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Evaluation of the efficacy of aqueous and ethanolic extracts of *Eucalyptus camaldolinsis* (Dehnhardt,1832) as a molluscicide compared to metaldehyde against *Monacha obstructa* (Pfeiffer, 1842)

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ABSTRACT

This study evaluates the molluscicide activity of the aqueous and ethanolic extracts of *Eucalyptus camaldulensis* Dehnhardt,1832 leaves against the ground snail *Monacha obstructa* Pfeiffer, 1842 at three concentrations (20, 25 and 30 mg/L) under laboratory conditions, for the purpose of comparison with metaldehyde as a primary molluscicide. Adult snails were randomly divided into four groups (ten snails per group), each receiving three doses of the extract, along with a control group. Mortality was recorded weekly for up to three weeks. We used one-way ANOVA and the LSD test at a significance level of $P \leq 0.05$ for statistical analysis. The study showed that The ethanolic extract exhibited significantly higher molluscicide effects than the aqueous extract at all tested concentrations ($P \leq 0.05$). The highest mortality rate was recorded at a concentration of 30 mg/L after three weeks of exposure. The ethanolic extract of Eucalyptus camaldolenesis showed significant effectiveness against slugs and molluscs; however, the pesticide metaldehyde was more effective. Further studies are needed to determine the feasibility of applying the current findings in the field.

Keywords: Eucalyptus camaldulensis, *Monacha obstructa*, metaldehyde, alcoholic plant extract, boiled aqueous extract.

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1.Introduction

The land snail (Monacha obstructa) belongs to the Gastropoda class, which represents the largest class in the Mollusca phylum of invertebrate animals, which includes large numbers of snails [1]. This snail is of great economic importance due to the damage and destruction it causes to alfalfa crops in particular and other types of agricultural crops in general, as it attacks crops in general, such as cotton, fruit, and vegetable farms. This is due to its high ability to adapt to any new environment, aided by the presence of a calcareous shell, which serves as a hiding place from animals and natural enemies. In addition, it can change the color of its shell to a transparent white to resist sunlight in hot regions. The mucus and the quantities of mucus secreted by the animal help raise the relative humidity of the atmosphere around the snail. These factors increase the snail's ability to adapt to new environments [2]. Therefore, the alfalfa glass snail is considered one of the largest agricultural pests, as it attacks various types of crops [3]. It has become imperative to resort to alternative mechanisms or strategies that contribute to the effective and safe control of snails. Hence, pesticides of plant origin, or what are known as plant extracts [4], have been used. Plant extracts are of great importance in pest control. Humans use them as safe and effective alternatives compared to chemical pesticides, which have a negative impact on the environment and human health, causing environmental problems, high toxicity, and ecological imbalance [5]. Plant pesticides can be extracted from all parts of the plant or from specific parts of the plant, depending on the effectiveness of the chemicals [6]. Many plant extracts have been studied on the mortality rate of insects and some types of snails, which are considered dangerous agricultural pests. Eucalyptus plant contains active compounds found in the aqueous extract such as glycosides, flavonoids, phenolic compounds, tannins, and resins, while the alcoholic extract does not contain proteins, saponins, or alkaloids. Also, eucalyptus leaves contain a group of minerals such as iron (Fe), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), and chromium (Cr) [7]. Eucalyptus also contains phytochemical compounds. These monoterpenes include 1,8-Cineole (30%), Borneol (17%), a-pinene (19%), Cuminaldehyde, Aromadendrene, Pinocarveola, Globulol, and a-terpenol (28%). These compounds are found in various parts of the plant and are biologically active. Eucalyptus leaves contain essential oils, which contain terpenoids. This essential oil contains numerous

