NTU Journal of Agricultural and Veterinary Sciences (2025) 5 (2) : 221-228
DOI: https://doi.org/10.56286/t4agqe51

IRAQI Academic Scientific Journals

JAVS NTU

P-ISSN: 2788-9890 E-ISSN: 2788-9904
NTU Journal of Agricultural and Veterinary Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JAVS/index

Evaluation of the Efficiency of the Plant *Urtica dioica* L. in Controlling Some Stages of the Greater Wax Moth (Lepidoptera: Pyralidae) *Galleria mellonella* L.

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Article Informations

Received:15–10–2024,
Accepted:23–04–2025,
Published online: 28–06–2025,

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Key Words:
keyword1, *Urtica dioica*
keyword2,*Galleria mellonella*
keyword3, alcoholic extract
keyword4, aqueous extract
keyword5.dry powder
keyword6, Evaluation and Efficiency

Abstract

The results of the study showed the superiority of both the alcoholic and aqueous extracts in recording the highest egg mortality rates at the third and fourth concentrations (100.00 and 93.33%, respectively), while the dry powder recorded (26.67 and 36.67%). As for the mortality of third and fifth instar larvae, the alcoholic extract at the third concentration after 96 hours of treatment, and at the fourth concentration after 72 and 96 hours, recorded a mortality rate of (100.00%). Regarding the mortality rates of the fifth instar, all treatments showed gradually increasing mortality rates, especially the alcoholic extract at the fourth concentration after 96 hours, which gave the highest mortality rate (96.67%). The aqueous extract and dry powder at the final concentration recorded (86.67 and 66.67%, respectively). The pupal mortality rate with the alcoholic extract was (80.00%), while the third concentration of the same treatment recorded (73.33%), which did not differ significantly from the aqueous extract treatment at both the third and fourth concentrations (73.33%). The lowest pupal mortality rate was recorded with the dry powder (26.67%).

Introduction

Plant-based pesticides are considered safe and low-risk pesticides. One of the most important plants in this category is *U. dioica*, (NETTLE)which is known for containing biologically active compounds and substances that have shown good results in repelling and killing insect larvae, similar to the active ingredients found in chemical pesticides. *U. dioica* is characterized by its stinging hairs, distributed across all parts of the plant, which cause irritation upon contact. The active compounds in this plant exhibit repellent and insecticidal properties (Maričic et al., 2021). Esposito et al., (2019) indicated that one of the characteristics taken into consideration when using *U. dioica* in pest control, including the greater wax moth, is its content of phenolic antioxidants in the plant tissues, which is utilized in controlling many stored-product pests from Coleoptera and Lepidoptera orders. Shazdeahmadi (2021) used the alcoholic extract of *U. dioica* to control tobacco pests. In Iraq, Al-Joary et al., (2021) found that using *U. dioica* powder in controlling the red flour beetle achieved a repellent rate of 30.46% against the pest. Meanwhile, Al-Jamil and Al-Bajari (2021) found that the alcoholic extract of *U. dioica* resulted in a mortality rate of 68.74% for nymphs and adults of the whitefly, respectively. Al Chalabi et al., (2023) demonstrated that using different concentrations of the ethanolic extract of *U. dioica* showed good results in repelling and killing the larvae of the greater wax moth, and their study results showed that the extract caused deformities in the pest's developmental stages. Given the scarcity of studies in Iraq and the Arab world on controlling the greater wax moth using *U. dioica* and its components, the idea for this study arose. The study aims to investigate the lethal effects of different concentrations of the alcoholic and aqueous extracts, as well as the dry powder of *U.dioica*, and to assess the mortality rates they achieve against some stages of the greater wax moth.

Materials and Methods:

The study was conducted during the 2023–2024 season in the laboratory and apiary of the Plant Protection Department and the Central Laboratory of the College of Agriculture and Forestry / University of Mosul to evaluate the efficiency of the alcoholic and aqueous extracts, as well as the dry powder of *U. dioica*, in controlling the egg stage, third and fifth larval instars, and pupal stage of the greater wax moth. The study included the following:

Preparation of the aqueous and alcoholic extracts and the dry powder of *U. dioica* From the agricultural fields in Nineveh Governorate:

A. Preparation of plant powder concentrations of Urtica dioica

Different weights of *Urtica dioica* powder, which was pre-prepared, were taken in four different weights (5 g, 10 g, 15 g, and 20 g) after drying it for use in the study. A sensitive digital balance (four digits after the decimal) of the Sartorius type was used to weigh the powder and prepare the treatments.

