

Evaluation of the effectiveness of the boiled extract, regular and nano oil of some medicinal plants on the eggs and larvae of the third stage of date moth *Ephestia Cautella* (Walk.) under laboratory conditions

Afrahan Denar Hadi¹, Majeed Miteb Dewan² and Thaer Mahmoud Taha³

^{1,2}Faculty of Agriculture/ University of Kufa/ Republic of Iraq

³Faculty of Education for Girls/ University of Kufa/ Republic of Iraq

Corresponding author, Email: afrahdonar222@gmail.com

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Abstract:

The experiment was conducted under laboratory conditions, in the insect laboratory of the Faculty of Education for women r, at the University of Kufa in 2021. The aim of this study was to test the effect of extracts, regular and Nano-oil of cinnamon, *Cinnamomum zeylanicu*, turmeric *Curcuma longa*, and *Elettaria cardamomum* on the eggs and third-stage larvae of date moth. Where the three Nano-oils excelled in mortality eggs, a mortality rate of 100% was observed compared to a significantly lower mortality rate with) the regular oil and the boiled extract. The Nano-oils resulted in the mortality rate of the third instar larva after undergoing treatment for 72 hours. The Nano cinnamon oil excelled in mortality larvae, where the mortality rate was 94.4%, compared to turmeric Nano oil and Nano cardamom oil, respectively, as THEY reached 89.2 and 61.8% at 2% concentration.

Keywords: plant extracts, essential oils, Nano oils, *Ephestia Cautella*.



Introduction:

Dates production has a significant contribution toward the national economy although dates have a high sugar content, they have a low glycemic index making them safe when consumed in moderation by diabetics. Sugar makes up 60-80% of the weight of the fruit, in addition to its content of mineral salts and trace elements of nutritional importance (19). Palm trees in Iraq are affected by many insect pests, spiders, and others. These pests infect palm trees in various parts, while others infect the fruits. These pests can cause great damage to the palm trees especially if left without pesticide treatment. (5). among the most important pests that infect dates in stores is the date moth *Ephestia cautella* (Walk), which is one of the most dangerous pests. Given the importance of this economic insect and the great losses it causes through its ability to feed inside the fruit and its resistance to chemical insecticides, it makes a pest that deserves great attention in the operations of combating and limiting its spread (13). Recently, numerous studies have been conducted in this field proving its potential significance in controlling the growth and spread, including the idea of searching for effective natural plant compounds to control insects, because “the plants contain secondary effective compounds that have a great impact” in the control of pests of the store, being fast-degrading and highly effective compounds against dangerous insects and have no effect on human and animal health and the environment (6). Plant extracts have been used in integrated management programs for insect pests, which are considered to be environment-friendly pesticides and have behavioral and

physiological effects on the pests of stored products (12) moreover, their rapid decomposition in the environment and its lack of effect on non-targeted organisms gives it an advantage over the conventional chemical pesticides (25). Plant essential oils also occupied an important role in combating pests *Officinalis* because they contain effective substances with a significant mortality rate for the pests. Nano-loaded oils were also used because they have high efficacy, where they act as feed repellants and growth inhibitors against a wide range of storehouse insects (26 and 18).

Materials and Methods:

1- Collecting and rearing the insect in the laboratory:

The sample of the date moth *E.cautella* was collected from the laboratories of the entomology department of the research center in Al-Zaafaraniya, bred on artificial food consisting of 81% wheat groats, 12% glycerin, 6% molasses, and 1% dry yeast (14 and 13). For the purpose of maintaining the colony of the insect, 250 g of artificial food was placed inside a plastic container with a diameter of 11 cm and a height of 12 cm, then 15 pairs (male and female) of the adults of the insect whose age ranged between 24-48 hours were released into it after closing the opening of the container with a dull cloth and fixed with a rubber band. Then it was placed in the incubator at a temperature of $25\pm 2^{\circ}\text{C}$, relative humidity of $60\pm 5\%$, and a period of illumination (light: dark) of 16:8 hours for a period of 25 days (24). Insect eggs were obtained by taking 10 pairs of adult date moths, aged 24-72 hours, and placed in a lantern bottle at the bottom of which is

a petri dish. It was moistened with a sugary substance at a concentration of 10% for the adults to feed on (7 and 15). The newly laid eggs were collected using a soft brush and placed in Petri dishes in the basin for the purpose of treatment with plant extracts, regular, and Nano oils. As for the other eggs, they were transferred to larval culture bottles to maintain the laboratory colony.

