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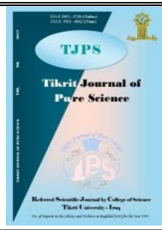

Effect of some plant extracts on the first and second larval instar of *Culex pipines pipines* L.

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Abstract

The present study aimed to study some alternatives chemical pesticides and observation their effect on the life of the first and second larva instar of *Culex pipiens pipiens* L. for this reason, this species of mosquitoes was raised in the breeding cage, which was locally produced. The plant extracts of the seeds of the *Nigella sativa, Azadirachta indica*, *Sesbania sesban* and *Lagenaria vulgaris,* the larvae of the first and second instar of mosquitoes were treated with plant extracts by concentrations of 100, 500, 1000, 5000, ppm. The study showed different results according to the concentration and duration of treatment. The treatment with plant extracts showed that the *Nigella sativa* seed extract is higher than the rest of the plant extracts. The mortality percentage was 93.3% in the concentration of 5000 ppm after 24 hours of treatment.The mortality percentage for the same extract was 100% at the concentration of 5000 ppm within 48 hours of treatment for the first larval instar, while the mortality percentage for the extract were 100% at 5000ppm concentration during 24 and 48 hours of treatment for the second larval phase.

While the *Lagenaria vulgaris* seed extract was the least affected from the rest of the extracts on the larvae, where the mortality percentage 26.66, 33.33% in the concentration of 5000 ppm percentage 24, 48 hours of treatment respectively for the first larval instar. For the second larvae, the results were 50% and 86.66% at 5000 ppm concentration after 24, 48 hours of treatment respectively for extract of pumpkin plant.

Introduction

The acquisition of disease-tolerant medicinal insects forms a major threat to human and animal health as well as its damage to insect control programs [1]. This problem has evolved over time, prompting researchers to find alternatives that are non-toxic to humans and animals [2],[3].

Mosquitoes are considered to be medical insects that have been resistant to all pesticides in many different places [4]. The human problem with mosquitoes has existed since ancient times. If humans did not know mosquitoes, An inconvenience and anxiety for his rest is enough to be considered a problem in itself.

Culex pipines pipines L. is one of the mosquito species known to be superior to humans and is located close to their habitat. It favors environments that contain a high concentration of nitrogen, such as wastewater, sewage, rich water and ponds, with their organic matter content [5]. The importance of mosquitoes to humans is medically responsible for the spread of many human and animal diseases, such as malaria, which kill about 3 million people annually" [6]. yllow fever, dengue fever, raftva fever filariasis, It is caused human [7],[8] Many plant extracts have been used as alternatives to chemical insecticides in the killing and control of insects where humans used plant flowers containing toxic substances in their leaves and seeds directly through the use of vegetable powder or after extraction with organic solvents.Increased insect control requires strategies to extend the use of effective vector control compounds, so using a combination of insecticides with other insecticides or with plant compounds is one of these strategies for mosquito control. And that the use of the mixture is more effective "than the use of manufactured insecticides or plant compounds alone [9].

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Materials and methods

- Collecting and breeding insects:

In this study, a strain of *Culex pipiens pipiens* L. larvae collected from the ponds was used for the aquifers of the crossing area in Al-Dur city, where the immature stages (larvae and Pupae) were collected by means of nets consisting of a wooden arm not exceeding a meter long with a circular metal mesh similar to a sieve its about 10 cm. The samples were transported by glass bottles to the breeding room, which is a wooden cage made up of 5 transparent plastic tubs. On the sides of the cage is a thin mesh that does not allow mosquitoes exit. The dimensions of the cage are 30 × 180 × 90 cm, the larvae are placed in plastic tubing capacity to show [10]. The method was used to place a Zajel-type bird in the cage, tied to its legs and wings. It was used for the purpose of feeding. For the purpose of obtaining eggs, and removed the feathers of the chest area to make it easier for mosquitoes to absorb blood, and continue feeding for 12 hours in the dark, and for the purpose of obtaining a pure farm eggs were transferred to the breeding site until hatching and get larvae [11].

- Collection of plant samples:

The required plant parts percentage in the present study percentage from Tikrit and Al-Dour city, which is the seed of the Niglla sativa, *Azadirachta indica*, , *sesbania sesban* and the *Lagenaria vulgaris*. These plants were classified into the herbarium of the College of Science - University of Tikrit under the taxonomic numbers (11,10,9) respectively. This plant was washed and cleaned of dust and dirt and dried in the laboratory at 25 ° C. dried plants were kept in bags until the extraction process began.

