Evaluating the efficiency of some plant extracts in controlling the different stages of the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract

This study examined the efficiency of cold aqueous extract of geranium, neem, and peppermint plants in mortality in different stages of the peach fruit fly, Bactrocera zonata. The results indicated that each of the three extracts had an effect on the third larval instar. The results showed that neem extract had the highest effect on the third larval instar. The cumulative corrected mortality rate after 72 hours reached 67.3%, while the corrected mortality rate of larvae due to the effect of peppermint extract was 58.6%. As for the geranium extract had the least effect among the three extracts on the larval stage, as the corrected cumulative mortality rate reached 52%. As for the effect of the aqueous extracts of the three plants on the stage of pupation, the neem extract was the most effective among the three extracts, as the highest rate of non-emergence of adults at a concentration of 7% was 30%. As for peppermint extract, the mortality rate was 10%, while for geranium extract, the mortality rate was 6%, There was no effect on the pupal stage at the concentration of 3%. These compounds also showed a clear effect in repelling the adult stage, as the geranium extract had a significant impact in repelling insects, as the insect repellency rate reached 72.16%. As for the neem plant extract, the insect repellent rate was 50.03%, while the peppermint plant extract showed a 62% repellent rate for B. zonata adults.

Keywords: aqueous extract, neem plant *Azadirachta Indica*, Peppermint plant *Mentha piperita,* Geranium plant, *Pelargonium zonale,* The peach fruit fly, *Bactrocera zonata*.

Introduction

Fruit trees are one of the basic elements in the economy of many countries of the world; cultivated areas have expanded significantly over the past two decades, as fruit production has occupied an advanced position in trade exchange between world countries, with the annual production rate estimated at 800 million tons[1]. Fruit production contributes significantly to the Iraqi economy, reaching 20% of agricultural economic income. Citrus trees rank third among orchard trees after palm trees and grapes in Iraq, and their spread is concentrated in the central governorates (Diyala, Baghdad, Karbala,

Wasit, Salah al-Din), the number of citrus trees is 7,768,290 trees, with a production rate of 142,717 tons[24]. The peach fruit fly *B. zonata* is an important economic insect that infects many fruits and vegetables and causes severe economic losses that may reach 40-46% in the fruits of deciduous trees and citrus fruits if no measures are taken to combat the insect, the loss rate may reach 100%, as the amount of losses reached $11.7 billion in America in 2022[2]. This insect is considered invasive in Iraq, as it was recorded in Wasit Governorate on citrus fruits in 2016 [3] and became an important pest in Karbala [4]. As for the damage caused by this insect, it causes direct damage represented by the deformation of the fruits as a result of laying eggs in addition to the larvae feeding on the pulp of the fruit, while indirect damage occurs as a result of the transmission of bacteria *Pseudomonas* sp. and *Pectobecterium* sp. with the egg-laying machine, which causes the fermentation and spoilage of the fruits[5]. Despite the great role of chemical pesticides and pheromones in controlling insects and their ease of application and speed of results [6] , they cause environmental pollution in addition to their residues in the fruits. Therefore, it was necessary to found for alternatives to chemical pesticides. The most important of these alternatives are plant extracts, especially since they are highly effective in controlling in
sects and do not pollute the environment. This research also aimed to use alternatives to pesticides to control this insect. Therefore, the current study aimed to evaluate the efficiency of different concentrations of cold aqueous extract of geranium, neen and peppermint plants on some life performance parameters of the peach fruit fly in the holy Karbala Province.

Materials and Methods

Laboratory rearing of insects

In order to obtain the different stages of the insect for use in conducting various laboratory studies, orange fruits infected with the peach fruit fly B. zonata were collected from Al-Hussainiya orchards affiliated with Karbala Province, and their infection was determined based on egg-laying scars and partial rot. The fruits were taken, transferred to the laboratory, and placed in plastic containers measuring 30 x 20 x 20 cm; a layer of sterile soil, 3 cm thick, was placed at the base of the container to prevent the larvae from growing. The fruits were placed in each container, 10 fruits, the containers were covered from the top with muslin cloth and secured with a rubber band. They were monitored daily to observe the emergence of third-instar larvae and their pupation in the soil.