2- Preparation of regular and Nano vegetable oils and extracts:

Preparation of the extract boiled water of cinnamon, turmeric, and cardamom plants:

Take 20 g of plant powder (cinnamon bark, turmeric powder, and cardamom seeds) after milling them. Place the content in a glass beaker with a capacity of 500 ml, add 400 ml of boiled distilled water and place the beaker on an electric shaker for 15 minutes. Afterward, the solution is filtered using a multi-layered meshed cloth, then take the filtered solution and filter it again using a Whatman No.1 filter paper. Then the filtrate should be taken and placed in glass tubes under a centrifuge at a speed of 3000 rpm for 10 minutes to obtain a clear solution. Then, the filtrate is placed in the electric oven at a temperature of 45 °C to obtain the dry matter. The dry matter should be kept in the refrigerator until use (13). Take 2 g of the dry matter and dissolve it in 98 ml of distilled water, thus the concentration of the resulting stock solution becomes 2% or equivalent to 20 mg/ml, and from it, the following concentrations were prepared (0.75, 1.5, 2 mg.ml⁻¹). As for the control treatment, it is represented by distilled water only.

Extraction of essential oils

The essential oils of the three plants (Cinnamon, turmeric, and cardamom) were extracted. The extraction process started with steam distillation using a (Clevenger) device, where 25-30 g of plant powder was placed in the device's beaker and 250-300 ml of distilled water was added to it. On the electric heater, the temperatures used for all plants were in the range of 60-70 °C. The oils obtained from the extraction process were placed in sealed tubes, wrapped in opaque paper, and kept in the refrigerator at a temperature ranging from 3-5 °C until use. (21). Three different concentrations of the above-mentioned plant oils were prepared 0.75, 1.5 and 2% by diluting 0.75, 1.5, and 2 ml of vegetable oils, in 100 ml of sterile and distilled water and a liquid soap emulsion of a concentration of 0.5 ml/liter (2). A preliminary experiment was conducted, and its results confirmed that the liquid soap emulsion with a concentration of 0.5 ml/liter of water had no negative effect on date moth larvae. The suspension was shaken for 20 minutes using a magnetic stirrer.

Nano-loaded essential oils:

The essential oils of cinnamon, turmeric, and cardamom, were loaded on Chitosan nanomaterial in closed containers, were obtained by (Prof. Dr. Manal Mohamed Adel), a researcher at the National Research Center in the Arab Republic of Egypt, and manufactured in the laboratory by chemical techniques using ultrasound (3, 22 and 23). The concentration of the base solution for oils was 10%, and the concentrations 0.75, 1.5, and 2 % were prepared in the same way that the regular oils were prepared in the previous paragraphs 3-10-2 and kept at 4°C. As for

the control treatment, it included sterile distilled water only. These nano-loaded oils were tested on the eggs and larvae of the date moth. The concentration of the base solution for oils was 10%, and the concentrations 0.75, 1.5 and 2 % were prepared using the same method that the regular oils were prepared in the previous paragraphs 3-10-2 and kept at 4°C. As for the control treatment, it included sterile distilled water only. These Nano-loaded oils were tested on the eggs and larvae of the date moth.

3- Effect of the extract, boiled water, regular and Nano oil of aromatic plants on date moth eggs.

The date moth eggs were treated, as 50 eggs were taken at one day age /replicate and three replicates were taken for each concentration, each separately, and the eggs were transferred using a soft brush to Petri dishes. These eggs were treated with 0.75, 1.5, and 2 ml of boiled extract concentration, regular, and Nano oil by spraying them using a small hand sprayer. The treated eggs were then transferred to the incubator at a temperature of $25 \pm 2^\circ\text{C}$, relative moisture of 70-60%, and duration of illumination (light: dark) of 16:8 hours. The mortality rate was recorded after 5 days of treatment. The mortality rate was corrected according to the Abbott formula (1).

Effect of the extract of boiled water, regular and Nano oil of aromatic plants on the third instar larvae of date moth.

Third-phase larvae were collected and treated with concentrations of 0.75, 1.5,

and 2%, in addition to three Petri dishes that were used as a control group, which were sprayed with distilled water. All the control and treatment dishes were transferred to the incubator at a temperature of $25 \pm 2^\circ\text{C}$ and a relative moisture humidity of $65 \pm 5\%$, the deaths were followed during 24, 48, 72 hours of the treatment. Larvae of the third and fifth phases were treated in the same methods above and in the same concentrations mentioned above and under the same conditions, and the mortality rates were recorded in the larvae and adjusted according to the method of Abbott formula (1).

