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EFFECT OF NANOCAPSULES AND TRADITIONAL ALCOHOLIC PLANT EXTRACTS OF GRAPE SEEDS *VITIS VINIFER* ON *CALLOSOBRUCHUS MACULATUS*

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Abstract

The grain legumes are the second most important economic crops that attacked by many of storage pests. One of these pests is the Cowpea seed beetle *(Callosobruchus maculatus*). This research aims to study the effect of saponin of grape seed (*Vitis vinifer)* extract (1000,1500,2000,2500and 3500 ppm) on eggs and larvae. The results showed that the alcoholic extract of the seeds recorded for the egg hatching were (60, 53.33,40,33.33and16.67%) respectively, while the results recorded for Larva mortality were (23.33,26.67, 46.67, 56.67, 76.67% respectively while the highest mortality rates was 76.67% after 72 hours of treatment at a concentration of 3500 ppm. And the result showed the egg hatching that were nanocapsules 6.67% at a concentration 500 ppm and the highest Larva mortality rates were recorded at 83.33% at a concentration of 500 ppm.

Keywords: nanocapsules, traditional alcoholic, grape seeds, *Vitis vinifer*, *Callosobruchus maculatus*

Introduction

Grain legumes are good source of proteins which offer a solution to malnutrition. they are the second most important group of crops worldwide after cereals., the cowpea serves as a source of dietary protein for human feeding, mostly in developing countries where a balanced diet is sometimes a problem. The dry seed contains about 25% protein and 67% carbohydrate. Cowpea also contains calcium, iron, vitamins, and carotene. the grain is extensively infested by a number of insect pests including *Callosobruchus maculatus*, which is a primary field to store pest causing considerably great losses to farmers [1] During early onset of infection, immature stages of insect are not normally seen, by the time, the visible symptoms occur, appearance of round holes in the grains due to the emergence of the insect through windows, the grain is completely damaged and lost infestation of *C. maculatus* can be up to 90% in markets and village stores besides storage, infestation can also occur in field which significantly reduces the quantity and quality of seeds reserved for sowing, food and trading purposes [2].

In recent years, researchers have been focusing on the secondary compounds of plant extracts to be used as alternatives for chemical insecticides [3] , botanical insecticides that degrade rapidly are safer than persistent synthetic chemical insecticides, less harmful to the environment, decrease production costs and are not likely to cause insecticide resistance among pests. [4] as plant-based pesticides are renewable, cheaper, very slow development of resistance to insect pests , etc. occupied a remarkable place in pest management . [5] Plants synthesize a wide range of phytochemicals (polyphenols, alkaloids, saponins, etc.) Indeed, phytochemicals seem to be able to interfere with the physiology and behavior of insects and cause their death. [6] botanical insecticides can cause mortality, infertile adults, slow growth, and a decrease in the egg viability of insects[7]. Grape seeds are considered one of the most important sources of phenolic and other compounds and are globally consumed for the biological value of thier active ingredients. [8] The seeds represent about 5% of the weight of whole Grape seed, a by-product of juices and wine production, are a biologically rich source of active compounds [9]. Saponins are among those secondary plant compounds. They may be steroidal or triterpenoids in nature and are characterized by a wide range of bioactivities [10]

Nanotechnology applications that are beneficial for agriculture include the encapsulation of pesticides in nanomaterials. [11] The size of nanocapsule ranged from 10 to 1000 nm. [12]

Nanoencapsulation techniques arise as suitable strategies to allow for the preservation and controlled release of plant components The use of nanoparticles has led to the development of effective approaches for pest control.[13] nanoencapsulation materials can enhance the bioavailability of active substances and reduce toxicity, improving the timed release of active substances and enabling the precise targeting of active substances by encapsulating the bioactive compounds.[14] Polymers and liposomes are renewable, biodegradable, and environmentally friendly, usually used as organic carriers to encapsulate pesticides [15].

Materials and methods

1-Insect culture

The laboratory culture of the southern cowpea beetle was prepared after obtaining a quantity of cowpea seeds infected with this insect from the local markets in Baghdad. placed in the incubator at a temperature of 28 ± 1 m and a relative humidity of 70 ± 5%, the culture was renewed after each generation.

2-Preparation of plant extracts

Grape seeds were collected from local markets in the city of Baghdad, then it washed, dried, and then ground to prepare plant extracts.

