Conclusions

Alcohol extracts from grape seeds had an impact on the egg hatching of southern lobby beetles, where the greater the concentration, the greater the effect, and there was an inverse relationship between egg concentration and the rate of hatching. Also, the nanocapsules from the same extract had the same effect. In the treatment of adults, there is a deadly effect from the use of alcohol extract and nanocapsules, which means that there is an absolute correlation between concentration and adult mortality.

Acknowledgment

We extend our sincere thanks to editor-in-chief and members of the editorial committee in Ibn Al-Haitham Journal for Pure and Applied Sciences.

Conflict of Interest

There are no conflicts of interest.

Funding

There is no funding for the article.

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