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active ingredients, including eucalyptol, α-pinene, lemonene, and p-cymene. Metaldehyde is an organic compound used to control land snails and slugs. Its common name is metaldehyde, and other names are metaacetaldehyde, cycomita, ariotis, halizan, and acetaldehyde. Metaldehyde is a solid, synthetic substance, first discovered by von Liebig in 1835. Metaldehyde is a dry alcohol, obtained by treating acetaldehyde with an acidic catalyst, such as hydrogen bromide. It is classified as a highly polar organic compound. It decomposes to acetaldehyde, and subsequently to water and carbon dioxide. Metaldehyde is soluble and relatively stable in water. Due to its insecticidal properties, metaldehyde is a solid polymer of acetaldehyde and is most commonly used against terrestrial gastropod pests [8]. The scarcity of studies on the use of aqueous and alcoholic extracts on snails in general and specifically on the studied snail, and the absence of such a study in Iraq [9]. This work is aimed to study the effect of the aqueous (boiled) and alcoholic (ethanol) plant extract of Eucalyptus camaldulensis at different concentrations on some life aspects of the terrestrial snail M. obstructa through its mortality rate, and to compare it with the molluscicide Metaldehyde.

2.Materials and Methods

Description of the Study Area

Karbala Governorate is located 105 km southwest of the Iraqi capital, Baghdad, on the edge of the desert west of the Euphrates River and on the left side of the Husseiniya Canal. The city lies at longitude 44 degrees 40 minutes and latitude 33 degrees 31 minutes. It is bordered to the north and west by Anbar Governorate, to the south by Najaf Governorate, and to the east and northeast by Babil Governorate. Al-Hur District is located (2 km) northwest of Karbala City, with an area of approximately (24) km2, and includes a large number of agricultural areas, the most important of which is the Kamaliya District.

1- Snail Sample Collection:

Adult land snails (M. obstructa) were collected manually from farms in the Kamaliya area of Karbala Governorate during November 2022 [10]. Plastic bottles were used to collect the snail samples under study. All information was recorded on them, including the location of collection, snail number, and collection date. The samples were then transported to the laboratory.

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2- Collecting plant samples:

Eucalyptus leaves were collected from the gardens of Karbala Governorate in September 2022. They were dried in well-ventilated areas away from sunlight, ground in a grinder, placed in opaque glass bottles, and refrigerated until ready to use.

3- Preparation of the boiled aqueous and alcoholic plant extract:

The boiled aqueous extract (eucalyptus leaves) was prepared according to the method [11] adapted from [12] with several modifications, including increasing the extraction time to (24) hours. 20g of the ground material under study was taken and placed in a (1000) mL glass beaker containing (400) mL of distilled water at room temperature (cold), and mixed using a hot plate device for 15 minutes and left the mixture for (24) hours to allow the plant parts to settle to obtain better extraction while covering it tightly to prevent the entry of impurities, then the mixture was filtered using dry filter papers (Whtman No. 1) and then the filtrate was transferred to a centrifuge at a speed of 3000 rpm for 10 minutes to obtain the filtrate. The filtrate was concentrated using a rotary evaporator at a temperature of (40-45) Celsius, then the sample was dried. The resulting dry extract was taken from this process and placed in a glass bottle and kept in the refrigerator until use. This process was repeated several times to obtain a sufficient amount of dry sludge for conducting experiments. The previous steps were repeated to prepare the boiled water extract. [13]

To prepare the required concentrations in the study experiments, (10) grams of dry dregs were taken for each extract and dissolved in 100 mL of distilled water to reach a concentration of 10% or 100 mg/ mL, which represents the Stoch Solution, the basic solution, from which the concentrations were prepared. The following concentrations were prepared: (20, 25, 30) mg/L for the eucalyptus boiled water extract. As for the control treatment, only distilled water was used. To prepare the alcoholic extract (eucalyptus leaves), alcohol (70%) was used according to the method of Al-Samarrai, 1983, modified from Harborne, 1973. The extraction process was carried out using the Soxhlet apparatus, by taking 20 grams of eucalyptus leaf powder extraction flask. 400 mL of alcohol was added to them, and the extraction process was carried out for 24 hours at a temperature of 40-459°C. The filtrate was concentrated at a temperature of 40-45°C using a Rotary Evaporator, then the extract was dried in an electric oven at a temperature of 40-

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45°C. The process was repeated several times until a sufficient quantity was obtained to be used in the required laboratory experiments.

4 - How to use the pesticide:

Metaldehyde (6%) powder was chosen as one of the pesticides licensed by the Iraqi Ministry of Health and used by farmers to control snails and ground slugs. This pesticide was used for comparison with the plant extract under study. The following concentrations of the pesticide were prepared (15, 20, 25) mg/L, according to the instructions on the package.