- preparation of the saponins

We put 50 gm of seeds powder, then 500 ml of petroleum ether was added to it in a ratio of 1:10 (plant: solvent), and it was placed in a Soxhlet device at a temperature of 60°C for 2-4 Hours, the seeds were left to dry, then Ethanol was added at a concentration of 99% and filtered, then the filtrate was taken and concentrated, then 125 ml distilled water was added to it gradually, then n-butanol was added by 125 ml gradually until two layers formed, an aqueous layer from the bottom and an organic layer to the top. The aqueous layer is removed, and 5 ml of ether is added to the organic layer to precipitate the saponin, which appears as a white precipitate [16].

- Saponin detection

a- Foam reagent: the contents were shaken vigorously until a thick foam was formed and left for one minute. Several drops of 1% hydrochloric acid were added. The absence of foam indicates the presence of saponin.

b- Mercury chloride reagent: Drops of $HgCl_2$ were added to the extract, where a white precipitate appeared and this indicates the positive detection. [17]

- Preparation of the concentrations used to treat the eggs hatches and larva mortality

The stock was prepared for saponins, by dissolving 1 gm of saponins with a little DMSO (Di Methyl Sulf oxide) and then completing the volume to 100 ml of distilled water to make (1000, 2000, 1500, 2500, 3500) ppm

The eggs were prepared by taking a pair of newly emerged male and female and placing them on 10 cowpea seeds and leaving them for 24 hours to lay eggs and with 3 replicates to treat the eggs for each concentration. The fourth instar larvae were removed from the infected seeds and every 10 larvae were placed on crushed cowpea seeds in a Petri dish with 3 replicates for each concentration.

3 -Preparation of saponin extract Nanocapsule.

The preparation Nanocapsule using the melt dispersion Polyethylene glycol (PEG) 6000 (50 g per part) was heated at 65 C. After being melted with 0.1 g of saponin extract .it was mixed with melted PEG and stirred lightly, with a glass rod to ensure even distribution of the mixture. The mixture was thoroughly ground in an electric grinder after naturally cooling at 25°C and then Sifted using a 200 mesh sieve. The powders were placed in airtight and resealable glass containers containing calcium chloride to prevent moisture absorption and stored at 25°C. This preparation is according to the method [18]. We prepare concentrations (100 , 250 ,500) ppm for saponin according to the law C1V1=C2V2 to treat egg and larva

4-Characterization of Nanocapsule.

4-1 Size and distribution of nanoparticles

The particle size was measured using a practical size analyzer (Horiba Scientific Nanopartica SZ-100). Droplet size was analyzed using dynamic light scattering (DLS) technique The particle size was measured at room temperature 25.1 °C.

4-2 Scanning electron microscope (SEM) The morphology of the nanoparticales was analyzed using (TESCAN MIRA3 FRENCH).

4-3 Fourier transform infrared spectroscopy (FT-IR)

It is a tool for identifying types of functional groups present in vehicles. The wavelength of light Absorbed is a feature of the visible chemical bond in the annotated spectrum. Infrared interpretation Absorption spectrum, chemical bonds in a molecule can be determined by usa PerkinElmer Spectrum Two N FT-NIR.

Statistical analysis

Statistical analysis was relative standard deviation (RSD) in the Microsoft Excel program and R version 4.0.4. R Core Team (2020). R: A language and environment for statistical computing.

Result and discussion

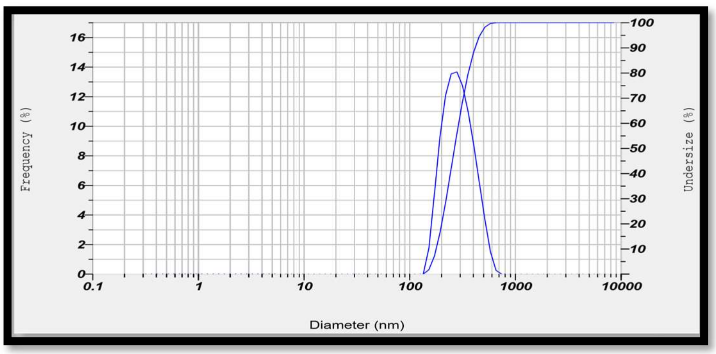
Dynamic Light Scattering (DLS)

The mean particle diameter and particle size distribution of the saponin nanocapsules were measured using dynamic light scattering techniques fig 1. According to the DLS findings, due to the saponin nanocapsules components. The size of particles using DLS analysis showed an average size of 277.3nm for nanocapsules.