B. Preparation of aqueous extract concentrations of U. dioica

Fifty grams of dry plant material were soaked in one liter of water for two hours on a shaker device in the laboratory. Then, the mixture was left for 24 hours in a dark place. The liquid was filtered in two stages: first, through a muslin sieve to remove large plant parts, and second, by filtering the liquid using filter paper(user in laboratories). The final product was a 100% concentration, which is the original (raw) concentration. Four final concentrations were prepared from this and used in the study (25%, 50%, 75%, 100%), following the method of Hjeij et al. (2018). The four extracts were placed in opaque bottles, labeled with the concentration, extraction date, and plant name, and stored in the refrigerator until use.

C. Preparation of alcoholic extract concentrations *of U. dioica*

After drying and grinding the *U. dioica* plant, 50 grams of dry plant material were soaked in 150 ml of ethanol for two hours on a shaker device in the laboratory. The mixture was then left for 24 hours in a dark place, and the liquid was filtered in two stages. The first stage was through a muslin sieve to remove large parts of *U. dioica*, and the second stage involved filtering the liquid using filter paper. The filtrate was placed on a rotary evaporator at 115 rpm at a temperature of 60°C until all the ethanol evaporated. The residue was then dissolved in 1–2 ml of ethanol, and distilled water was added to reach the original volume, which was used to prepare four final dilutions for the study. These dilutions were (first concentration 5 ml, second 10 ml, third 15 ml, and fourth 20 ml per liter), following the method of Hjeij et al. (2018). The four alcoholic extracts were placed in opaque bottles, labeled with the concentration and extraction date, and stored in the refrigerator until use.

1. **Study of the Lethal Effect of the Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the Eggs, Larvae, and Pupae of the Greater Wax Moth**

   Preparation of the Insect Stages:

The adult insect and its various stages were obtained from the wax discs infected with the pest after sending samples of the insect and its stages to the Natural History Museum, University of Baghdad, for entomology to confirm the diagnosis of the insect. The diagnosis was confirmed according to the museum's book numbered (30) on 4/1/2024

Different larval stages of the greater wax moth, along with adults, were collected from pre-prepared

and maintained breeding colonies. The larvae were collected using a soft brush and placed in plastic rearing containers of 500 cm³ capacity, with a diameter of 10 cm and a height of 5 cm, covered with muslin cloth. These containers were supplied with pre-sterilized old beeswax, which had been frozen for 24 hours, for larval feeding (Kareem, 2011). The larvae were monitored until they entered the pupal stage. To establish a continuous culture, the pupae were transferred to pre-sterilized plastic containers of size 10×10×30 cm, covered with muslin cloth and tightly secured with a rubber band to prevent the larvae and adult insects from escaping and to ensure good ventilation. The pupae were monitored until the adults emerged. Pieces of cotton soaked in a sugar solution were placed in the plastic rearing containers to feed and stimulate the adults to lay eggs. Black cardboard pieces were also placed inside the containers for the insects to lay their eggs after mating. The eggs were collected every 24 hours for further experiments. To obtain sufficient numbers of each larval stage for the study, enough eggs were isolated to obtain third and fifth instar larvae. This was done by isolating sufficient numbers of larvae from the previous instar and transferring them to rearing containers, monitoring them continuously until they molted to the desired larval stage. To obtain the pupal stage, a sufficient number of last instar larvae were isolated in rearing containers and monitored during cocoon formation and pupation. The required number of pupae was taken for the experiments, while others were left to complete pupation, from which young adults emerged for subsequent experiments.

Preparation of Extract Concentrations and Treatments for the Alcoholic and Aqueous Extracts and Dry Powder *of U. dioica*:

In this experiment, four concentrations were used for each of the alcoholic and aqueous extracts, as well as the dry powder, as described in Section (2) of the Methods. The control treatment was left untreated. The direct spraying method was used for applying the extracts and dry powder to both the egg and pupal stages, while for the third and fifth larval instars, both direct spraying and food spraying methods were used (Mallah and Al-Juboori, 2012).

