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Effect of Alcoholic Extract and Nano-capsules of *Bacopa Monnieri* Plant on Eggs of the *Callosobruchus maculatus*

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Abstract

This study used wild barbines (*Bacopa monnieri*) to control the southern cowpea beetle (*Callosobruchus maculatus*) and their effects on cowpea beetle eggs. Alcoholic extracts and nanocapsules were used at different concentrations, with the alcohol extract being 1000, 2000, and 3000 PPM. Eight out of ten eggs were unhatched, and two hatched, indicating that the 3000 concentrations had the highest egg toxicity rate. Regarding saponins at the same concentrations, the 3000 concentrations had the best results, whereas the 1000 and 2000 concentrations produced poorer results. Additionally, it was discovered that saponins had a stronger effect than terpenes. The nanocapsule concentrations that affected the *Callosobruchus maculatus* beetle's eggs were 80, 100, 120, and 140. The results showed that the alcoholic extract at different concentrations and times had a greater effect than the nanocapsules. The nanocapsules also had an effect, but it was slightly slower than the alcoholic extract, and the effect increased with increasing time. The effect was found to be highest at a concentration of 140 and lowest at 80 on the eggs of *Callosobruchus maculatus*.

Keywords: Alcoholic extract, nano capsules, *Callosobruchus maculatus*, *Bacopa monnieri*.

1. Introduction

The *Callosobruchus maculatus* beetle belongs to the *Bruchidae* family and the *Coleoptera* order, which attacks stored legumes [1]. Africa is the original home of the southern cowpea beetle, and infestations by this beetle are widespread in tropical and subtropical regions [2]. This insect is also widespread in the central and southern regions of Iraq [3]. This insect is known as the cowpea weevil or the cowpea seed beetle [4]. The damage caused by insect infestations is not limited to the field only. The southern cowpea beetle is widespread among other legume beetle species. The female lays between 76 and 107 eggs at temperatures up to 30°C and a relative humidity of 70%. The eggs hatch within 4 to 6 days, producing larvae that develop inside the seed. They feed on the contents until they reach the surface, then pierce a hole with their gnawing jaws to emerge as a full-grown insect and begin their life cycle anew [5]. This insect has been observed infecting the seeds of at least 20 legume species, causing damage that negatively impacts germination as the larvae feed on the seed contents and leave behind waste and decaying matter. The larvae consume 29 to 45.6% of the seed weight, and the damage increases significantly after the larval stage. The southern cowpea beetle is an important storage pest [6,7].

There are several methods used to control this insect, including biological, chemical, and physical methods. In the field of physical control, refrigeration has been used as an effective means of controlling the cowpea beetle [8].

In terms of chemical control, the use of pesticides against insect pests in stored food can cause health and environmental damage. Although the use of chemical pesticides at controlled concentrations can yield positive results, their excessive use can lead to environmental and food chain pollution, potentially resulting in chronic poisoning among consumers [9]. However, because herbicides are inexpensive and have minimal environmental impact, their use has increased. These pesticides eliminate harmful insects without polluting food or the environment. Biological methods are considered safe and effective alternatives to chemical pesticides. They rely on the use of natural enemies of pests, such as predators, parasites, and pathogens, to reduce pest numbers to levels that do not cause economic losses, making them environmentally friendly [10]. Biological control also includes the use of biopesticides, which are pesticides extracted from natural sources such as microorganisms, plants, or biochemical compounds. These pesticides are divided into three main categories and offer significant benefits in both agriculture and public health programs [11, 12].

