



## EVALUATION OF THE EFFICIENCY OF SOME AQUEOUS AND ALCOHOLIC PLANT EXTRACTS IN CONTROLLING THE SOUTHERN COWPEA BEETLE (*CALLOSOBRACHUS MACULATUS* .F)

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### ABSTRACT

Article information  
Article history:  
Received: 16/05/2025  
Accepted: 29/03/2026  
Published: 31/03/2026

#### Keywords:

Southern cowpea beetle (*Nerium oleander*.) (*Melia azedarach*), (*Eucalyptus camaldulensis*)  
Plant extracts.

DOI:

[10.33899/mja.2026.160372.1594](https://doi.org/10.33899/mja.2026.160372.1594)

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The results of the study showed that the alcoholic extract of *Nerium oleander* leaves was superior, recording the highest Mortality rate of 34.14%, which did not significantly differ from the average Mortality rate of *Melia azedarach* extract, which amounted to 33.05%. Meanwhile, the aqueous extract of *Eucalyptus camaldulensis* leaves achieved the lowest Mortality rate of 15.55%. The interaction effect between the extract and the concentration used also had a significant impact, as the alcoholic extract of *Nerium oleander* achieved the highest Mortality rate of 65.56% at a concentration of 3.5%. Also, regarding the interaction effect between the extracts studied and the treatment time, we found that the alcoholic extract of *Nerium oleander* leaves was more effective, achieving a Mortality rate of 43.33% after 72 hours of treatment. As for the interaction effect between the concentrations used and the time used on the Mortality rate, the concentration achieved 3.5% after 72 hours. The highest mortality rate was 66.66% for the treatment, while the lowest mortality rate was at a concentration of 1.5% after 24 hours of treatment. Therefore, the results confirmed that the alcoholic extract of *Nerium oleander* leaves is effective and has a significant impact on the mortality rate of the southern cowpea beetle, as it is considered an environmentally friendly natural pesticide.

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## INTRODUCTION

The southern cowpea beetle (*Callosobruchus maculatus* . F.) is one of the most important pests of legume seeds, especially in many tropical and subtropical regions around the world, causing qualitative and quantitative damage during storage (Jehajo, Anjorin, 2018, Din, 2020). The host range of this insect is wide, as it infects the seeds of many legume crops, including chickpeas, broad beans, lentils, and peas, in addition to its main host, cowpea. It causes severe crop losses, reaching 90% during the storage period, which ranges from three to six months under ideal storage conditions of temperature, humidity, and lack of light (Agour *et al.*, 2022, and Taggar, 2021). According to the World Health Organization statistics of 2009, Iraq is among the countries producing and consuming cowpeas, which makes it necessary to pay attention to combating and controlling pests that affect it (Mowery, 2012 and Syaekhu , 2023). The importance of this insect comes from its feeding and the development of its larvae inside the seeds and consuming all their contents, thus

increasing the percentage of seed damage and reducing their nutritional value and germination rate (Mounika *et al.* 2025). To avoid the damage of this insect, several methods were used, the most important of which are synthetic chemical pesticides (Ebadollahi, *et al.* 2020), in addition to fumigating stores with toxic gases such as methyl bromide (Kang *et al.* 2020). However, these pesticides caused environmental problems, as they created unwanted residues in food and toxic effects on humans, in addition to increasing production costs (Boneer *et al.* 2017). As a result, the search for plants developed as a cheap and sustainable alternative for pest control (Bachrouch, 2025). The use of plant extracts has received special attention due to their safety, wide consumer acceptance, environmental friendliness, very low or no toxicity, and biodegradability for controlling various stored insect pests (Farag *et al.*, 2021). These extracts have been shown to have cytotoxic, antioxidant, antifungal, and insecticidal properties (Zimmermann *et al.*, 2021). Aqueous extract of neem seeds has been used to protect crops from pest infestation (Akbar, 2022; Androustopolou *et al.*, 2021).

### **MATERIALS AND METHODS**

1- Plant Collection: *Eucalyptus camaldulensis*, *Nerium oleander*, and *Melia azedarach* leaves were collected from the University of Mosul gardens in November 2023 and stored at laboratory temperature until dry, with occasional stirring. The dried leaves of Eucalyptus and Oleander plants and the dried seeds of Melia azedarach were ground in an electric grinder, placed in nylon bags, and stored in the laboratory until ready for use.