2. Study of the Lethal Effect of the Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the Egg Mortality Rate of the Greater Wax Moth:

After confirming the mating of adults and egg laying on pieces of cardboard, 24-hour-old eggs were collected, with 10 eggs used per treatment for each concentration, in three replicates. The number of eggs was counted under a microscope, and the cardboard pieces containing the eggs were placed in 10 cm diameter Petri dishes. The eggs were sprayed with the concentrations used in the study using a hand sprayer for the liquid materials, from a height of approximately 20 cm, ensuring that the sprayer nozzle was properly adjusted.

For the dry powder, a baby powder container was used to treat the eggs after ensuring it was clean and sterilized, and it was filled with *U. dioica* powder. The experiment was repeated three times for each concentration and treatment. The control treatment was left without applying the materials used in the study. The eggs were monitored until they hatched, and the mortality rate of the eggs was calculated according to the method of Mallah and Al-Juboori (2012).

3. Study of the Lethal Effect of the Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the Third and Fifth Larval Instars of the Greater Wax Moth in the Laboratory:

A laboratory experiment was conducted to determine the lethal effect of the alcoholic and aqueous extracts, as well as the dry powder of *U. dioica*, by using a piece of natural beeswax measuring 10 cm by 10 cm. The beeswax was sprayed with the study materials according to the concentrations mentioned in Section (2), in three replicates for each treatment. The control treatment was left without treatment with the extracts or powder of *U. dioica*. The wax pieces were placed in plastic containers of 20 cm length and 20 cm height. After treatment with the extracts and powder, an artificial infection was induced by adding 10 larvae of the third and fifth instar separately, with each instar being treated and replicated independently. The plastic containers were covered with muslin cloth and secured with a rubber band to prevent the larvae from escaping. The mortality rate of the third and fifth instar larvae was calculated after 24, 48, 72, and 96 hours of treatment using Abbott's formula (1925) as mentioned by Mallah and Al-Juboori (2012):

$$\text{Corrected Mortality Percentage} = \left(\frac{Mortality\ in\ Control\% - Mortality\ in\ Treatment\ \%}{Mortality\ in\ Control\ \% - 100}\right) \times 100$$

4. Study of the Effect of Alcoholic and Aqueous Extracts and Dry Powder of *U.dioica* on the Mortality Rates of the Pupal Stage

A sufficient number of pupae of the greater wax moth were collected from the insect rearing containers for conducting the laboratory experiment, with an average of 5 pupae for each concentration and treatment, in three replicates. The pupae were placed in plastic dishes of 500 cm³ volume, with a diameter of 9-10 cm and a height of 5 cm, containing blotting paper inside. The pupae were placed on top of the blotting paper after being treated with the extracts and dry powder of *U. dioica,* as described in Section (2), following the experimental design. The dishes were covered with muslin cloth in anticipation of adult emergence. The treatments were placed in an incubator at 30°C and 70% humidity. The pupae were monitored until their transformation into adults, and the mortality rate was calculated.

Design of the study experiments

The experiments for the laboratory study were designed according to the completely randomized design for factorial experiments (CRD), while the field and warehouse studies were designed according to the randomized complete block design for factorial experiments (RCBD). Their results were analyzed according to the analysis of variance table using the SAS electronic program. The averages were compared using Duncan's test at a probability level of 5%. (Al-Zubaidy and Al-Falahy, 2016)

Results and Discussion

1. Effect of Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the Mortality Rates of Eggs of the Greater Wax Moth *Galleria mellonella* L.