Bacopa monnieri (wild barbines) is an annual, aquatic, herbaceous plant called in Latin *Bacopa monneiere* Linn. And its English name is Monnie's Waterhyssop. It belongs to the genus Bacopa, which consists of 70 to 100 species of aquatic plants. This plant belongs to the order Lamiaceae, part of the 13th family of Gazelles and the Apiaceae family. It is widely distributed in wetlands or swamps, particularly in Asia, such as southern and eastern India, Nepal, Sri Lanka, China, Pakistan, Taiwan, and Vietnam. It is also native to Australia, Europe, Africa, and North and South America. It grows along the banks of the Qurna and Shatt al-Arab rivers in Iraq. There are numerous names for this plant, including water hyssop, herb of grace, wild barberry (*Bacopa monnieri*), and brahmi in India. In Indian medicine, it is among the most significant medicinal plants. system since ancient times [14]. Chemical analysis of the ethanol extract, methanol extract, and aqueous extract of *Bacopa monnieri* found that in all of these extracts, the powder of this plant contains numerous major compounds such as carbohydrates and proteins, as well as minor compounds such as steroids and glycosides, such as cardiac glycosides [15]. Saponins are a subclass of glycosides and are well known in the scientific literature for their soap-like properties. Most saponins are terpenoids or steroids due to the linkage of hydrophilic polysaccharide chains (glycosides). Due to the carbon I structure of the aglycone, saponins are antimicrobial, insecticidal, and hemolytic. Thus, insect growth and reproduction are directly influenced by multiple saponins, as these bioactive compounds repel herbivores from target host plants. However, if insect pests feed on these defensive host plants, these herbivores lose further nutrition and motility, leading to lethargy and eventual death due to the high toxicity of saponins [16, 17]. These saponin molecules indirectly affect the beneficial gut microbiota within the digestive tract of insect pests by forming various bonds with multiple digestive enzymes. Thus, due to their strong binding to specific enzymes, saponins damage the mucosal lining of many digestive tract cells. Similarly, these saponin molecules bind to a cholesterol compound and cause cytotoxicity [18]; thus, this combination of saponins and various enzymes leads to the failure of insects to reproduce [19]. Therefore, most herbivores avoid feeding on plants rich in saponins, as we have shown here that saponins negatively impact insect survival. Therefore, implementing an integrated pest management program using various types of saponins is highly effective in controlling various insect pests in different environments [20]. Plants produce a wide range of chemical compounds known as terpenes. Among these natural products, terpenes have been shown to have significant potential for insect control. Terpenes are secondary metabolites widely distributed in the plant kingdom [1–5]. Plants produce a mixture of terpenes, which can be easily isolated by hydrolysis into oils [21]. Their toxicity against many insects makes them excellent candidates for the development of environmentally friendly insecticides [21]. Nanomaterials are used to affect many insects because they are very small objects that can affect their survival or life cycle [22]. Nanotechnology is an application of various physical, chemical, biological, engineering, medical, and pharmaceutical sciences, harnessing them to design and manufacture chemicals capable of affecting and penetrating the bodies of living organisms, causing birth defects or death. Therefore, these materials are used as nanocapsules to influence the growth and life cycle of insects [23]. We will use these nanochitosan capsules to influence the southern bean beetle under the experiment. In this research, we will study the effect of alcoholic extract of *Bacopa monniri* and nanocapsules on the Egg hatching rate [24].

2. Materials and methods

2.1. Collection and rearing of *Callosobruchus maculatus*

A quantity of beans infected with this insect was collected from the stores of local markets in Baghdad. This insect was diagnosed as *Callosobruchus maculatus* by the Natural History Museum/University of Baghdad. A new colony was prepared by adding pairs (males and females) of bean beetles to healthy bean seeds and ensuring that the seeds were not infected. They were placed in glass bottles and covered with gauze and a rubber band to prevent the insect from coming out. The bottles were placed at room temperature [20].

2.1.1. Collecting samples of *Bacopa monnieri plants*

The plant was collected from the edges of Shatt al-Arab in Basra, and was identified in the College of Science for women /University of Baghdad by Zainab Abdul Aoun. The plant was washed well of dust and dirt and was spread on newspapers until it became dry. The plant was crushed using an electric grinder, and it was stored in glass bottles until use. Preparation of alcoholic extract of wild berberine plant [19,2].

2.2. Terpene extraction method

150 g of plant powder was placed, and 250 ml of acetone and 500 ml of hexane were added to it at a temperature of 22 and left for 24 hours, then filtered and packed in glass dishes until the solvent evaporated, leaving behind the isolated terpenes, and preserved until use [18].