2- Preparation of the alcoholic extract: 20 g of the ground leaf and seeds extract were taken and placed in a 500 ml glass beaker. ml of 70% ethyl alcohol was added to the mixture. The mixture was placed in a shaker for 24 hours, after which it was filtered using a fine sieve cloth. The filtrate was placed in a centrifuge at 3000 rpm for 15 minutes. The filtrate was then collected in glass dishes and dried in an oven at 40°C until the alcohol evaporated. The powder was placed in glass bottles and stored in the laboratory until use (Ali Shtayeh, 1999).

3- Preparation of the aqueous extract: 20 grams of ground leaf and seeds extract were taken and placed in a 500 ml glass beaker. 200 ml of distilled water was added to the extract (usually for 72h) and then placed in a centrifuge at 5,000 rpm for 10 minutes. The filtrate was then evaporated in an oven at 40°C until the water evaporated. The powder was placed in bottles and stored until use (Gökçe, 2012).

4- Preparation of aqueous and alcoholic extract concentrations: Concentrations of 1.5, 2.5, and 3.5% were prepared for the aqueous and alcoholic plant extracts by taking 10g of each plant extract powder separately and dissolving it in 100ml of distilled water for the aqueous extracts and 100ml of ethyl alcohol for the alcoholic extracts, to obtain a 10% stock solution. The above concentrations were then prepared. The control treatment was performed with distilled water and alcohol alone (Gökçe, 2012).

5- Insect rearing: Cowpea seeds infested with the southern cowpea beetle were obtained from local markets and added to 250 g of sterilized seeds in an electric oven at 60°C for two hours, to obtain a pure colony of the insect. The seeds were then placed in clean glass bottles, covered with a muslin cloth lid, tied with a rubber band,

and placed in an incubator at 28°C and 60% relative humidity. The colony was replenished regularly.

6- The effect of aqueous and alcoholic plant extracts on mortality rates. Sterilized, intact cowpea seeds were placed in Petri dishes, 20 seeds per dish, in three replicates. They were treated with aqueous and alcoholic extracts at concentrations of 1.5, 2.5, and 3.5%. The seeds were immersed in the extracts for 5-10 seconds. The seeds were placed on filter paper to dry. The control treatment involved treating the aqueous extracts with water only and the alcoholic extracts with alcohol. A plate containing 10 southern cowpea beetle larvae was then added to the control treatment. The effect of the extracts is determined by feeding the larvae on the extract treated cowpea seeds. The seeds were placed in an incubator to calculate mortality rates 24, 48, and 72 hours after treatment.

7- Statistical Design and Analysis: All experiments were analyzed according to a completely randomized design (C.R.D.) model, and means were compared using the least significant difference (LSD) test using the SAS program. The percentage of Mortality values were corrected according to the Abbott formula (Abbott 1925).

$$\text{Percentage of Mortality} = \frac{\text{Percentage of mortality in treatment} \times \text{percentage of mortality in control}}{100 - \text{percentage of mortality in treatment}} \times 100$$

## RESULTS AND DISCUSSION

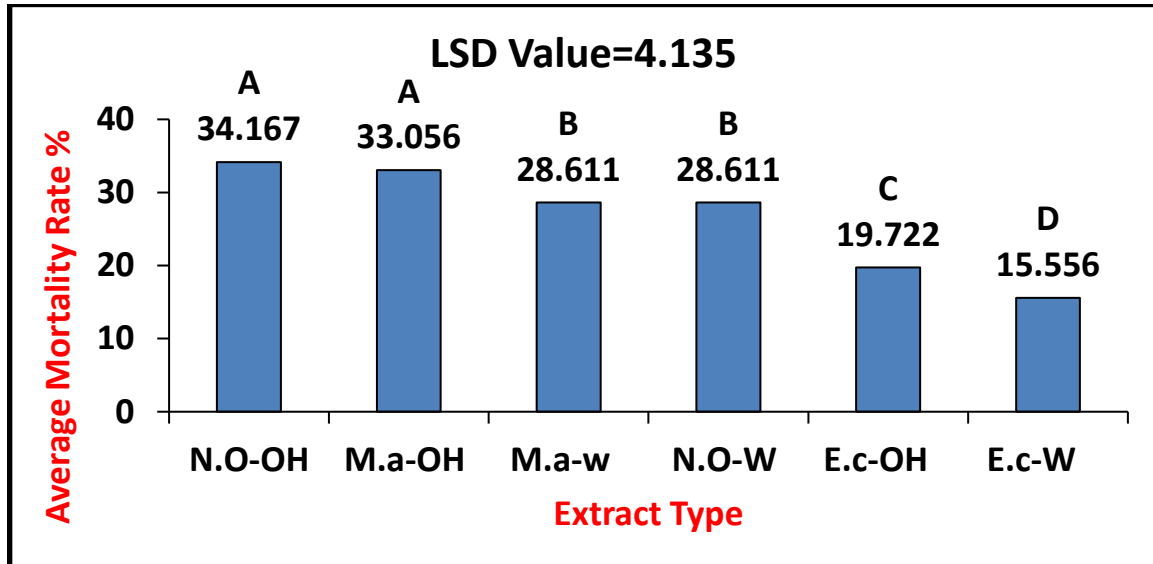
1- The effect of aqueous and alcoholic plant extracts on the average mortality rates of adult cowpea beetle *Callosobruchus maculatus*. F