The results of statistical analysis and Duncan's test at a significance level of 0.5% (Table 1), regarding the means of interactions between treatments and concentrations, indicated significant differences between treatments in egg mortality rates depending on the type of treatment. The mortality rate of the insect eggs increased gradually with increasing concentration. Thus, both the alcoholic and aqueous extracts were superior, recording the highest cumulative egg mortality at the third and fourth concentrations, with percentages of (100.00 and 93.33%) respectively, while the dry powder recorded egg mortality rates at the same concentrations of (26.67 and 36.67%) respectively. The mortality rates varied between treatments according to the type of treatment and the concentration used, with the lowest mortality rate of Galleria mellonella eggs observed in the dry powder treatment, with an average of (13.33%). The difference in egg mortality rates may be due to the nature and form of the pesticide preparation and the type of solvent used. It is worth noting that no egg mortality was observed in the control treatment, with a percentage of (0.00%).

As for the overall mean of the treatments, the results of Table (1) showed the significant superiority of the alcoholic extract treatment in recording the highest egg mortality rate, with an average of (69.33%), compared to the aqueous extract treatment, which recorded a good and satisfactory mortality rate, with an average of (62.00%). In contrast, the dry powder treatment of *U. dioica* recorded the lowest egg mortality rate, with an average of (18.67%).

Table 1. The Effect of Interaction Between Treatments and Concentrations of *U. dioica* on the Mortality Rates of *Galleria mellonella* Eggs.

Concentrations	Coefficients			Overall Average Concentration
	Alcoholic	Dry	Aqueous	
First Concentration	66.67 d	13.33i	56.67e	45.56c
Second Concentration	80.00 c	16.67h	66.67d	54.44b
Third Concentration	100.00 a	26.67g	93.33b	73.33a
Forth Concentration	100.00 a	36.67f	93.33b	76.67a
Comparison	0.00j	0.00j	0.00j	0.00d
Overall Average of Equations	a69.33	c18.67	b 62.00	

The values followed by similar letters for each attribute do not differ significantly according to Duncan's multiple range test at the probability level ($P \leq 0.05$).

As for the overall mean of concentrations, the results of statistical analysis in Table (1) showed significant differences in the mortality rates of eggs for some treatments, which gradually increased across all treatments with increasing concentration. The third and fourth concentrations recorded the highest egg mortality rates, with averages of (73.33 and 76.67%) respectively, while this percentage decreased to (54.44%) when the second concentration was used. The first concentration recorded the lowest egg mortality rate, with an average of (45.56%). No egg mortality was observed in the control treatment, with an average of (0.00%). The results indicated a positive correlation between the concentrations and egg mortality, with significant differences between treatments in the study. This finding aligns with the results of (Al-Ghalabi, 2023), who found that the toxicity of ethanolic extracts *of U. dioica* increases with higher concentrations. Moreover, (Hussein, 2022) found that the alcoholic and aqueous extracts of *Myrtuscommunis* leaves caused increased mortality rates of Galleria mellonella eggs, with a positive correlation between the concentrations of the extracts used and egg mortality rates.

2. Effect of Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the Percentage Mortality of Third-Instar Larvae of the Greater Wax Moth

The results of statistical analysis and Duncan's test at the 0.5% probability level (Table 2), concerning the means of interaction between treatments, time intervals, and concentrations for the percentage mortality of third-instar larvae, showed significant differences depending on the treatment type, time interval, and concentration used. All treatments achieved good mortality rates after 72 and 96 hours of treatment, particularly the alcoholic extract treatment at the third concentration after 96 hours, and the fourth concentration after 72 and 96 hours, which recorded the highest mortality rate of (100.00%). The study results also showed that the aqueous extract at the same concentration and time interval achieved good results, with mortality rates averaging (93.33 and 96.67%) respectively. Meanwhile, the dry powder treatment of *U. dioica* recorded mortality rates at the same concentration and time intervals of (73.33 and 76.67%)

respectively. Cumulative mortality rates of third-instar larvae varied between treatments depending on the concentration used and the type of treatment. The dry powder treatment *of U. dioica* recorded the lowest mortality rate at the first concentration after 24 and 48 hours, with no significant difference between them, averaging (16.67%).

As for the overall mean of the treatments, the results of Table (2) showed the significant superiority of the alcoholic extract in recording the highest mortality rate for third-instar larvae, with an average of (63.67%). The aqueous extract recorded a mortality rate with an average of (56.50%), while the dry powder *of U. dioica* recorded the lowest mortality rate for third-instar larvae, with an average of (39.50%). The difference in these percentages may be due to the nature of the solvent used in the study and the form of pesticide preparation, which may have influenced the chemical composition and elements present in the alcoholic, aqueous extracts, and dry powder of *U.dioica*, affecting the third-instar larvae of the greater wax moth.