2.3. Saponin extraction method

Saponin was prepared according to the method of Alwash [25]. 50 g of powder of all parts of the wild berbin plant were taken, then 500 ml of the solvent petroleum ether was added to it at a ratio of 10:1 (plant: solvent) and placed in a Soxhlet device at a temperature of 60 m for 4-6 hours, after which ethanol was added at a concentration of 99%. After Completing the extraction process, the extract was filtered using filter papers of type (wattman.no.1), then the filtrate was taken and concentrated, then 125 ml of distilled water was added to it gradually, then n-butanol was added in an amount of 125 ml gradually until two layers were formed, an aqueous layer at the top and an organic layer at the bottom in the separation funnel. The aqueous layer was disposed of, and 5 ml of ether was added to the organic layer to precipitate the saponin; then the saponin was poured into glass containers until use.

2.4. Method of preparing nano capsules for the alcoholic extract of the *Bacopa monnieri* plant

The ion glycation method was used to prepare nano capsules with some modifications, where chitosan was used and dissolved using a solution of acetic acid with a concentration of 2%, then it was mixed and blended for 24 hours to dissolve the chitosan. After dissolving, a stop was used with mixing and blending, then the terpene extract was added in an amount of 0.9 g and the saponin in an amount of 1.8, then the pH was adjusted to 6.5. By adding sodium hydroxide 1 molar, then the mixing and blending process continued until the components were homogeneous to obtain a homogeneous solution. To separate the capsules, the centrifugation process was carried out to separate the capsules that were in the sedimentary part; then they were collected and dried and placed in Petri dishes, then placed in a thermal oven at a temperature of 40-50 until they became completely dry, then they were ground with an electric grinder, then placed in a container, and a part of it was taken to conduct tests [26-28].

2.5. Study of the effect of the alcoholic extract and nano capsules of *Bacopa monnieri* on some roles of *Callosobruchus maculatus* and its development

2.5.1. The direct effect of the alcoholic extract of *Bacopa monnieri* on the eggs of the *Callosobruchus maculatus*.

Twenty-four-hour-old eggs were obtained by placing 10 healthy cowpea seeds in Petri dishes and releasing a pair of southern cowpea beetles (male and female). On the second day, the seeds containing eggs were isolated, and the eggs on the seeds were destroyed, leaving one egg per seed. Three replicates were used for each concentration, in addition to a control treated with distilled water. The eggs were then treated with concentrations of 1000, 2000, and 3000 PPM using a hand sprayer, ensuring that the eggs were well covered. They were incubated at 28°C and 60°C relative humidity. The hatching rate was recorded after seven days, and the percentage of egg hatching was calculated.

2.5.2. The direct effect of the nano capsules of *Bacopa monnieri* on egg of the *Callosobruchus maculatus*

Early-emerging insects were isolated at 24 hours of age, and 10 adults were placed in a Petri dish containing filter paper, with three replicates for each concentration, in addition to a control treatment consisting of distilled water. The egg was then treated with concentrations of 80, 100, 120, and 140 using a hand sprayer, ensuring good insect coverage [28]. They were incubated under standard incubation conditions of 28°C and 60°C. The mortality rate was recorded for 5 days, and the percentage of egg mortality was calculated and corrected using the Abbott equation.

2.5.3. Statistical analysis

Factorial experiments were carried out according to the Completely Randomized design (CRD) and the differences between the means of the coefficients were compared according to the value of the least significant difference (LSD) and at a probability level of 0.05 [28]. The data were analyzed using the statistical program 12.

3. Result

The active compounds are mild in nature. Interestingly, nano-encapsulated treatments did not interfere with germination even though they persisted longer on seed surfaces, suggesting selective toxicity—an important attribute for sustainable pest management. These findings reinforce the utility of nano-biopesticides in integrated pest management (IPM) strategies where the objective is to minimize collateral effects on crops or beneficial organisms.