The results of Figure (1) showed the effect of aqueous and alcoholic plant extracts and their interaction on the mortality rates of the southern cowpea beetle. The alcoholic extract of *Nerium oleander* leaves achieved the highest mortality rate, reaching 34.167, which did not significantly differ from the mortality rate of the alcoholic extract of *Melia azedarach* seeds, which reached 33.056, compared to the control treatment, which was sprayed with distilled water only, where the mortality rate was zero. Meanwhile, the aqueous extract of *Eucalyptus camaldulensis* leaves achieved the lowest mortality rate, reaching 15.556, which significantly differed from the alcoholic extract of the leaves of the same plant, which reached 19.722. This confirms what Alhadidy (2023) reached when studying the effectiveness of alcoholic and aqueous extracts of *Nerium oleander*, *Ocimum bacilicum*, *Melia azedarach*, and *Cyperus* leaves. (Royundus) against the cowpea beetle. *Nerium oleander* leaf extract outperformed the other extracts used in the study in terms of the studied characteristics this means that the presence of alcohol may have increased the toxicity of the leaf and seed extracts of the plants used in the experiment .

In a study by (Alhadidy 2023) on the effectiveness of alcoholic and aqueous extracts of *oleander*, basil, and Chinese mulberry leaves against the cowpea beetle, the study showed that *oleander* and mulberry leaf extracts outperformed basil and Chinese mulberry leaf extracts in terms of the studied characteristics.

- In a study by (Haidri, 2021) on the biological effectiveness of *A. indica* and *M. koenigi*, it was found that *A. indica* caused the highest mortality rate, reaching 79%, compared to *M. koenigi*, which reached 67%. Nisar (2022) also found in his study on the effectiveness of neem seed powder and extracts on the beetle, the cowpea beetle, that all treatment levels showed toxic activity,

prevention of infection, and rapid results, as is the case with the inorganic pesticide darksh. The basis upon which the comparisons are built is to understand the effect of the substances contained in the parts of the plants used, whether in the leaves or the seeds.