Table (1) Plants seeds using in the study

Part used	The scientific name	English Name
Seeds	*Nigella sativa*	Nigella
Seeds	*Azadirachta indica*	Neem
Seeds	*Sesbania sesban*	Acacia
Seeds	*Lagenaria vulgaris*	Pumpkin

- Preparation of plant extracts:

Plant extracts were prepared in the laboratory of College of Agriculture, University of Tikrit. The seeds of the plants used in the current study were grinded by Miller Electric and took 100 g of black seed, neem, seespan and pumpkin seed powder separately and placed in a special thimble [12]. The 500 mL flask has 250 ml of petroleum ether at 95% concentration and the extract period lasted 48 hours and at 55-60 °C. after extraction, the extract it was concentrated in the vacuum rotary evaporator at the Central Research in Tikrit university, at 55-60 ° C temperature. to get rid of the residue of the solvent used and to obtain a gelatinous fluid, repeat the process was repeat for a number of times until To obtain a sufficient amount of concentrated extract, the extract was subsequently placed in dark glass bottles and stored in the refrigerator to be ready for use.[13].

- Preparation required concentrations

The required concentrations were obtained by taking 1 g of the seed extract of the plant mentioned above . each part was placed in a 100 ml glass container with 99 ml of distil water, and then Polysorbate was added in the form of drops on the mixture Distil water and extract) until the mixture is suspended in a way that is easy to mix and brush, a concentration solution of 1% or 10,000 ppm was abtained. According to the dilution formula of C1V1 = C2V2, the other required concentrations of 5000, 1000, 500, 100 ppm The control treatment used only distilled water.

- Studying the effect of plant extracts on the destruction of the first and second larvae of *Culex pipiens pipiens* L.

I took the larvae of the first and second instar of *Culex pipiens pipiens* L. were took to know the deadly effect of plant extracts where the larvae of these two instars before the transition to the third instar were took and placed in glass containers of 500 ml and containing 100 ml of plant extract with required concentrations by three replicates for each concentration, and 10 larvae of each repeater, as well as the case for control treatment, and should be taken into consideration the calculation of the amount of water preeding when preparing the required concentrations and added to the repeaters 0.01 of feed rabbits for the purpose of feeding the larvae, then the larvae were observed and the results in this test were recorded after 24 and 48 hours of treatment.

- Statistical analysis:

The results were statistically analyzed by using ANOVA in the implementation of experiments. The Duncan Multiplication test was carried out at a 5% probability level to ascertain the differences between the various parameters [14].

Results and discussion

1- The plant extracts efficacy of the first larval instar after 24 hours of treatment.

The result in table 2 shows the percentage mortality of plant extracts after24 hours the treatment of the first larval instar of mosquitoes. Pipiens pipiens L. in the concentrations of 100, 500, 1000, 5000, ppm, where the results showed that there is an effect of the plant extracts used in this study on the death of larvae of the first instar of mosquitoes.

The results of the statistical analysis showed that there are significant differences in the toxicity of plant extracts based on the type of plant extract used in the study, where the percentage mortality increase with a direct increase of concentration and that there is a difference "between the plants to make the impact so that the superiority of a plant on another plant, this is due to the difference in the quality and quantity of active compounds contained in the plants that affect the nervous system of the insect, that paralyzes its movement, leading to death or affect the mechanism of action of the necessary enzymes responsible for important biological processes, causing the cessation of metabolism and death [15].The results in Table (2)

showed that the *Nigella sativa* seed extract gave higher effect in the insect than the rest of the plant extracts, with the highest mortality percentage of 93.3% at the concentration of 5000 ppm and the lowest mortality percentage of 40% in concentration. this is corresponding with [16]. among the show cause of the larvae is due to the effect of certain plant compounds in the killing of the epithelial cells of the central digestive tract of the insect fed to these compounds or that these compounds have high toxicity affecting in the nerve tissue of the larvae and cause paralysis and death.

Also the results of Table 2 showed that the *Azadirachta indica* extract came after the *Nigella sativa* extract in effect on the insect. The highest mortality percentage was 90% at the concentration of 5000 ppm and the lowest mortality percentage was 23.3% at 100 ppm. This study was agreed with a study by [17]. On the use of Neem seed oil extract in the control of mosquitoes and the concentration (0.01%) led to 100% killing within 24 hours. The results showed that the extract of Seesban was followed the Neem extract in terms of effect. The highest mortality percentage was 50% at 5000ppm concentration and the lowest mortality percentage was 10% at 100 ppm.The results showed that the extract of the seeds of the plant pumpkin is the lowest plant extracts impact in the insect with the highest mortality rate 26.66% at the concentration of 5000 ppm and the lowest mortality percentage of (0) at 100 ppm concentration (Figure 1-A).