After the larvae had hatched, the fruits were removed, and the soil was taken and sieved using a 1 mm sieve to isolate the pupae from the soil; the pupae were placed in 9 cm diameter glass dishes with a thin layer of sterile soil at the base. To obtain the adult insect, the uncovered glass dishes were transferred to wooden cages measuring 30 x 20 x 20 cm, with a front made of muslin cloth containing a circular hole with a diameter of 15 cm surrounded by muslin cloth fixed in a conical shape. Its end was tied

with a rubber band for easy handling on the farm. Daily monitoring of the cages was carried out to ensure the emergence of adult insects.

Design a special container to obtain eggs to obtain the larval stage:

A special container was designed to obtain peach fruit fly eggs. It consists of a 300 ml plastic jar, the upper half punctured with a 1 mm diameter pin; a plastic container was prepared, approximately half the size of the first container, and pierced from all sides with the aforementioned pin. The lid of the small container was fixed inside the lid of the large box with glue. A piece of orange was placed inside the small box to provide an attractive scent, and then the container was closed with its lid attached to the lid of the large container. It put a piece of dark-colored paper napkin so that it would be easier to see the laid eggs and transfer them to the artificial medium for feeding the larvae, placing the entire plastic container inside the breeding cage containing the adult insects (females and males). The insect inserts the egg-laying device through the holes in the large container and lays eggs that fall onto the paper napkin. Eggs were collected every 24 hours for use in subsequent experiments, and the orange fruit was replaced every 24 hours.

Preparing the artificial nutrition medium for breeding larvae in the laboratory:

To obtain the third larval stage to study the effect of different concentrations of some plant extracts, the nutritional medium was prepared, consisting of 500 g of fine wheat bran, 125 g of yeast (Yeast Hydrolysate Enzymatic), 125 g of ground table sugar, 5 g of citric acid, 5 g of sodium benzoate (C_6H_5COONa), and 750 ml of distilled water. All materials were mixed in a 2000 ml plastic container and the mixing process continued for 15 minutes using a fine wooden spoon [7].

Preparation of cold aqueous extract

The leaves of geranium, neem and peppermint plants were collected from the Al-Abbas's (p) Holy Shrine nursery, washed and left to dry with continuous stirring to prevent fungal infection until completely dry. The leaves were ground using an electric grinder to obtain a fine, dry powder and then placed in dry, airtight bottles in the refrigerator at a temperature of 4°C until use. The cold aqueous extract was prepared according to the method of [8], where 10 g of dry powder were taken and mixed with 200 ml of distilled water using an electric mixer and left for 24 hours at room temperature, after which the mixture was filtered using several layers of medical gauze to get rid of suspended particles then, centrifugation was carried out at a speed of 3000 rpm for ten minutes. After that, the extract was filtered using filter paper to obtain a clear extract. Then, the extract was dried at 40°C. After that, the extract was stored in the refrigerator until use.

Treatment of the third larval and pupal stages of the peach fruit fly B. zonata with different concentrations of cold aqueous extract of neem, geranium and peppermint plants:

Three concentrations of each extract were prepared, which included (3%, 5% and 7%) and placed in small sprayers with a capacity of 10 ml to be sprayed on sterile soil placed in a glass dish with a diameter of 9 cm, at a rate of 2 ml for each dish. Then, the

third larval instar was transferred to the treated dishes at a rate of 10 larvae per dish, and each treatment had 5 replicates in addition to the control treatment, where only water was used. The same concentrations used with the larvae were used in the pupal stage treatment, where 9 cm diameter glass dishes were used to place sterile soil in them. Then, the dishes were sprayed with different concentrations of each plant extract at a rate of 10 larvae per dish, and each treatment had 5 replicates, in addition to the control treatment.