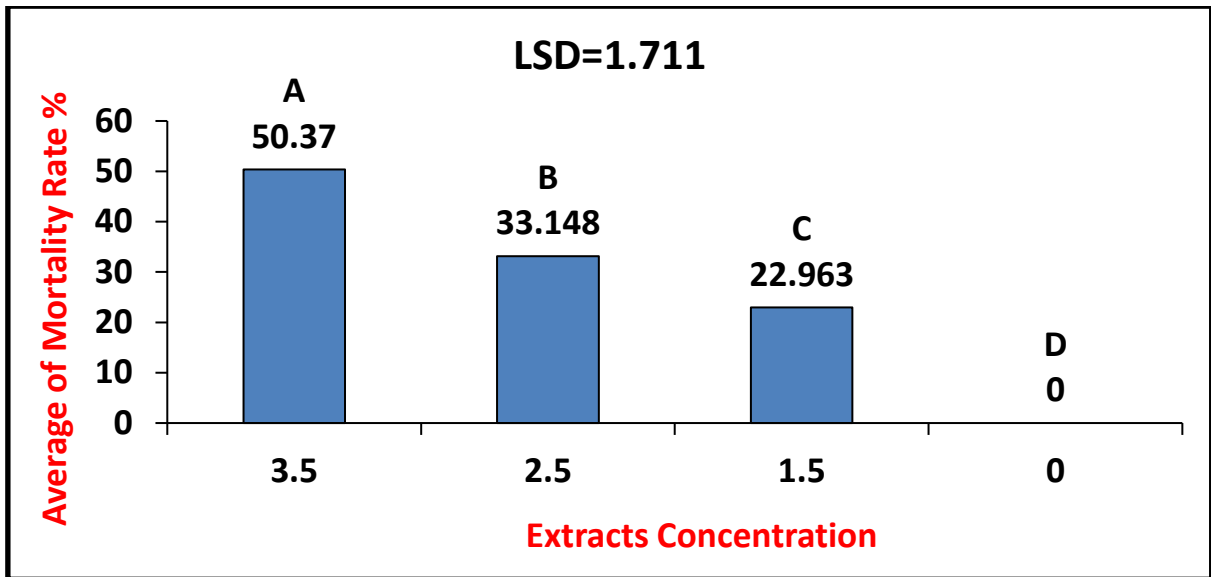


Means with non-identical letters indicate significant differences at the 5% level according to the LSD test.

**Figure (1) Effect of aqueous and alcoholic plant extracts on the Average Mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* . F).N.O=*Nerium Olender*----M.a=*Melia azedarach*---- E.c=*Eucalyptus camaldulensis*--- OH= alcohol----W= aqueous**

2- The effect of the concentrations used on the average mortality rates of the southern cowpea beetle (*Callosobruchus maculatus*, F.)

The results of the study, shown in Figure (2), confirmed that the concentrations used in the study had a clear effect on the mortality rates of the southern cowpea beetle. The 3.5% concentration resulted in the highest mortality rate of 50.37, which differed significantly from the 2.5% and 1.5% concentrations, which resulted in mortality rates of 33.14 and 22.96, respectively, compared to the control treatment, which did not result in any mortality rate, which was zero. This was confirmed by Akbar's study (2024), which showed that the *A. indica* plant extract achieved the lowest infection rate of 16.65% and the highest expulsion rate of 100% at a concentration of 3%, 48 hours after the cowpea beetle treatment.

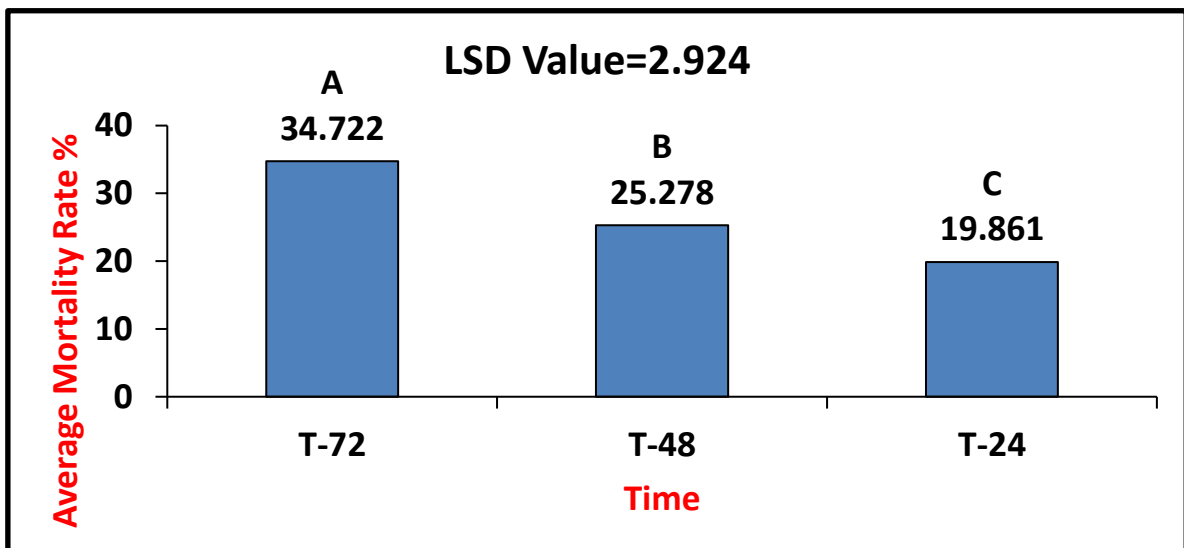


Means with non-identical letters indicate significant differences at the 5% level according to the LSD test.