Table (2) The mortality percentage of first larval instar treated with plant extracts after 24 hours.

Mortality percentages of concentration in ppm					**Extracts**
Average plant	5000	1000	500	100	
75.8 A	93.3 a	90 bc	80 D	40 F	*Nigella sativa*
64.9 B	90 bc	86.6 cd	60 E	23.33 G	*Azadirachta indica*
24.9 C	50 cd	26.66 G	13.3 I	10 H	*Sesbania sesban*
19.9 D	26.66 g	13.33 H	0 I	0 I	*Lagenaria vulgaris*
	64.9 a	54.1 B	38.3 C	18.3 D	**Average concentration**

*Similar small letters in one line (horizontally) mean no significant differences.

**Similar capital letters in one column (vertically) mean no significant differences.
(Using Dankin test)

2- The plant extracts efficacy of the first larval instar after 48 hours of treatment.

The results of Table 3 showed that the *Nigella sativa* extract was superior to the rest of the others in its effect on the larvae, with the highest mortality percentage of 100% at the concentration of 5000 ppm and the lowest mortality percentage of 43.33% in the concentration of 100ppm. After 48 hours of treatment due to the duration of the exposure that caused an increase in mortality percentage. the increasing of the duration of exposure to the active agent, leads to increase of mortality percentage. These results are agreed with the results of [18]. which showed that the duration of exposure to the active substance has the greatest dose effect.The results indicated in Table (5) that the extract of the *Azadirachta indica* came after the *Nigella sativa* extract in the effect on the larvae with the highest mortality percentage 96.66% at the concentration of 1000 ppm and the lowest one 30.33% at 100 ppm concentration. This study is consistent with the findings of [19]. in the treatment of the *Schistocerca gregaria* with the *Azadirachta indica* extract. The percentage of mortality increased to 75%. The treatment of locusts with neem oil reduced their ability to fly and the locusts could not return to its normal state after months of treatment. The results of Table (3) showed that the extract of *Sesbania sesban* came after the Neem extract in terms of its effect on the insect with the highest mortality percentage 40% in the concentration 5000 ppm and the lowest one was 16.66% in the concentration 100 ppm. The results showed that the *Lagenaria vulgaris* extract is the lowest plant extracts in the larvae, with the highest mortality percentage being 33.33% at the concentration of 5000 ppm and the lowest mortality percentage of 10% in the concentration of 100 ppm (Figure 1-B).The reason of the low effect of the plant extract on the larvae compared with the other extracts is due to The quantity and quality of the active substances present in the extracts, which can be accessed through the cotyledon of the insect body, reaching the vital target that can affect it, leading to an end to the paralysis of the insect and then its death.

Table (3) The mortality percentage of first larval instar treated with plant extracts after 48 hours.

Mortality percentages of concentration in ppm					Extracts
Average plant	5000	1000	500	100	
81.6 A	100 A	96.66 Ab	86.66 C	43.33 e	*Nigella sativa*
66.7 B	96.66 Ab	90 a	50 E	30.33 f	*Azadirachta indica*
30.2 C	40 e	33.33 f	30.33 F	16.66 Hg	*Sesbania sesban*
22.4 D	33.33 f	26.66 g	20 Fg	10 H	*Lagenaria vulgaris*
	67.4 a	61.6 b	46.7 C	25.2 d	**Average concentration**

*Similar small letters in one line (horizontally) mean no significant differences.

**Similar capital letters in one column (vertically) mean no significant differences.
(Using Dankin test)

3- The plant extracts efficacy of the second larval instar after 24 hours of treatment.

Table (4) shows the mortality percentage of plant extracts after 24 hours treatment for second instar larvae. *Culex Pipiens pipiens* L. In the concentrations of 100, 500, 1000, 5000, ppm, where the results showed a mixed effect on the type and concentration of plant extracts used in the study on the mortality of larvae of the second instar , the results showed the sensitivity of the larvae to the plant extracts used in the study. It was found that there was a direct correlation between the mortality percentage and the concentration where the concentration is raised the mortality percentage. The exposure of mosquito larvae to the extracts results to paralysis in the insect body and its inability to feed. This is due to that Plant extracts have a high toxicity effect on the nervous system (neurotoxins) or may interfere with it [20].