Chemotropometer (Figure 1) adapted from Folso's apparatus [25]

The device consists of a wooden box 48 cm long, 20 cm wide and 20 cm high. The device has a movable cover, and a transparent glass tube runs through this box; it used a neon lamp bottle to make it. It passes through two opposite holes with a diameter equal to the diameter of the bottle from the outside. The length of the bottle is 100 cm, and its diameter is 3 cm. The glass tube has a hole in the middle to insert the insects. The glass tube is divided into centimetres by small lines and fixed in place; pieces of cotton are placed in the side holes, one of them moistened with distilled water and solvent and the other with the extract to measure the percentage of attraction and repellent and their 5strength for the extract to be studied.

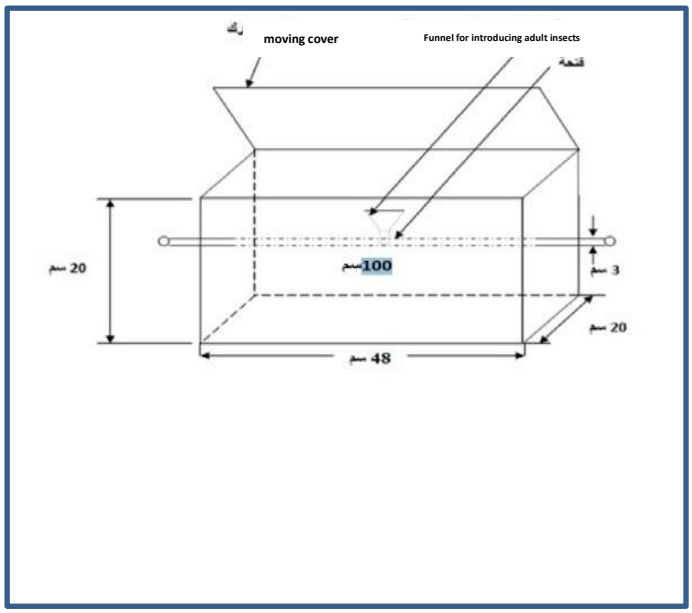

Figure (1): Chemotropometer

Study of the percentage and repellent force of plant extracts on adults of the peach fruit fly *B. zonata*

The force and rate of repellent of plant extracts were studied using a Chemotropometer device, where a piece of cotton soaked in plant extracts was placed in one direction of the tube, taking care not to leave hole or space in the cotton fabric to prevent air from passing through. In contrast, at the other end of the tube, a piece of cotton soaked in distilled water only was placed. Insects were introduced into the glass tube through the middle hole at a rate of 10 insects per replicate, with three replicates for each treatment for each extract and each concentration of the concentrations used (3%,

5% and 7%). The insects were left after the movable cover was closed for an hour, and the results were calculated after 12 hours by recording the number of insects at each end of the tube that had moved 25 cm away from the middle hole. Then the experiment was repeated after 24 hours, 48 hours, 72 hours and 96 hours, and this data was recorded. From it, the repellent rate and its strength were calculated according to the following equations [9].

$$percentage\ of\ repellent = \frac{Number\ insects\ moving\ opposite\ the\ test\ material\ and\ travelling\ 25\ cm\ from\ the\ center}{Total\ number\ of\ insects} \times 100$$

$$\text{repellent force} = \frac{Total\ distances\ traveled\ by\ insects\ in\ opposite\ direction}{Number\ of\ replicates}$$

Spraying cold aqueous extracts of (neem, geranium and peppermint) on orange trees to protect them from infestation by the peach fruit fly *B. zonata:*

The experiment evaluated the efficiency of the cold aqueous extract of neem, geranium, and peppermint plants. Two concentrations of each extract were made (5% and 7%) using a 20-liter backpack sprayer. Small, fruitful orange seedlings were sprayed in 5 kg plastic anvils, 5 anvils for each concentration, and 5 seedlings for comparison, after the replicates were sprayed with plant extracts and the control trees were sprayed with water only. Adult insects (ten males and ten females) were released on the trees for each treatment. Then, the fruits and the number of scars resulting from the insect's egg-laying device were monitored daily in order to determine the effect of the plant extracts on protecting the fruit from the insect.