**Figure (2) Effect of the concentrations used on the Average of mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* . F).**

3-The effect of time on the average mortality rate of the southern cowpea beetle adults. (*Callosobruchus maculatus*. F).

The results of Figure (3) illustrate the effect of time on the mortality rate of the southern cowpea beetle. The 72-hour period produced the highest mortality rate of 34.72%, which was significantly different from the mortality rates after 48 hours and 24 hours, which reached 25.27% and 19.86%, respectively. In a study by Adams (2022) on the effect of mint leaf powder, neem leaf powder, and camphor on cowpea beetle infestation in storage, the study demonstrated the success of the treatments in protecting seeds from infestation during the 8-week storage period. Therefore, neem and mint leaf powders can be used as an alternative source of pesticides for pest control.



Means with non-identical letters indicate significant differences at the 5% level according to the LSD test.

**Figure (3) Effect of time on the average mortality rate of the southern cowpea beetle adults(*Callosobruchus maculatus*).**

4. The results of table (1) indicated that the alcoholic and aqueous extracts of the studied plants showed a varying effect on the mortality rate of the southern cowpea beetle adults depending on the concentrations used in the study. The results of the statistical analysis showed significant differences in the mortality rate depending on the type of aqueous and alcoholic extract and the concentration used. The highest mortality rate was for the alcoholic extract *Nerium oleander* which reached (65.56) at a concentration of 3.5%, followed by the alcoholic extract *Melia azedarach* which reached (58.89) at a concentration of 3.5%, which differed significantly between them and from the rest of the other treatments. The lowest mortality rate was for the aqueous extract of the leaves of the plant *Eucalyptus camaldulensis* which reached (12.22) at a concentration of 1.5%. This was confirmed by the study of Akbar (2024). *A. indica* plant extract achieved the lowest infection rate of 16.65% and the highest repellency rate of 100% at a concentration of 3% after 48 hours of treatment with the beetle. Therefore, this study suggests incorporating these plant extracts into integrated pest management programs.

**Table (1): Effect of the interaction between the type of aqueous and alcoholic plant extracts and the concentrations used on the average mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* F.). N.O = *Nerium oleander*, M.a = *Melia azedarach*, E.c = *Eucalyptus camaldulensis*, OH = Alcoholic extract, W = Aqueous extract.**

Extract*Concentration	Mortality %	LSD Group
M.a-OH 3.5	65.56	A
N.O-OH 3.5	58.89	AB
M.a-W 3.5	55.56	BC
N.O-W 3.5	48.89	CD
N.O-OH 2.5	43.33	DE
M.a-OH 2.5	41.11	DEF
E.c-OH 3.5	40.00	EF
N.O-W 2.5	37.78	EF
M.a-W 2.5	36.67	EF
N.O-OH 1.5	34.44	FG
E.c-W 3.5	33.33	FGH
N.O-W 1.5	27.78	GHI
M.a-OH 1.5	25.56	HI
E.c-OH 2.5	23.33	IJ
M.a-W 1.5	22.22	IJ
E.c-W 2.5	16.67	JK
E.c-OH 1.5	15.56	JK
E.c-W 1.5	12.22	K

Means followed by different letters differ significantly at the 0.5% probability level according to the LSD test.

5- The effect of interaction between the type of aqueous and alcoholic extracts with the time used on the Average of Mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* . F).

The results of the study in table (2) indicate that the interaction between the studied aqueous and alcoholic extracts with the treatment time differs in their effect on the average mortality rate of the southern cowpea beetle adults. Based on the results of the statistical analysis, we find that the alcoholic extract of *oleander* leaves was the most effective extract on the insect adults, as the mortality rate reached 43.33% after 72 hours of treatment, which differed significantly from the rest of the extracts used and at the same treatment time. While the aqueous extract of *eucalyptus* leaves achieved the lowest mortality rate of 10% after 24 hours of treatment, which in turn differed significantly from all extract treatments. This confirms what Ahmed (2020) found in his study on the biological effectiveness of three plant extracts against the southern cowpea beetle, that the mortality rate is directly proportional to time.