3.Results and Discussio

1- The effect of the boiling aqueous extract of eucalyptus on the mortality of adult snails.

The results of the experiment shown in Table (1) for the boiled aqueous extract of eucalyptus at three concentrations (20, 25, 30) mg/L, respectively, in addition to the control concentration, show that the eucalyptus extract had the least effect on the death of the land snail M. obstructa, with an average of (3.33) mg/L at a concentration of (20). Concentration (30) had the highest effect on snail death, with a death rate of (13.33) for the boiled aqueous extract. There were clear significant differences between the three concentrations used, with the control concentration below the significance level of 0.05, as shown in Table (1).

Table1 . The effect of the boiling aqueous extract of eucalyptus on the mortality of adult snails.

Concentration	Time			Concentration rate
	Week 1	Week 2	Week 3	
0	0.00	0.00	0.00	0.00
20	0.00	0.00	10.00	3.33
25	0.00	10.00	20.00	10.00
30	10.00	10.00	20.00	13.33
Time rate	2.50	5.00	12.50	
LSD	Concentration	Time	Inter action	
	2.5456	3.5155	6.0611	
Moral level	0.05	0.05	0.05	

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There is a relationship between the concentration of boiled aqueous extract and the mortality rate of the snail M. obstructa. As the concentration increased, the mortality rate also increased, with the highest mortality rate observed at a concentration of 30 mg/ mL of the boiled eucalyptus aqueous extract during the third week. This finding is consistent with the study by [14] , which indicated that increasing the extract concentration accelerates the mortality of the organism due to various reasons, including the indirect physiological effects caused by the toxic compounds on the snail's tissues, which leads to a disruption in neural secretion. Additionally, there is a direct impact of toxic compounds on specific tissues referred to as target tissues, or due to toxic compounds that inhibit the activity of digestive protein enzymes. These substances are highly toxic due to their ability to penetrate body cells and hinder the production of necessary proteins for biological functions, thereby destroying cells in all body organs. Furthermore, the results align with the study by The results indicate that the toxicity of this eucalyptus leaf extract may be partly due to its content of active compounds such as terpenes and volatile oils, particularly cineole and camphor. These compounds negatively affect the nervous system and vital functions of snails. Studies [11, 14] confirmed that the chemical composition of eucalyptus found in Iraq may differ somewhat from that found in other regions, attributable to varying environmental conditions. This suggests a difference in the type and concentration of active compounds. This is reflected in the variation in toxicity and LD50 values. Sometimes, local plants possess higher proportions of different bioactive compounds compared to other regional plants, which explains the discrepancies in the results of different studies [15] .

1- **The effect of the alcoholic extract of eucalyptus on the mortality of adult snails**

The results showed that the alcoholic extract of eucalyptus at three concentrations (20, 25, 30) mg/L was the most effective on snails compared to the other concentrations. Pesticide concentrations (20, 15, 25) were the most lethal to snails compared to the plant extract concentrations in general, with the highest concentration of pesticide (25), causing a 100% mortality rate, as shown in Table 2.

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Table 2. The effect of the alcoholic extract of eucalyptus on the mortality of adult snails.

Concentration	Time			Concentration rate
	Week 1	Week 2	Week 3	
0	0.00	0.00	0.00	0.00
20	0.00	10.00	10.00	6.66
25	10.00	10.00	20.00	13.33
30	10.00	10.00	40.00	20.00
Time rate	5.00	7.50	17.50	
LSD	Concentration	Time	Inter action	
	1.5452	1.2132	2.7584	
Moral level	0.05	0.05	0.05	

The mortality rate was determined at concentrations (30, 25, 20), and the alcoholic extract of eucalyptus had a stronger effect at the highest concentration compared to the lower concentrations. A study by [16] investigated the effect of eucalyptus oil and the ethanolic extract of castor bean seeds Ricinus. communis on the reproductive biology of the land snail Theba pisana. The snails were exposed to different concentrations of these plant extracts for six weeks, which led to changes in some physiological parameters, including some hormones. Moreover, increasing the concentrations of the extracts, especially the alcoholic ones, resulted in greater changes in those parameters, and a 2% concentration of R. communis extract caused a significant decrease in the activities of antioxidant enzymes The results indicate that the toxic effects of eucalyptus leaf extract are linked to the presence of active compounds such as cineole and camphor. These compounds have direct effects on the nervous system and vital functions of snails. Studies (11,14) also showed that variations in environmental conditions between local and regional eucalyptus species may lead to differences in the type and concentration of these compounds, which could affect the toxicity of the extract.