3.1. The direct effect of the alcoholic extract of *Bacopa monnieri* on the eggs of the *Callosobruchus maculatus*, C. maculatus

Twenty-four-hour action. The figure illustrates the impact of various concentrations (1000, 2000, and 3000 ppm) of the alcoholic extract on the eggs of the southern cowpea beetle, while monitoring this effect over a period of 5-7 days. The figure shows that the alcoholic extract of the saponins recorded higher results at a concentration of 300, while the results were lower at concentrations of 1000 and 2000. As for the terpenes, we note that the rate of egg toxicity increases with increasing concentration [29]. The results of the statistical analysis showed significant differences in the rate of egg toxicity when using the alcoholic extract, as the rate increases with increasing concentration, as shown in **Figures 1** and **2**.

3.2. The direct effect of the nano capsules of *Bacopa monnieri* on the eggs of the *Callosobruchus maculatus, C. maculatus*

Twenty-four-hour. **Figures 3** and **4**. The figure shows the effect of different concentrations (80, 100, 120, and 140 ppm) of two types of ten eggs of *Bacopa monnieri* nanocapsules and the effect of the alcoholic extract on the eggs of the southern cowpea beetle, monitoring the effect for 5–7 days. The figure shows that the alcoholic extract of the saponins recorded the highest results at a concentration of 140, while the results were lower at concentrations of 100 and 120. As for the terpenes, we observed that the rate of egg toxicity increased with increasing concentration. The results of the statistical analysis showed significant differences in the rate of egg toxicity when using the alcoholic extract, with the rate of egg toxicity increasing with increasing concentration, as shown in **Figures 3** and **4**.

The direct effect of *Bacopa monnieri* nanocapsules on *C. maculatus* eggs. Twenty-four hours. **Figures 3** and **4** show the effect of different concentrations (80, 100, 120, and 140 ppm) of two types of *Bacopa monnieri* nanocapsules and the effect of the alcoholic extract on the eggs of the southern cowpea beetle, with the effect monitored over 5-7 days. The figure indicates that the alcoholic extract of the saponins recorded the highest results at a concentration of 140, reaching 90%, while the results were lower at concentrations of 100 and 120. As for the terpenes, we note that the rate of egg toxicity increases with increasing concentration. The results of the statistical analysis showed significant differences in the rate of egg toxicity when using the alcoholic extract, with the rate of egg toxicity increasing with increasing concentration, as shown in **Figures 3** and **4**

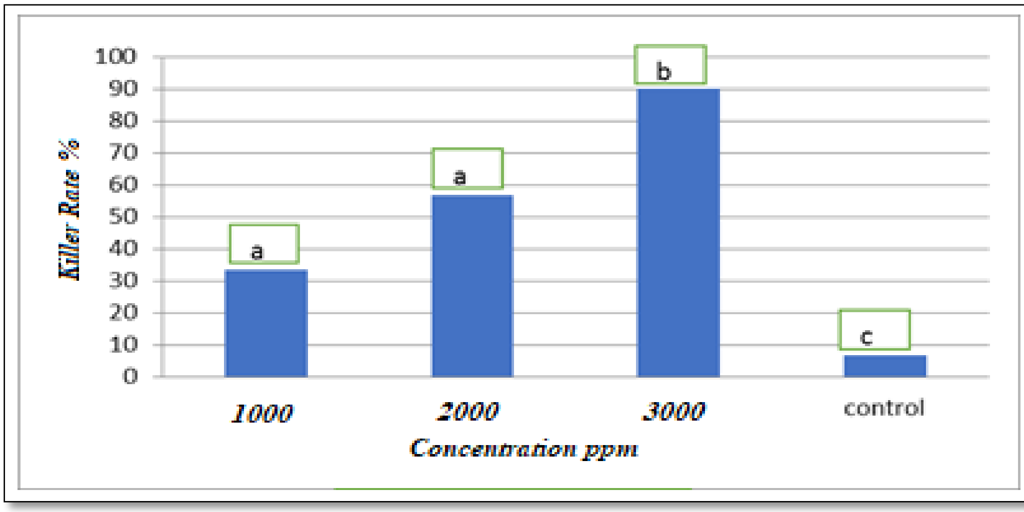

Figure 1. Shows the statistical analysis of the effect of saponin on Egg hatching rate.