**Table (2). Effect of the interaction between the type of aqueous and alcoholic extracts and the exposure time on the average mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* F.). N.O = *Nerium oleander*; M.a = *Melia azedarach*; E.c = *Eucalyptus camaldulensis*; OH = alcoholic extract; W = aqueous extract.**

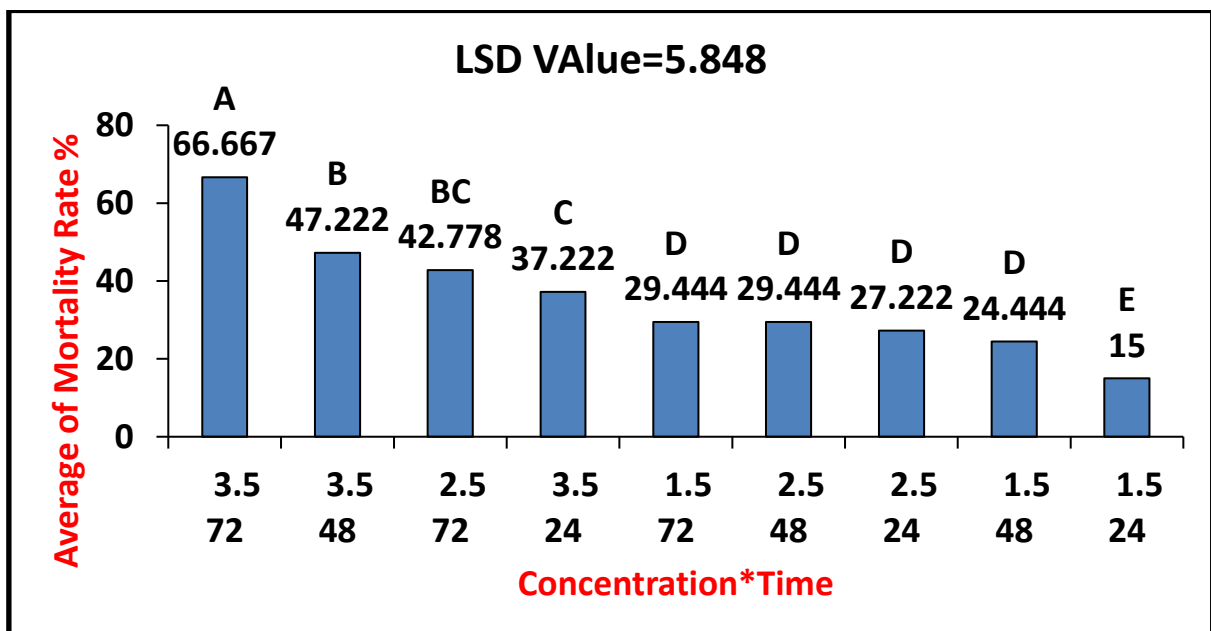
Extract × Time	Mean	LSD Group
N.O–OH 72	43.33	A
M.a–OH 72	40	AB
N.O–W 72	39.167	AB
M.a–W 72	34.167	BC
M.a–OH 48	31.667	CD
N.O–OH 48	31.667	CD
M.a–W 48	30	CDE
M.a–OH 24	28.333	CDEF
E.c–OH 72	27.5	CDEF
N.O–W 48	26.667	DEFG
E.c–W 72	23.333	EFGH
M.a–W 24	21.667	FGH
N.O–W 24	20	GHI
E.c–OH 48	18.333	HIJ
E.c–OH 24	13.333	IJK
E.c–W 48	12.5	JK
E.c–W 24	10	K

Means followed by different letters differ significantly at the 0.5% probability level according to the LSD test.

6- The effect of the interaction between the concentration used and the time used on the Average of average mortality rate of adult cowpea beetles (*Callosobruchus maculatus*, F.).

The results of the study in Figure (4) demonstrated that the interaction between the time used and the concentration used affected the mortality rate of adult cowpea

beetles. The 3.5% concentration achieved the highest mortality rate of 66.66% after 72 hours of treatment, which differed significantly from the same concentration after 48 hours of treatment, which reached 47.22%. The lowest mortality rate was at the 1.5% concentration, 24 hours after treatment, which in turn differed significantly from the other concentrations used and the time used, which reached 15%. This means that the mortality rate increases directly with increasing concentration and time. This confirms the findings of (Mohammed, 2024) that treating the food of the southern cowpea beetle with an alcoholic extract led to an increase in the mortality rate with increasing concentrations used. Likewise, what was found by Husein and Ali (2024) in their study was that the extract *Heliotropium bacciferum* achieved the highest percentage of Mortality adults and eggs at a concentration of 10% and after 96 hours of treatment, meaning that the percentage of Mortality is directly proportional to the concentration and time.