Results and Discussion

The efficiency of plant extracts of neem, peppermint and geranium leaves in controlling different stages of the peach fruit fly B. zonata was tested at three concentrations for each of the extracts mentioned above, the results in Table (1) showed that the neem extract gave the highest percentage of mortality in the third larval instar individuals, as the corrected cumulative mortality rate reached 67.3%. As for the peppermint extract gave a mortality rate of 58.6%, while the geranium extract had the least effect on the larval stage, as the corrected cumulative mortality rate reached 52%%. This is consistent with what was mentioned previously [10,11,12].

The results also showed that the percentage of effect on the third larval stage at a concentration of 7% reached (78%, 68% and 66%) respectively (neem, peppermint and geranium). The results also showed that the least effect on the larval stage for all plant extracts under study was at a concentration of 3%, as the corrected percentages of death after three days reached (52%, 46% and 40%) respectively, for each of (neem, peppermint and geranium). The results showed that the neem extract was superior to other extracts at all concentrations. The reason for this is that the neem extract contains more than one active substance, each of which significantly affects the insect. The isodrachtin substance present in the neem extract is one of the substances that causes a mortality rate that may reach 71.44% at a concentration of 7.5% on mosquito larvae [13]because this substance greatly affects the ecdysone hormone and thus prevents the

insect's moulting process, leading to the insect's death [14]. The effect of peppermint extract is due to the extract containing menthol, which causes symptoms similar to those of the insect treated with the pesticide, as it affects the process of nerve transmission in the insect by affecting the nicotine acetylcholine receptors (NAChR) found in the membranes of nerve fibers in the insect's peripheral, central nervous system and the process of binding the menthol compound with some proteins of these receptors leads to the continued transmission of nerve signals, causing paralysis and death of the insect[12],stated that the mortality rate might reach 69.20% for stages of the whitefly insect at a concentration of 8%. The effect of the geranium extract is due to geraniol, a neurotoxin that causes symptoms of paralysis and tremors in the insect exposed to it, It has been mentioned by [15] that the use of geranium extract led to symptoms of tremors, paralysis, and then death, as the death rates reached 80% at a concentration of 5% of Culex pipiens mosquito larvae.

Table (1): Effect of cold aqueous extract of neem, peppermint and geranium plants on the corrected mortality percentages of the third larval stage of the peach fruit fly B. zonata.

Extract	Concentration	Percentage of third instar larval mortality					
		After 12 hours	After 24 hours	After 48 hours	After 72 hours	GPA for concentration	GPA of the extract
Neem Extract	%3	%14	%10	%16	%12	%52	%67.3
	%5	%22	%12	%28	%10	%72	
	%7	%36	%12	%20	%10	%78	
Peppermint extract	%3	%14	%8	%14	%10	%46	%58.6
	%5	%18	%10	%32	%2	%62	
	%7	%16	%20	%26	%6	%68	
Geranium extract	%3	%8	%6	%18	%8	%40	%52
	%5	%14	%8	%24	%4	%50	
	%7	%14	%12	%28	%12	%66	
Control		%0.00	%0.00	%0.00	%0.00	%0.00	%0.00
Average		%15.6	%9.8	%20.6	%7.4		
LSD	2.637 : to extract		2.047 for time:		5.382 to interactions:		3.301 to concentration :

The results of Table (2) showed that the neem extract had the highest effect on pupae and non-emergence of adults, as the percentage reached (30% and 12%) respectively, for the concentration of 7% and 5%, while the concentration of 3% did not show any effect on pupae. A similar study on mosquito pupae [13] stated that the percentage of non-emergence of adults from pupae may reach 49.20% at a concentration of 5 ml/L. In another study[10] stated that neem extract affects the role of the virgin insect *Liriomyza huidobrensis* in the soil, with a non-emergence percentage of 35% at a concentration of 10% as for the peppermint extract, it gave a mortality rate of up to 10% at a concentration of 7%, while it had no effect at a concentration of (3% and 5%). In a similar study conducted by[16], it was mentioned that the Peppermint extract affects the pupae of the house fly Musca domestica by 11.51%, while the geranium extract has

the least effect among the three extracts on the pupae, as the highest percentage of non-emergence reached 6% at a concentration of 7%, while no effect was shown for both concentrations (3% and 5%).