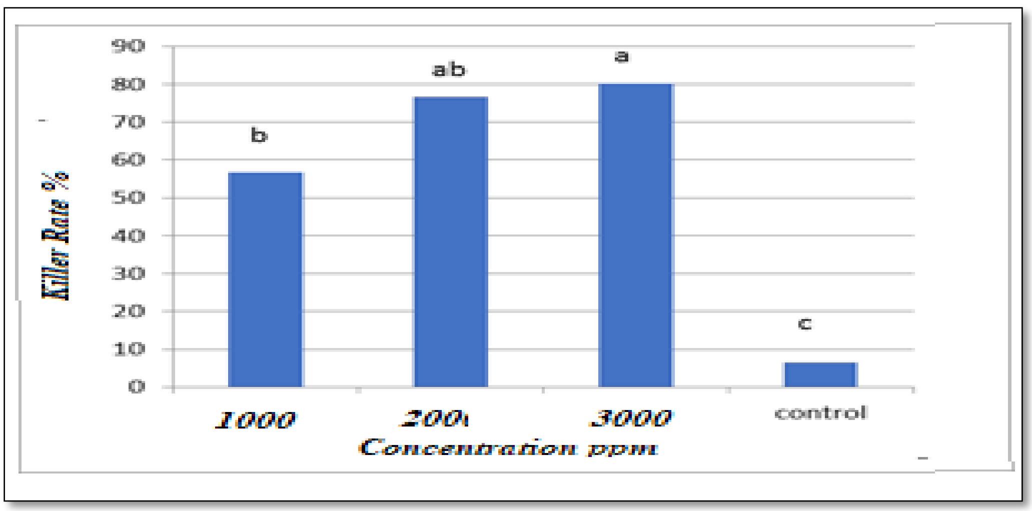

Figure 2. Shows the statistical analysis of the effect of terpenes on Egg hatching rate.

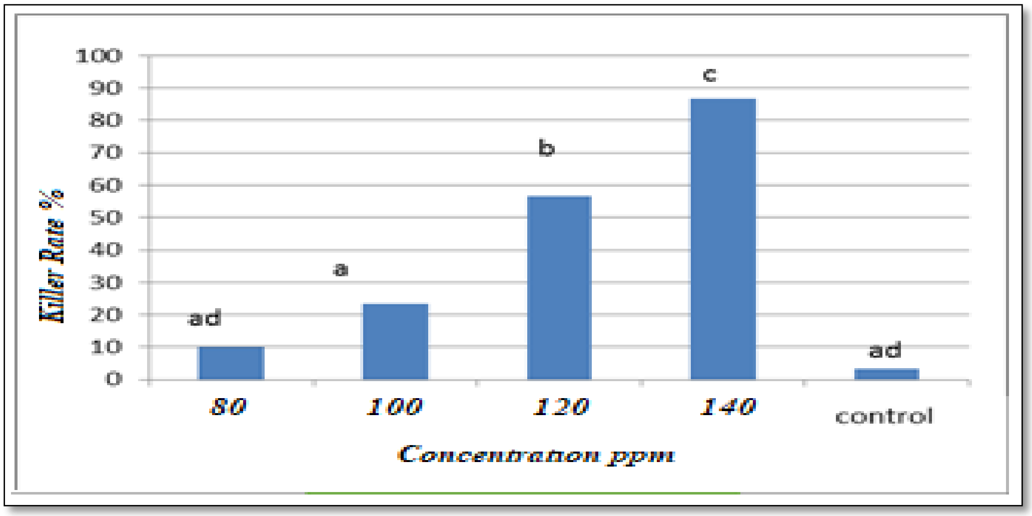

Figure 3. shows the statistical analysis of the effect nano capsules of saponin on Egg hatching rate.