Figure 1. saponin nanocapsules droplet size by Dynamic Light Scattering

SEM Test

The Scanning electron microscope is an important way to analyze the surface morphology of



saponin nanocapsules, as it provides a high- resolution view of the in situ structure of the saponin nanocapsule. Figure 2 shows the surface morphology of droplets that appeared as bright circles, and the castor oil .The morphology of saponin nanocapsule analyzed in SEM with 200,000 &50,000 magnifications, revealed the particles were arranged in irregular shapes with nanocular surface of the porous structure.

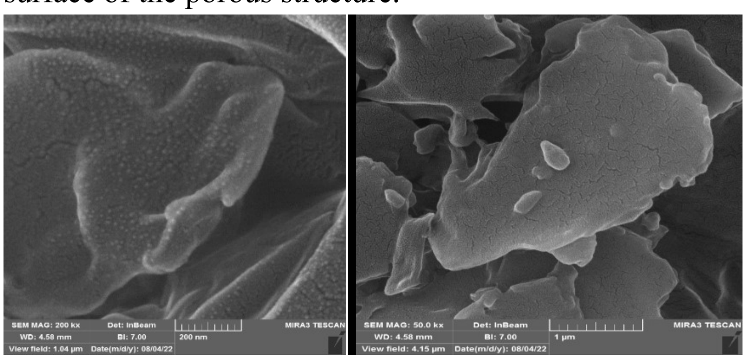


Fig-2 SEM image of saponin nanocapsule

FTIR characterization

The IR spectra of (Figure 3) showed -O–H stretch, free hydroxyl peak range from 3850.26-3645.54cm1, C-H peak range from 3353.27–2855.04cm1, C=O stretch peak at 1770.71–1651.80 cm-1, –C=C– stretch peak at 1682.85–1634.35 cm-1, N–H bend peak at 1651.80–1575.05 cm-1, C–C stretch (in–ring) peak at 1615.54–1575.05 cm-1, C–H bend peak at 1464.80–1456.24cm-1, C–H wag peak at 1341.26–1142.44 cm-1, C-O-C peakat 1059.13-1104.03 cm-1 and C–Cl stretch peak at 841.56–470.89.

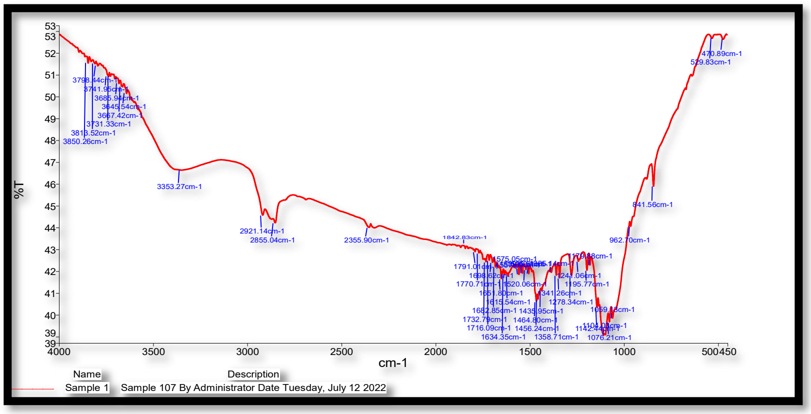


fig- 3 FT-IR of saponin nanocapsule

2. Effect of saponin of grape seed (*Vitis vinifer)* extract on eggs hatch

Table (1) shows that effect of saponin on eggs hatching at all concentrations, which means that the saponin has an influence on embryo development. Eggs of *Callosobruchus maculates* are very sensitive after five days of treatment the 1000,1500,2000,2500 and 3500 ppm concentrations were the most effective for egg hatching , which was 60% followed by the concentration of 1000 ppm, which the concentration 3500 ppm caused a lower percentage of egg hatching 16.67%.

Table 1 :Effect of saponin extracted by Ethanol on egg hatch of *Callosobruchus maculatus*

Concentration Saponin	**Effect on egg hatch after 5 days**
0	96.67±0.333
1000	60.00±1.000
1500	53.33±0.577
2000	40.00±1.000
2500	33.33±1.155
3500	16.67±0.577

Saponins play an important role in increased mortality and decreased reproduction in pest insects. The mode of action of saponins in insects demonstrates a block of the uptake of sterols. The insects cannot synthesize sterol structures by themselves [19] and another possibility is that saponins are toxic to insects because of their membrane per mobilizing. is increase the permeability of plasma membranes, and they are known to reason dissociation [20]. Reducing hatchability may be due to the extract preventing air from entering the egg and thus preventing the fetus from breathing.[21]

Table 2 shows the effect of nanocapsule saponin extract on egg hatching after 5 days, where the concentration of 500ppm shows an effect on hatching eggs, which amounted to 6.67% . As for the concentrations 100, 250 ppm and gave percentages of 46.67, 30% respectively.