Results and Discussion:

Effect of extracts of boiled water, regular oil, and Nano-oils of aromatic plants the on date moth eggs

The results in Table (1) show of killing the eggs of the date moth were 100% at the treatments of concentrations 0.75, 1.5, and 2 mg/ml for Nano-oils of turmeric and cinnamon for each of them, respectively, as well as a concentration of 2 mg/ml for cardamom Nano oil. While the concentration well as 2 mg.ml^{-1} significantly excelled as it showed to have the highest average of eggs mortality which reached 58.26 %.As for the effect of the extract and oil type on the eggs' mortality, the cinnamon extract significantly excelled on the cardamom extract, as it gave 51.10% and 37.83% for each of them, respectively, while there was no significant difference between the cinnamon extract and the turmeric extract, which in turn gave a death rate of 48.35%

Table 1. Effect of boiled water, regular oil, and Nano-oil extracts of aromatic plants on date moth eggs

Concentrations %	Type of extract and oil									
	Nano cardamom oil	cardamom oil	cardamom extract	Nano Cinnamon oil	Cinnamon oil	Cinnamon extract	Nano Turmeric oil	Turmeric oil	Turmeric Extract	concentration effect
0	6.67	13.3	10.0	16.67	13.3	10.00	13.33	16.6	13.33	9.99
0.75	90.00	15.6	7.03	100.00	41.63	17.93	100.00	42.6	15.27	37.54
1.5	95.00	23.3	18.33	100.00	49.5	40.00	100.00	36.1	26.56	45.54
2	100.0	42.6	32.2	100.00	72.3	63.33	100.00	59.7	56.66	58.26
Effect of the type of extract or oil		37.83			51.10			48.35		
L.S.D _{0.05}		Extract	6.974 = concentration			4.649 = interaction	13.948 =			

The mortality of eggs is due to the entry of the volatile oils of these plants into the egg through the opening of the hilum or through the eggshell, and thus resulting in the death of the fetus or the incomplete development of it. Some of these compounds disrupt the process of gas exchange inside the egg. (9 and 11) indicated that the extracts and oils entered the hilum opening and caused a vital disturbance inside the eggs, which led to the larvae not being able to tear the egg membrane and exit normally. (8) A study was conducted to evaluate the effect of the tested essential oils of rosemary, lemongrass and eucalyptus on the eggs of the fig moth *E. cautella*. The corrected percentage of egg mortality was 7.1, 22.4 and 23.4% for each of rosemary, lemon and eucalyptus, respectively, at a concentration of 5%, As well as 40.3, 57.1 and 67.8%, respectively, at a concentration of 10%, as well as 53.5, 60.7 and 89.3, respectively at a concentration of 15%. It was concluded from this that the different concentrations of the essential oils of the three plants led to a reduction in the rate of hatching of the eggs of the date moth, and

this can be attributed to the fact that the high concentrations of the essential oils led to the penetration of the eggshell which led to the poisoning of the fetus before hatching. The mortality rate of the third larval instar of date moth treated with boiled extract, regular and Nano oil of cinnamon, turmeric and cardamom at a concentration of 0.75, 1.5 and 2% The results in table (2) include the effect of three concentrations of boiled cinnamon extract, regular cinnamon oil and Nano cinnamon oil after 24, 48 and 72 hours, indicating that the boiled cinnamon extract had a significant effect on the percentage of third instar larvae mortality of date moth, which reached the highest percentage. The loss of 39% at the concentration of 2% compared to the control treatment(0%), which amounted to 4.6%, Also, the time factor had a significant effect on the mortality rate, as it was found that the longer the period, the higher the mortality rate, the 72 hour period was the best, where the mortality rate was 31.1% compared to the period 24 hours, which reached 14.7, As for the interaction, it had a significant effect, and the treatment

with boiled cinnamon extract at a concentration of 2% for a period of 72 hours obtained the best result, where the mortality rate was 46.6% compared to the control treatment, which amounted to 6.7%. It was also found from the study of the same table that the use of regular cinnamon oil affected the percentage of larval mortality, and the concentration of 2% was the best, as the percentage of mortality was 34% compared to the control treatment, which amounted to 1.1%. As for time, it had a significant effect on the mortality rate, where the period of 72 hours was the best, where the mortality rate was 37.0% compared to the period of 24 hours, where the mortality rate was 15.8%. As for the interaction between the two factors of the study, it was significant on the mortality rate, and the treatment with a concentration of 2% for a period of 72 hours was the best, where the mortality rate was 58.9% compared to the control

treatment, which amounted to 3.33%. The results of the table also indicated that the addition of Nano cinnamon oil had a significant effect on the mortality rate of date moth of third instar larvae. The treatment with a concentration of 2% was superior in recording the highest rate of larval mortality of 94.4% compared to the control treatment, which was 0%. The time period had a significant effect on the mortality rate, where 72 hours was the best, where the mortality rate was 65.8, compared to 24 and 48 hours. The mortality rate is 55% and 62.5%, respectively. As for the interaction between the study factors, it had a significant effect on the mortality rate, where the treatment with Nano cinnamon oil at a concentration of 2% for a period of 72 hours was the most effective compared to the control treatment, which amounted to 0% under the same conditions.