Table (2): The effect of cold aqueous extract of plants (neem, geranium and peppermint) on the corrected percentages of pupal mortality of the peach fruit fly B. zonata.

Extract	Concentration	Percentage of influence on the role of the pupae									concentration average
		After (1) day	After (2) days	After (3) days	After (4) days	After (5) days	After (6) days	After (7) days	After (8) days	After (9) days	
Neem Extract	%3	%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
	%5	%0	%0	%0	%0	%0	%0	%4	%8	%0	%12
	%7	%0	%0	%0	%0	%0	%0	%2	%28	%0	%30
Peppermint Extract	%3	%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
	%5	%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
	%7	%0	%0	%0	%0	%0	%0	%2	%8	%0	%10
Geranium Extract	%3	%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
	%5	%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
	%7	%0	%0	%0	%0	%0	%0	%1	%5	%0	%6
Control		%0	%0	%0	%0	%0	%0	%0	%0	%0	%0
Average		%0	%0	%0	%0	%0	%0	0.9 %	4.9 %	%0	
LSD	1.036to extract :		1.897 for time :			:3.751 to interactions			1.036 to concentration		

The results of Table (3) for the comparison between the leaf extracts of the three plants at concentrations (3%, 5% and 7%) indicated that the extract of geranium and Peppermint plants recorded the highest repellent force (72.16% and 62%) respectively and in a similar study, [17] stated that the percentage of housefly repelling using mint extract ranged from (61-96%) while the neem extract recorded the lowest repellent force among the three extracts, with a repellent force of 56.03%. The results of the statistical analysis at a probability level of 0.05 indicated significant differences between the studied plants at different concentrations as well as for the time difference, as the highest rate of repellent force reached 16.15% after 48 hours,and then began to decrease, as the repellent force decreased after 4 days of treatment to 9.36%. In a similar study, [18]mentioned when studying the effect of neem extract in repelling the diamondback moth *Plutella xylostella*, that the neem extract showed effectiveness in repelling the insect of 60.8%.

Table (3): Repellent force averages of cold aqueous extract of three plants (geranium, neem, peppermint) at different concentrations under laboratory conditions.

Extract	Concentration	After 12 hours	After 24 hours	After 48 hours	After 72 hours	After 96 hours	GPA for concentration	concentration average
Neem extract	%3	%5	%8	%10.5	%10	%2	%38.5	%56.03
	%5	%07	%10.5	%12.5	%12	%8	%55	
	%7	%10	%13.5	%18	%15	%11.6	%75.1	
Peppermint extract	%3	%4	%8	%10.8	%10	%3	%38.8	%62
	%5	%8	%11.5	%18	%15	% 10	%62.5	
	%7	%11	%15	%20	%17.5	%14.2	%84.7	
Geranium extract	%3	%10	%8	%12.6	%10	%7.5	%51.1	%72.16
	%5	%13	%13	% 20	%15.3	%13	%74.3	
	%7	%15	%18.5	%23	%19.6	%15	%91.1	
Average		%9.2	%12.9	%16.15	%13.82	%9.36		
LSD	5.43 to extract :		2.211for time :		8.771 to interaction:		4.120to concentration :	

Table (4) shows the repellent effectiveness of the three plant extracts (neem, geranium and peppermint) against the peach fruit fly *B. zonata*. The highest repellent force was (69.8%, 60% and 50.7%) for each (geranium, peppermint and neem) at a concentration of 7% after 96 hours. In a similar study [19], the percentage of geranium extract-repelling *Callosobruchus maculatus* was 70.56% at a concentration of 7.5. In a similar study [20],indicated that mint extract can repel houseflies by 54.5%. In a study conducted by [21], to measure the effect of peppermint leaf extract on nymphs and adults of the *Aphis craccivora*, the scientist stated that the extract showed high effectiveness in controlling the insect, as the death rate reached 84.37%.