Means with non-identical letters indicate significant differences at the 5% level according to the LSD test

Figure (4) Effect of interaction between the concentration used and the time used on the Average Mortality rate of adults of the southern cowpea beetle (*Callosobruchus maculatus* . F).

### CONCLUSIONS

- 1- The alcoholic extract of the leaves of the plant (*Nerium oleander*) was superior, recording the highest Mortality rate of 34.14% compared to the extracts studied.
- 2- The 3.5% concentration achieved the highest Mortality rate of 50.37%, which was significantly different from the 2.5% and 1.5% concentrations
- 3- Regarding the time used after treatment, the time 72 hours after treatment achieved the highest Mortality rate of 34.72%, which differed significantly from the Mortality rate 48 and 24 hours after treatment.
- 4- The interaction effect between the extracts and the concentrations used was significant on the killing rate, as the alcoholic extract of the leaves (*Nerium*

*oleander*) at a concentration of 3.5% achieved the highest Mortality rate of 65.56%, which differed from the extracts studied at the same concentration.

5- As for the effect of the interaction between the extracts studied and the treatment time, it was found that the alcoholic extract of the leaves of the plant (*Nerium oleander*) achieved the highest Mortality rate after 72 hours of treatment, reaching 43.33%.

6- The effect of the interaction between the studied extracts and the concentrations used with the treatment time was great on the Mortality rates. The alcoholic extract of the leaves of the plant (*Nerium oleander*) achieved the highest Mortality rate at a concentration of 3.5% and after 72 hours of treatment, it reached 66.66%

Therefore, the results showed that the alcoholic extract of the leaves of the plant (*Nerium oleander*) is effective and has a significant impact on the Mortality rate of the southern beetle, as it is considered a natural and environmentally friendly pesticide.

### ACKNOWLEDGMENT

The authors extend their gratitude to the University of Mosul and the College of Agriculture and Forestry Department of Plant Protection, and the University of Telafer and the College of Agriculture for their support in making this work.

### CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

### تقييم كفاءة بعض المستخلصات النباتية في مكافحة حشرة خنفساء اللوبيا الجنوبية (*Callosobrachus maculatus*)

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#### الخلاصة

أظهرت نتائج الدراسة تفوق المستخلص الكحولي لأوراق الدفلة (*Nerium oleander*) مسجلاً أعلى معدل هلاك بلغ 34.14%، وهو معدل لم يختلف بشكل كبير عن متوسط معدل هلاك مستخلص *Melia azedarach* الذي بلغ 33.05%. في حين حقق المستخلص المائي لأوراق *Eucalyptus camaldulensis* أقل معدل هلاك بلغ 15.55%. كما كان للتفاعل بين المستخلص والتركيز المستخدم تأثير كبير، حيث حقق المستخلص الكحولي لأوراق الدفلة (*Nerium oleander*) أعلى معدل هلاك بلغ 65.56% عند تركيز 3.5%. وفيما يتعلق بتأثير التفاعل بين المستخلصات المدروسة ومدة العلاج، فقد وجدنا أن المستخلص الكحولي لأوراق الدفلة (*Nerium oleander*) كان أكثر فعالية، محققاً معدل هلاك بلغ 43.33% بعد 72 ساعة من العلاج. أما بالنسبة لتأثير التفاعل بين التراكيز المستخدمة والمدة على معدل الوفيات، فقد حقق التركيز 3.5% بعد 72 ساعة. وبلغ أعلى معدل وفيات 66.66% للمعاملة، بينما كان أقل معدل وفيات عند تركيز 1.5% بعد 24 ساعة. لذلك، أكدت النتائج فعالية المستخلص الكحولي لأوراق الدفلة (*Nerium oleander*) وتأثيره الكبير على معدل وفيات خنفساء اللوبيا الجنوبية، حيث يُعتبر مبيدًا طبيعيًا صديقًا للبيئة. الكلمات المفتاحية: خنفساء اللوبيا الجنوبية، بوكالبتوس، دقله، سبجح، مستخلصات نباتية.

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