Table 2. the mortality rate of the third larval instar of date moth treated with boiled extract, regular and Nano-oil of cinnamon at different concentration

Concentrations %	Boiled Cinnamon extract				concentratio n effect	Regular Cinnamon oil				concentratio n effect	Nano Cinnamon oil				concentratio n effect
	24 hou r	48 hou r	72 hou r			24 hour	48 hou r	72 hou r			24 hou r	48 hou r	72 hou r		
0	3.3	3.7	6.7		4.6	0	0	3.33		1.1	0	0	0		0
0.75	7	17.4	31.8		18.8	13.3	26.7	38.1		26	63.3	70	73.3		68.9
1.5	17.4	28.5	39.2		28.4	23.3	33.3	47.8		34.8	70	83.3	90		81.1
2	31.1	39.2	46.6		39	26.7	43.3	58.9		43	86.7	96.7	100		94.4
average time	14.7	22.2	31.1			15.8	25.8	37.0			55	62.5	65.8		
L.S.D _{0.05}	concentration = 7.75 days = 6.72 interaction = 13.43					concentration = 9.06 days = 7.84 interaction = 15.69					concentration = 6.07 days = 5.25 interaction = 10.51				

Table 3. The mortality rate of the third larval stage of date moth treated with boiled extract, regular and Nano oil of turmeric at different concentration

Concentrations %	Boiled turmeric extract				Turmeric oil regular				Turmeric oil nano			
	24 hou r	48 hou r	72 hou r	concentratio n effect	24 hou r	48 hou r	72 hou r	concentratio n effect	24 hou r	48 hou r	72 hou r	concentratio n effect
0	6.7	6.7	6.7	6.7	3.3	6.67	6.67	5.6	3.3	6.7	6.7	5.6
0.75	3.7	14.4	21.5	13.2	3.3	17.8	24.4	15.2	54.8	64.4	74.8	64.7
1.5	10.4	24.8	31.8	22.3	17	31.8	42.9	30.6	55.5	74.8	81.8	70.7
2	21.1	28.1	42.6	30.6	27.4	42.6	53.3	41.1	82.6	92.5	92.5	89.2
average time	10.5	18.5	25.6		12.8	24.7	31.8		49.1	59 6	63.9	
L.S.D _{0.05}	concentration = 8.45 days = 7.31 interaction = 14.63				concentration = 8.77 days = 7.59 interaction = 15.18				concentration = 9.04 days = 7.83 interaction = 15.66			

Table (3) shows that the boiled turmeric extract had a significant effect on the mortality rate of the third instar larvae of the date moth, where the highest mortality rate was 30.6% at the 2% concentration compared to the control treatment, which amounted to 6.7%, also the time factor had a significant effect on the mortality rate. Where it was found that the higher the period, the higher the mortality rate, the better the 72 hours period, as the mortality rate reached 25.6%, compared to the period of 24 and 48 hours, which reached the mortality rate 10.5 and 18.5%, respectively. As for the interaction, it had a significant effect, and the treatment with boiled turmeric extract at a concentration of 2% for a period of 72 hours obtained the best result, where the mortality rate was 42.6% compared to the control treatment, which amounted to 6.7%. As for the regular turmeric oil, it affected the mortality of the third larval instar, where the mortality rate was 41.1% compared to the control treatment, which amounted to 5.6%, The time coefficient also affected

the mortality rate, where the period of 72 hours was the best, where the mortality rate was 31.8% compared to the period of 24 hours when the mortality rate was 12.8%. As for the interaction between the two factors of the study, it was significant on the mortality rate, and the treatment with a concentration of 2% for a period of 72 hours was the best, where the mortality rate was 53.3% compared to the control treatment, which amounted to 6.67%. The turmeric Nano oil also affected the third instar of the date moth larvae, where the mortality rate reached 89.2% at a concentration of 2% compared to the control treatment, which amounted to 5.6%, and the effect of time was significant on the mortality rate, as it reached 63.9% at 72 hours compared to 24 hours, which amounted to 49.1%. As for the interaction between the treatments, it had a significant effect, as it reached a concentration of 2% and in 72 hours, 92.5%, compared to the control treatment, which amounted to 6.7%.