Table (4): The repellent average of the extract of (neem, geranium and peppermint) at a concentration of (3%, 7% and 5%) for the adults of the peach fruit fly *B. zonata*.

Extract	Concentration	After 12 hours	After 24 hours	After 48 hours	After 72 hours	After 96 hours	Concentration average	Extract average
Neem extract	%3	%3	%7	%10	%10	%3	%36	%50.7
	%5	%5	%9.3	%11.5	%10.5	%7	%48.3	
	%7	%8	%11	%17.2	%13.6	%11	%67.8	
Peppermint Extract	%3	%4	%7	%10	%10	%4	%38	%60
	%5	%7	%11	%16	%13	% 10	%62	
	%7	%10	%14	%18.5	%16.5	%14	%80	
Geranium extract	%3	%8	%7	%12	%10	%7	%47	%69.8
	%5	%10	%12	% 18	% 15	%12.7	%72.7	
	%7	%13	%16	%20.5	%19.2	%14	%89.7	
Average		%7.55	%10.47	%14.85	%13.12	%9.18		
LSD	4.02 to extract :		2.45 for time :		7.33 to interaction :		to concentration:3.20	

The results of Table (5) showed that plant extracts have an effect plant extract have an effect on adults and on laying eggs on the host. The results showed that spraying trees with plant extracts (neem, peppermint and geranium) played a major role in

preventing the insect from laying eggs and protecting the fruits from infection; this is because these extracts played a major role in influencing the sensory receptors in the antennae of insects, thus confusing the insect and its failure to recognize the plant host to lay eggs, in addition, these extracts are considered insect repellents, as it was shown that spraying the trees protected the fruits from the peach fruit fly *B. zonata* for not less than 5 days. The geranium extract had the greatest effect, as the number of scars on the fruits reached (1.2 and 0.8) scars, respectively, for the concentration (5% and 7%); this is consistent with a similar study conducted by [19], on the *Sitophilus zeamais*, which stated that the percentage of adult insect repellent may reach 91.67% . The peppermint extract came after it in terms of effect, as the number of scars after 7 days reached (2.50 and 2.00) for each of the 5% and 7% concentrations. In a similar study, [22], mentioned that peppermint extract greatly affects insect repelling, reaching 82% when sprayed with 0.353 µg/cm^3. As for the neem plant extract, it had the least effect on the peach fruit fly adults, as the number of scars after 8 days reached 3.40 and 2.60 for both the concentrations 5% and 7%. It was mentioned in a similar study [23], that the neem plant extract showed high efficiency against the *Sitophilus granarius*, as the insect repelling rate reached 60% at a concentration of 8% for no less than 3 days.

Table (5): Number of scars resulting from egg laying by *B. zonata* on fruits after treatment with plant extracts (neem, geranium and peppermint) at a concentration of (7% and 5%).

Extract	Con.	(1) day	(2) day	(3) day	(4) days	(5) days	(6) days	(7) day
Geranium extract	%0.5	0.00	0.00	0.00	0.00	0.40	1.00	1.20
	%0.7	0.00	0.00	0.00	0.00	0.00	0.20	0.80
Neem extract	%0.5	0.00	0.00	0.00	1.20	2.00	2.40	3.40
	%0.7	0.00	0.00	0.00	0.00	1.40	1.80	2.60
Peppermint extract	%0.5	0.00	0.00	0.00	0.00	1.00	1.60	2.50
	%0.7	0.00	0.00	0.00	0.00	0.6	1.20	2.00
Control		1.40	2.40	3.20	3.80	4.00	4.60	4.60
Average		0.20	0.34	0.45	0.71	1.34	1.97	2.44
LSD	to concentration :0.200		0.302for time :		0.680to interaction :		0.331to extract :	

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