Table 2. The Effect of Interaction Between Treatments, Time Intervals, and Concentrations on the Mortality Rates of Third-Instar Larvae of the Greater Wax Moth *Galleria mellonella*

Concentrations	Readings	Coefficients			Overall Average between Concentration and Readings	Overall Average of Readings	Overall Average of Concentrations
		Alcoholic	Dry	Aqueous			
First	24	43.33 k–m	16.67 n	36.67 l m	32.22g	42.44d	44.72d
	48	46.67 j–m	16.67n	36.67 l m	33.33g		
	72	66.67 f–h	36.67 l m	56.67 h–j	53.33 f		
	96	73.33e–g	43.33 k–m	63.33h i	60.00 h		
Second	24	63.33h i	33.33 m	53.33 I –k	50.00 f	47.33 c	64.17c
	48	66.67f–h	36.67 m	56.67 h–j	53.33 f		
	72	86.67b–d	50.00j k	76.67 d –f	71.11d		
	96	93.33a–c	66.67f–h	86.67b –d	82.22 c		
Third	24	73.33e–g	43.33 k–m	63.33 h i	60.00 h	59.11 b	73.89 b
	48	83.33d e	43.33k–m	73.33e –g	66.67 d		
	72	96.67a b	63.33h i	86.67b–d	82.22 c		
	96	100.00a	70.00 f g	90.00 a –c	86.67 a–c		
Fourth	24	83.33d e	53.33 i–k	73.33e –g	70.00 d	64.00 a	83.33a
	48	96.67a b	66.67 f–h	86.67b –d	83.33 b c		
	72	100.00 a	73.33 e g	93.33a–c	88.89 a b		
	96	100.00a	76.67d–f	96.67 a b	91.11a		
Comparison	24	0.00 o	0.00 o	0.00 o	0.00 h		0.00e
	48	0.00 o	0.00 o	0.00 o	0.00 h		
	72	0.00 o	0.00 o	0.00 o	0.00 h		
	96	0.00 o	0.00 o	0.00 o	0.00 h		
Overall Average of Pesticides		63.67 a	39.50 c	56.50 b			

The values followed by similar letters, for each attribute, indicate no significant differences according to Duncan's multiple range test at the probability level ($P \geq 0.05$).

As for the overall mean of the time intervals, Duncan's multiple range test at the 0.5% probability level showed a clear significant difference between the readings in the mortality rates of third-instar larvae. The results indicated that mortality rates gradually increased with the advancement of the time interval after larval treatment. The last reading after 96 hours of treatment recorded the highest mortality rate of (64.00%), significantly surpassing the average mortality rates of all other time intervals. The third and second readings, taken after 72 and 48 hours of treatment, recorded mortality rates of (59.11 and 47.33%) respectively, while the first reading after 24 hours of treatment had the lowest mortality rate, with an average of (42.44%).

As for the overall mean of the concentrations, the results of Table (2) confirmed that the mortality rates increased with the concentration used across all treatments. There was a positive correlation between the mortality rates and the increase in concentration. The highest mortality rate was recorded at the fourth concentration, with an average mortality of (83.33%). The third and second concentrations recorded mortality rates of (73.89 and 64.17%) respectively, while the lowest rate was observed at the first concentration, with an average of (44.72%). No mortality of third-instar larvae was observed in the control treatment, with an average of (0.00%).

3. Effect of Alcoholic and Aqueous Extracts and Dry Powder of *U. dioica* on the

Percentage Mortality of Fifth-Instar Larvae of the Greater Wax Moth

The results of statistical analysis and Duncan's test at the 0.5% probability level, Table (3), regarding the means of interaction between treatments, time intervals, and concentrations for the percentage mortality of fifth-instar larvae, showed significant differences between treatments based on treatment type, reading, and concentration used. All treatments recorded gradually increasing mortality rates with the increase in concentration and time intervals, especially the alcoholic extract treatment at the fourth concentration after 96 hours, which recorded the highest mortality rate for fifth-instar larvae, with an average of (96.67%). The mortality rate at 72 hours was (93.33%). The aqueous extract and dry powder treatments at the highest concentration and last reading also recorded satisfactory mortality rates, with averages of (86.67 and 66.67%) respectively.