Table 4. The mortality rate of the third instar larvae of date moth treated with boiled extract, regular and Nano oil of cardamom at different concentration

Concentrations %	Boiled cardamom extract				regular cardamom oil				nano cardamom oil			
	24 hou r	48 hou r	72 hou r	concentratio n effect	24 hou r	48 hou r	72 hou r	concentratio n effect	24 hou r	48 hou r	72 hou r	concentratio n effect
0	0	3.3	3.3	2.2	0	0	0	0	3.3	3.3	3.3	3.3
0.75	3.3	10	20.4	11.2	3.3	16.7	23.3	14.4	34.4	51.8	58.9	48.4
1.5	6.7	17.4	24.4	16.2	6.7	23.3	26.7	18.9	45.2	55.5	65.5	55.4
2	10	20.4	31.1	20.5	13.3	26.7	36.7	25.6	48.1	61.8	75.5	61.8
average time	5	12.8	19.8		5.8	16.7	21.7		32.8	43.1	50.8	
L.S.D ₀₀₅		concentration = 6.98 days = 6.04 interaction = 12.08				concentration = 6.88 days = 5.96 interaction = 11.92				concentration = 7.04 days = 6.10 interaction = 12.20		

Table (4) shows that the boiled cardamom extract had a significant effect on the percentage of mortality of the third instar larvae of the date moth, where the highest mortality rate was 20.5% at 2% concentration compared to the control treatment, which amounted to 2.2%. Also, the time factor had a significant effect on the mortality rate, where it was found that the longer the period, the higher the mortality rate, the 72-hour period was the best, where the mortality rate reached 19.8% compared to the period 24 and 48 hours, which reached the mortality rate 5 and 12.8%, respectively. As for the interaction, it had a significant effect, and the treatment with boiled cardamom extract at a concentration of 2% for a period of 72 hours had the best result, where the mortality rate was 31.1% compared to the control treatment, which amounted to 3.3%. As for the regular cardamom oil, it affected the mortality of the third larval stage, where the mortality rate was 25.6% compared to the control treatment, which amounted to 0%. The time factor also affected the mortality rate,

where the period of 72 hours was the best, where the mortality rate was 21.7%, compared to the period of 24 hours when the mortality rate was 5.8%. As for the interaction between the two factors of the study, it was significant on the mortality rate, and the treatment with a concentration of 2% for a period of 72 hours was the best, where the mortality rate was 36.7% compared to the control treatment, which amounted to 0%. The cardamom nano oil also affected the destruction of the third stage of date moth larvae, where the mortality rate reached 61.8% at a concentration of 2%, compared to the control treatment, which amounted to 3.3%. and time had a significant effect on the mortality rate, as it reached 50.8% at 72 hours, compared to 24 hours, which amounted to 32.8%. As for the interaction between the treatments, it had a significant effect, as it reached a concentration of 2% and in 72 hours, 75.5% compared to the control treatment, which amounted to 3.3%. The results of the current study indicate the nano-oils excelled on regular oils and extracts of boiled water, where the

Nano-loaded cinnamon oil excelled on turmeric oil and Nano-loaded cardamom oil. It is possible to compare the results of our study with the previous studies in terms of the effect only and according to the nature of the plant.

The findings of the current study are consistent with those of Sabbour and Abd El-Aziz (20) when they studied the evaluation of the efficiency of the essential oils (regular and Nano) of purslane, mustard and castor plants on the eggs and larvae of the date moth *E. cautella*. Whereas, Nano-oils excelled on regular oils by increasing the concentrations of oils. The mortality in the larvae is due to the closure of the respiratory stomata and bronchioles and thus affecting the breathing efficiency of insects (26), or these substances prevent the larvae from feeding, which leads to the mortality of large numbers of them (17 and 4). Kavallieratos *et.al.* (16) Indicated that nanomaterials vary in their effectiveness due to the difference in the basic components of each nanomaterial, or nanomaterials may differ in their effectiveness depending on their adhesion to the insect's body wall. The Nano cinnamon oil excelled on turmeric and cardamom Nano oil due to the fact that cinnamon contains cinnamaldehyde, carvacrol, eugenol, cinnamic acetate and thymol compounds. These compounds have broad-spectrum effects as antibiotics and antioxidants (10).

Conflict of Interest

The authors have no conflict of interest.

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