The results of table (4) showed that the plant extract of the *Nigella sativa* was the most effective from the rest of the plant extracts in terms of its effect on the insect where the highest mortality percentage was 100% of concentration 5000 ppm and the lowest mortality percentage 43.33% in the concentration 100 ppm, The results showed that "Neem extract come after from the *Nigella sativa* extract in terms of its effect on larvae of the second stage. The highest mortality percentage was 96.66% at the concentration of 5000 ppm and the lowest killing rate was 33.33% at 100 ppm concentration. Neem in its effect on the larvae, the highest rate of killing 60.00% at the concentration of 5000 ppm, and the lowest mortality percentage 20,00% at 100 ppm concentration. The results showed that the extract of pumpkin plant is the lowest plant extracts effect on the larvae, giving the highest mortality percentage 50% in the concentration of 5000 ppm and the lowest mortality percentage 10.00% in the concentration 100 ppm after 24 hours of treatment (Figure 1-C).

Table (4) The mortality percentage of second larval instar treated with plant extracts after 24 hours.

Mortality percentages of concentration in ppm					Extracts
Average plant	5000	1000	500	100	
79.9 A	100 A	90 bc	86.66 D	43.33 f	*Nigella sativa*
69.1 B	96.66 b	86.6 cd	60 E	33.33 g	*Azadirachta indica*
38.4 C	60 d	43.33 ef	30.33 G	20 fg	*Sesbania sesban*
24.1 D	50 e	23.33 hg	13.33 H	10 H	*Lagenaria vulgaris*
	76.6 a	60.8 B	47.5 C	26.6 d	**Average concentration**

*Similar small letters in one line (horizontally) mean no significant differences.

**Similar capital letters in one column (vertically) mean no significant differences.
(Using Dankin test)

4- The plant extracts efficacy of the second larval instar after 48 hours of treatment.

The results of table 5 showed that the *Nigella sativa* extract was the most effective on the larvae of the second instar of mosquitoes compared with the rest of the extracts. The highest mortality percentage was 100% at the concentration of 5000 ppm and the lowest mortality percentage was 80% at 100 ppm concentration. The results showed that there was a significant increase in the lethal effect of plant

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extracts after 48 hours of larval treatment, where the mortality percentage of the *Nigella sativa* extract was higher than the mortality percentage after 24 hours of treatment, this is due to the duration of exposure. the results were in line with the study [21]. which showed that the highest percentage of larva death e was 66.7% at the concentration of 250 ppm and the lowest mortality percentage of larvae reached 15.4% at 50 ppm concentration. the results showed that the *Azadirachta indica* extract came after the *Nigella sativa* extract in terms of the effect on the larvae. Table (5) was the highest mortality percentage 96.66% at the concentration of 5000 ppm and the lowest mortality percentage 66.66% at 100 ppm concentration, the results of the study were agreed with the results of [22]. which is used a 5% Naem emulsion to control mosquitoes in India and it gave good results**.** The results showed that the extract of the Seesban plant was came after the Neem extract in terms of the effect on the larvae of the second stage. The highest mortality percentage was 93.33% at the concentration of 5000 ppm and the lowest mortality percentage was 50% at 100 ppm concentration. On the larvae, giving the highest mortality rate of 86.66% in the concentration of 5000 ppm and the lowest mortality rate of 33.33% in the concentration of 100 ppm**,** The extract of the pumpkin plant has a little effect on mosquito larvae the cause due to the amount of active substances found in plant extracts that can be accessed through the insect body. (Figure 1-D).our study showed the mortality percentage of larvae increased within 48 hours of treatment compared to treated within 24hours. We conclude that the duration of exposure has a significant effect on the efficacy of the extracts used, which led to an increase in mortality rates.

Table (5) The mortality percentage of second larval instar treated with plant extracts after 48 hours.

Mortality percentages of concentration in ppm					**Extracts**
Average plant	5000	1000	500	100	
92.5 A	100 A	100 bc	90.00 D	80.00 f	*Nigella sativa*
84.1 B	96.66 b	93.33 cd	80.00 E	66.66 g	*Azadirachta indica*
72.4 C	93.33 d	80.00 ef	66.66 G	50 fg	*Sesbania sesban*
57.4 D	86.66 e	66.66 hg	43.33 H	33.33 H	*Lagenaria vulgaris*
	89.1 a	84.9 B	69.9 C	57.4 d	**Average concentration**

*Similar small letters in one line (horizontally) mean no significant differences.