The study results indicated that the mortality rates decreased gradually with decreasing concentration for all treatments. The treatments showed significant differences among them, with the lowest mortality rate for fifth-instar larvae observed in the dry powder treatment at the first concentration for both the first and second readings after 24 and 48 hours, with an average of (10.00%). The study confirmed that the mortality rates for third-instar larvae were higher than those for fifth-instar larvae across all treatments at the same concentration and reading. This may be attributed to the smaller size of the third-instar larvae compared to the fifth-instar larvae, which have fewer defense mechanisms and a thinner chitin layer in their body wall, making them more sensitive to the active ingredient *of U. dioica* used in the study, particularly in the alcoholic and aqueous extract treatments. (Ghani, 2021) found that the mortality rates for third-instar larvae were higher than those for fifth-instar larvae treated with plant powders from oleander, garlic, and cinnamon.

As for the overall mean of the treatments, the results of Table (3) showed the superiority of the alcoholic extract treatment, which recorded the highest mortality rate for fifth-instar larvae, with an average of (51.17%). The aqueous extract treatment recorded a mortality rate of (44.17%), while the dry powder treatment recorded the lowest mortality rate, with a percentage of (27.00%).

Table 3. The Effect of the Interaction between Treatments, Readings, and Concentrations on the Mortality Rates of Fifth-Instar Larvae of the Greater Wax Moth

Concentrations	Readings	Coefficients			Average Interaction between Concentration and Readings	Overall Average of Readings	Overall Average of Concentrations
		Alcoholic	Dry	Aqueous			
First	24	26.67 o–q	10.00 r s	20.00 p–r	18.89 i	27.33 d	.3167 d
	48	30.00 n–p	10.00 r s	26.67 o–q	22.22 i		
	72	43.33 k –m	26.67 o–q	36.67 m–o	35.56 g h		
	96	60.00 f–i	40.00 l–n	50.00 i–L	50.00 f		
Second	24	43.33 k–m	13.33 r	36.67 m–o	31.11 h	33.33 c	46.11c
	48	46.67 j–m	20.00 p–r	40.00 L–n	35.56 g h		
	72	66.67 e g	36.67 m–o	56.67 g–j	53.33 f		
	96	80.00 cd	43.33 k–m	70.00 d–f	64.44 d e		
Third	24	53.33 h–k	16.67 q r	43.33 k–m	37.78 g	46.89 b	58.89 b
	48	63.33 f–h	26.67 o–q	53.33 h–k	47.78 f		
	72	86.67 a –c	46.67 j–m	76.67 c d	70.00 c d		
	96	93.33 a b	63.33 f –h	83.33 b c	80.00 a b		
Fourth	24	63.33 f –h	30.00 n–p	53.33 h –k	48.89 f	55.56 a	.6722 a
	48	76.67 c –e	40.00 l–n	66.67 e–g	61.11 e		
	72	93.33 a b	50.00 i–l	83.33bc	75.56 b c		
	96	96.67 a	66.67 e–g	86.67 a–c	83.33 a		
Comparison	24	0.00 s t	0.00 s t	0.00 s t	0.00 j		0.00e
	48	0.00 s t	0.00 s t	0.00 s t	0.00 j		
	72	0.00 s t	0.00 s t	0.00 s t	0.00 j		
	96	0.00 s t	0.00 s t	0.00 s t	0.00 j		
Overall Average of Pesticides		51.17a	27.00 c	44.17 b			

The values followed by similar letters for each attribute do not differ significantly according to Duncan's multiple range test at the probability level ($P \leq 0.05$).

As for the overall mean of the readings, the statistical analysis results showed significant differences between the readings, with an increase in mortality rates as the time period progressed. The final reading after 96 hours of treatment recorded the highest mortality rate for fifth-instar larvae, with an average of (55.56%). The second and third readings had lower mortality rates, with averages of (46.89 and 33.33%) respectively. The first reading after 24

hours recorded the lowest mortality rate, with an average of (27.33%).