This means that the effect of increasing concentrations of nanocapsule saponins affects egg hatching, as there is an inverse relationship between increasing concentrations and hatching . It is similar to a study [22] Ovicidal test of Ni-Pd NPs against C. maculatus eggs at various concentration and various intervals inferred that none of the eggs were hatched at higher concentrations.

Table 2 :Effect of nanocapsule for saponin on egg hatch of *Callosobruchus maculatus*

Concentration Saponin Ppm	**Effect on egg hatch after 5 days**
	Mean ±Std. Deviation
0	93.33±1.155
100	46.67±1.155
250	30.00±1.000
500	06.67±1.155

3 . Effect of treatments on Larva mortality *Callosobruchus maculatus*

The results reported in Table 3 show the efficacy and toxicity of five different concentrations of saponin extracted from grape seeds in the fourth larval stage of *Callosobruchus maculatus*. The mortality rate reached an average of (23.33, 26.67, 45.67,56.67 , and %76.67) at concentrations of (1000, 1500, 2000, 2500 , and 3500 ppm) respectively after 72 hours of treatment, with a significant difference between the concentrations at 0.05. According to table 3, a concentration of 3500ppm resulted in a 76.67% mortality rate, while a concentration of 1000ppm resulted in a 23.33% mortality rate. The results showed the importance of the time factor in increasing the rates of mortality with a significant difference. The results also revealed that the interaction between the studied factors had significant. This means that the effect of increasing concentrations of saponins affects on Larva mortality as there is a positive relationship between increasing concentrations and Larva mortality. Plant extracts affect metabolism, which affects the life of the insect and may interfere with chemicals in the hormones. [21] Fenugreek saponins presented insecticidal activity against bean weevil (*Acanthoscelides obtectus*). Topical applications of seed (6 mg per insect) and leaf (30 mg per insect) extracts caused mortality and reduced the fecundity of the bean weevil(Bruchinae) within 2 days. A powder of fenugreek leaves enriched with diosgenin(steroidal saponin) was mixed in the stored grains of Phaseolus vulgaris, and it was observed that there was considerable mortality of cowpea weevil in powder treated peas as compared to untreated cowpeas. This powder also inhibited the larval growth of weevils as well as adult emergence.[23]

Table 3: Toxicity of saponin extracted by Ethanol on 4^{th} larval stage of *Callosobruchus maculatus*

Concentration Saponin	***Fourth Larva instar Mortality within 72 hours***
0	3.33±0.577
1000	23.33±1.155
1500	26.67±1.155
2000	46.67±0.577
2500	56.67±1.528
3500	76.67±1.528

The results in Table 4 indicate the efficacy and toxicity of three different concentrations of saponin nanocapsule, as the mortality rates reached an average of (26.667 , 53.333 and 83.333) at concentrations of (100 , 250 ,and 500 ppm) respectively after 72 hours of treatment, with a significant difference between the concentrations , the concentration (500 ppm) showed the highest mortality rates (83.33 %) , while the concentration (100ppm) show the lowest (26.667 %) , the results showed the importance of the time factor in the increasing the rate of mortality with a significant difference as well .

Table 4: Toxicity of saponin nanocapsules on 4th larval stage of *Callosobruchus maculatus*

Concentration Saponin nanocapsule	Fourth Larva Instar Mortality within 72 hours
0	03.333±0.577
100 ppm	26.667±0.577
250	53.333±0.577
500	83.333±0.577

This means that the effect of increasing concentrations of saponins effects on Larva mortality as there is a positive relationship between increasing concentrations and Larva mortality. This study is similar to[24] where the higher doses of AgNPs that are synthesized using aqueous extracts of *Solanum nigrum L.* facilitate more uptakes of nanoparticles by the larval body Mosquito *Culex quinquefasciatus* and *Anopheles stephensi* , and causes their death accordingly. Previously it was reported that penetration of AgNPs through treated larval membrane caused an interaction with cell molecules resulting in death of larvae. In addition, after AgNPs reach their midgut epithelial membrane, the enzymes are inactivated and generate peroxide leading to cell death.

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