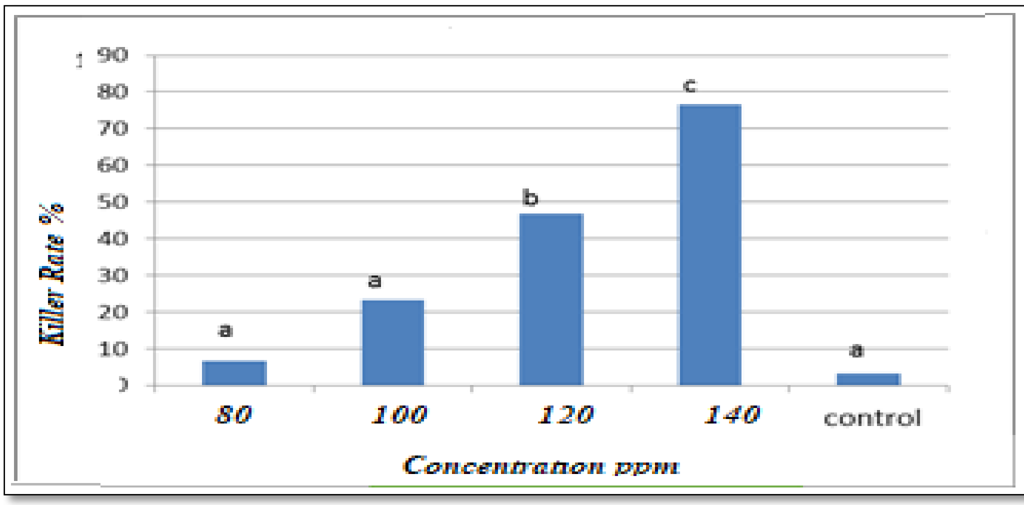

Figure 4. shows the statistical analysis of the effect nano capsules of terpenes on Egg hatching rate.

4. Discussion

This study has shown that *Bacopa monnieri's* alcoholic extract and its nano-capsules are both highly effective in inhibiting egg hatchability in *Callosobruchus maculatus*, but that efficacy is greatly affected by both concentration and formulation used to produce each of the compounds. In addition, the 3000 ppm concentration of *Bacopa monnieri's* alcoholic extract produced the largest percentage of eggs that didn't hatch compared to all other treatments (91%) and was most likely due to the effects of biologically active compounds, such as saponins and terpenes, interfering with normal development during the embryonic stage by damaging egg membranes and disrupting normal physiological functions[3,12, 15]. Saponin activity was observed in this study as superior to terpenes, consistent with earlier results that showed the binding of saponins to sterols and digestive enzymes that can lead to the production of cytotoxic effects and inhibit growth and reproduction of insects[13, 14,30]. While the nano-capsule formulations produced a slower onset of action than the alcoholic extracts, the ability of the nano-capsule formulations to continue to provide a larger percentage of the maximum response with increasing concentrations until a maximum effect was achieved at 140 ppm may be attributed to their ability to continuously release the active constituents in a controlled manner while providing extended release times for their actions, as seen with other types of nanoparticle formulations[18,24]. An extended mode of action results in increased persistence of nano-capsules on the surfaces of seeds with reduced initial toxicity, which was emphasized by studies on other nano-based botanical insecticides[25, 26, 31]. Also, the selective toxicity of such nano-capsules (the fact that they did not affect germination negatively on treated seeds) would make them suitable for protecting stored products. These results are in agreement with earlier findings that support the use of plant-derived extracts, combined with the use of nanotechnology-based formulations, as safe, environmentally-friendly substitutes for conventional chemical pesticides when controlling insects that infest stored products[1, 6].

5. Conclusion

The results showed the effect of the alcoholic extract and nanocapsules on fresh *Callosobruchus maculatus* eggs at 24 hours of age. The alcoholic extract of terpenes at different concentrations (1000, 2000, 3000) showed the highest toxicity to eggs at 3000, with 8 out of 10 eggs not hatching and 2 hatching. As for saponins at the same concentrations, the result was zero hatching at 3000, while the concentrations of 1000 and 2000 were lower, showing that the effect of saponins was greater than that of terpenes. As for the nanocapsules, they were available at different concentrations (80, 100, 120, 140) for terpenes and the same concentrations for saponins. The effect was highest at 140 and lowest at 80 on *Callosobruchus maculatus* eggs.

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Conflict of Interest

This research was conducted with full transparency and integrity, and the authors declare that they have no conflicts of interest.

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