As for the overall mean of the concentrations, the results of Table (3) confirmed that the increase in the concentrations used was positively correlated with the cumulative mortality rates of fifth-instar larvae. The fourth concentration recorded the highest cumulative mortality rate, with an average of (67.22%). The mortality rates decreased at the third and second concentrations, with averages of (58.89 and 46.11%) respectively, while the first concentration, the lowest across all treatments, recorded the lowest mortality rate, with an average of (31.67%). The increase in larval mortality, particularly for the fifth instar, may be attributed to the higher concentration used, indicating that the increase in the active substance weakens the insect's immune system. The larvae's immune system can only defend the body up to certain concentrations, and beyond that, it loses its efficiency.

4. Effect of Alcoholic and Aqueous Extracts and Dry Powder on the Percentage Mortality of Pupae of the Greater Wax Moth

The results of Table (4) showed significant differences between treatments in the cumulative mortality rates of the pupae of the Greater Wax Moth depending on the type of treatment and concentration used, according to Duncan's multiple range test. The mortality rates gradually increased with the increase in concentration. The alcoholic extract treatment at the fourth concentration recorded the highest cumulative mortality rate for the pupae, with an average of (80.00%). The third concentration of the same treatment recorded a good cumulative mortality rate of (73.33%), which did not differ significantly from the aqueous extract treatment at both the third and fourth concentrations, with an average of (73.33%). The dry powder treatment recorded a cumulative mortality rate of (53.33%) for the pupae at the fourth concentration, which did not differ significantly from the alcoholic extract treatment at the second concentration, which had the same average mortality rate of (53.33%).

The mortality rates varied between treatments depending on the type of treatment and the concentration used. The lowest mortality rate for the pupae was recorded in the dry powder treatment at the first and second concentrations, with an average of (26.67%). No pupal mortality was observed in the control treatment.

As for the overall average of the treatments, the statistical analysis results indicated a significant superiority of both the alcoholic and aqueous extracts over the dry powder treatment, recording the highest cumulative mortality rate of pupae, with an average of (49.33%) respectively, compared to the dry powder treatment, which recorded the lowest mortality rate with an average of (32.00%).

Table (4): The Effect of the Interaction between Treatments and Concentrations on the Mortality Rates of Pupae of the Greater Wax Moth

Concentrations	Coefficients			Average Concentrations
	Alcoholic	Alcoholic	Alcoholic	
First	40.00e f	26.67f	46.67d e	37.78d
Second	53.33c–e	26.67f	66.67a–c	48.89c
Third	73.33a b	53.33c–e	60.00b–d	62.22b
Fourth	80.00a	53.33c –e	73.33a b	68.89a
Comparison	0.00g	0.00g	0.00g	0.00h.
Average Equations	49.33a	32.00b	49.33a	

The values followed by similar letters, for each attribute, indicate no significant differences according to Duncan's multiple range test at the probability level (($P \geq 0.05$)).

Regarding the overall average of the concentrations, the statistical analysis results showed a significant difference in the cumulative mortality rates of the pupae, which gradually increased in all treatments with the increase in the concentration used. The highest mortality rate was recorded in the fourth concentration treatment, with an average of (68.89%), while the mortality rate decreased to (62.22%) when using the third concentration, then (48.89%) with the second concentration, compared to the lowest mortality rate of (37.78%), which was recorded in the first concentration treatment. The reason for the decrease in the effectiveness of the treatments with the decrease in concentration could be attributed to the presence of cocoons spun by the larvae of the greater wax moth in the pupal stage, which act as a hydrophobic environment. Additionally, the pupal cuticle is more rigid than that of the larval stages, as chitin reaches its highest level in the pre-pupal stages.

The results of this study agree with what was mentioned by (Ghani, 2021), that the appearance of differences between treatments and concentrations is due to the inability of the extract or powder to penetrate the silk tissue and the pupal cuticle layer. There is a direct relationship between the concentrations used in the treatments and the mortality rate of the pupae, and the decrease in the effectiveness of each treatment and concentration is due to the presence of the cocoons they spin and the cuticle layer, which act as a hydrophobic environment.

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