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3- **The effect of metaldehyde pesticide on the mortality of adult snails.**

The results of the experiment shown in Table (3) indicated that when using the pesticide metaldehyde at three concentrations (15, 20, 25) mg/ mL respectively, in addition to the control concentration (0), we observe that the pesticide had the most significant effect at a concentration of (25), resulting in a mortality rate of (100.00) for the land snail M. obstructa, while the effect of the pesticide at a concentration of (15) was the least effective, with a mortality rate of (66.6%) .

Table 3. The effect of metaldehyde pesticide on the mortality of adult snails.

Concentration	**Time**			**Concentration rate**
	Week 1	Week 2	Week 3	
0	0.00	0.00	0.00	0.00
15	40.00	60.00	100.00	66.66
20	60.00	40.00	100.00	66.66
25	100.00	100.00	100.00	100.00
Time rate	50.00	50.00	75.00	
LSD	Concentration	Time	Inter action	
	0.9727	0.8426	1.4441	
Moral level	0.05	0.05	0.05	

The results of the current study agreed with what [17] reported that the treatment with the molluscicide metaldehyde for the land snail Eobania. vermiculata led to rapid mortality in the snail according to the concentrations used [18,19]. The reason is attributed to the fact that metaldehyde acts to destroy the connective tissue and cause pathological histological changes and a wide gap in the cytoplasm of the digestive cells, as well as necrosis in the epithelium lining the digestive tubes through microscopic examination, irregular thickening in the muscular layer of the external covering, thinning of the basal layer, and the appearance of histological changes in the kidneys where there is degeneration of renal cells and an increase in the number and size of aggregates in the cellular cells. Metaldehyde showed cytotoxic effects on all snail organs, which in turn leads to failure of the digestive, reproductive, and excretory systems.

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4- Compared with metaldehyde pesticide and Eucalyptus

The results of the experiment using metaldehyde pesticide at three concentrations (15, 20, 25) mg/ mL showed that there were significant differences between the concentrations below the significance level of 0.05 (P≤) [20,21]. The minimum differences were found in the first concentration, reaching (0.9727). As for the time factor, the first week also had a percentage of (0.8426). Table (4) shows that the third concentration, (25) mg/ mL, was the most lethal to the snails in the study, and from the first week, the mortality rate reached 100%, followed by the other two concentration levels used.

Table 4. Compared with metaldehyde pesticide and Eucalyptus

Treatment	Time			Treatment rate
	Week 1	Week 2	Week 3	
Control	0.00	0.00	0.00	0.00
Boiled Eucalyptus 30	40.00	60.00	100.00	66.66
Alcoholic Eucalyptus 30	60.00	40.00	100.00	66.66
Metaldehyde 25	100.00	100.00	100.00	100.00
Time rate	50.00	50.00	75.00	
LSD	Treatment	Time	Inter action	
	0.9727	0.8426	1.4441	
Moral level	0.05	0.05	0.05	

The current study agreed with what was stated in the study by [22] regarding the control of the land snail M. cartusiana using eucalyptus extract as a natural and environmentally safe molluscicide [23]. The concentration of ethanol eucalyptus at 20 mg/ mL caused a mortality rate of 10% after one day of exposure to the extract and 66.7% after a 28-day exposure period. The mortality rate of snails was rec [24] orded at 90% after treatment with a concentration of 40 mg/ mL of eucalyptus extract, which resulted in increased levels of antioxidant enzymes in the

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treated snails compared to the untreated snails. It also caused hemolysis, increased luminal secretions, cellular distortions in the digestive gland, and rupture of cellular membranes [25].

Conclusions

The alcoholic plant extract is more lethal to the snail Monacha obstructa as it is more effective than the boiled aqueous plant extract. The ethanolic extract showed significant effectiveness in controlling snails, but its effectiveness did not exceed that of the insecticide. Further toxicology and field studies are recommended.

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