**Similar capital letters in one column (vertically) mean no significant differences.
(Using Dankin test).

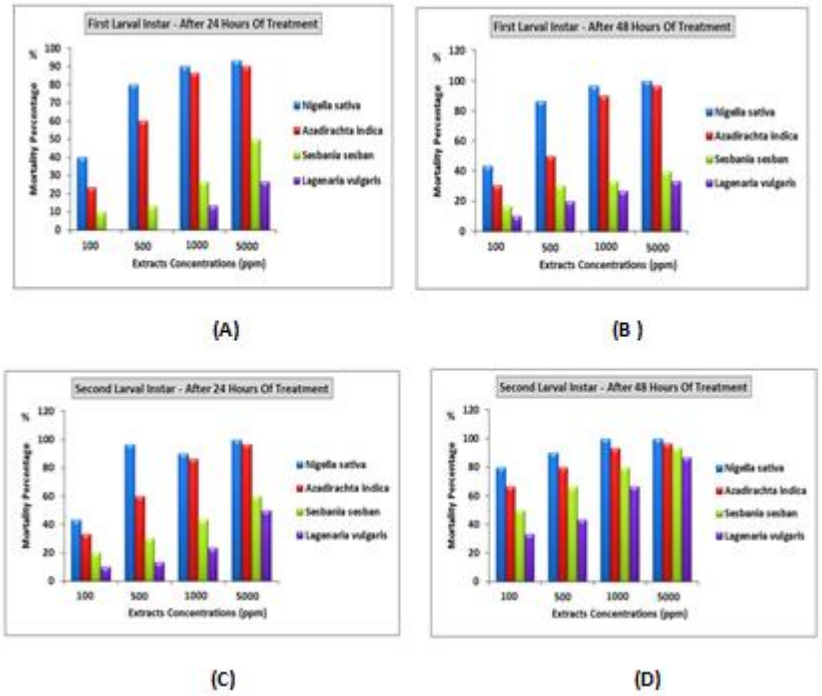

Figure (1) The First and Second Larval instar Mortality Percentage treated with plant extracts.

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تأثير بعض المستخلصات النباتية على الطور اليرقي الأول والثاني لبعوض *Culex pipiens pipiens* L.

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الملخص

هدفت الدراسة الحالية إلى بحث بعض بدائل المبيدات الكيمياوية وملاحظة تأثيرها على حياتية يرقات الطور الأول والثاني للبعوض المنزلي من نوع *Culex pipiens pipiens* L. ولأجل ذلك تم تربية هذا النوع من البعوض في قفص التربية والذي صنع محليا" ومن ثم تم تحضير المستخلصات النباتية لبذور نباتات الحبة السوداء، النيم، السيسبان واليقطين، اذ تمت معاملة يرقات الطور الاول والثاني للبعوض بالمستخلصات النباتية وبالتراكيز 100، 500، 1000، 5000، ppm. وتوصلت الدراسة إلى نتائج اختلفت باختلاف التراكيز والمدة الزمنية للمعاملة، حيث أظهرت المعاملة بالمستخلصات النباتية تفوق مستخلص بذور الحبة السوداء على باقي المستخلصات النباتية، اذ حقق نسبة قتل93.3% في التركيز 5000 ppm خلال 24 ساعة من المعاملة.

وكانت نسبة القتل لنفس المستخلص هي 100% عند التركيز 5000 ppm خلال 48 ساعة من المعاملة بالنسبة للطور اليرقي الأول في حين كانت نسبتي القتل للمستخلص هي 100% عند التركيز 5000ppm خلال 24 و48 ساعة من المعاملة بالنسبة للطور اليرقي الثاني، بينما كان مستخلص بذور اليقطين هو الأقل تأثيرا" من بقية المستخلصات على اليرقات حيث حقق نسبتي قتل 26.66، 33.33 % في التركيز 5000 ppm خلال 24، 48 ساعة من المعاملة على التوالي بالنسبة للطور اليرقي الأول. اما بالنسبة للطور اليرقي الثاني فكانت النتائج هي 50% و86.66 % في التركيز 5000 ppm خلال 24، 48 ساعة من المعاملة على التوالي بالنسبة لمستخلص نبات اليقطين.