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Evaluation of Toxicological Effects of Some Plant Oils and Diesel Oil on Some Biological Aspects of *Culex pipiens* (Diptera: Culicidae) larvae.

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Abstract: The *Culex Pipiens* mosquito is considered one of the most important vectors of pathogens to humans, as it transmits malaria and yellow fever to humans all around the world, and due to the harmful effects that traditional chemical pesticides cause on the environment, there was an urgent need to search for natural alternatives to control insects and limit their damage. In this work, the effect of the following treatment with plant oils and diesel oil was studied to compare their effect on some biological aspects of *Culex Pipiens* mosquitoes. It was found that the value of IC_{50} for *C. limon* oil was 9.67, while it was equal to 21.38 ppm in the case of *M. piperita* oil, as recorded 29.82 ppm for *L. angustifolia* oil, as the value of IC_{50} for *A. indica* oil was 38.06 ppm, while IC_{50} value was 80.89 ppm when treating mosquito larvae with effective concentrations of diesel oil. The effect on egg production was recorded in the treatments with the four plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) and diesel oil respectively 97.4, 102.5, 127.1, 107.2, 124.4 eggs compared to the average number of eggs / female in the control, which recorded 141.6 eggs. These results confirm that the treatment of larvae of *C. pipiens* with *C. limon* oil has caused 31.2% a decrease in eggs production in females who lived from the treatments followed by *M. piperita* oil 24.6%, then *A. indica* oil 24.3% and *L. angustifolia* oil 10.2%, while it recorded diesel oil 12.1 % decrease in egg production. These results confirm that the larval treatments with the tested plant oils and diesel oil led to a decrease in the levels of egg hatching in the treatments by about (12.5, 14.7, 5.2, 15.3 and 7.4)%, respectively, compared to the control. These results confirm that the treatment of larvae *C. pipiens* with the tested oils had a negative effect on longevity in males by (13.4, 10.7, 6.7, 5.4 and 15.4)%, respectively, and in females by (29.5, 20.5, 24.6, 26.9 and 25.7)% on ranking in comparison to average longevity in male and female mosquitoes in the control groups, the results of the statistical analysis revealed that the differences in the average longevity between males resulting from the treatments and the control were not significant, while the differences were significant when comparing the average longevity between the females resulting from the treatments and the females of the control group.

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Keywords: Mosquito, *Culex pipiens*, Plant Oils, larvae, Diesel, pesticide, egg production, *Mentha piperita, Lavandula angustifolia, Citrus limon*, *Azadirachta indica,* adult longevity, hatchability.

1. Introduction

Mosquitoes *Culex pipiens* (Linnaeus, 1758) belong to the rank of Diptera and the Culicidae family and it is considered one of the most important blood-sucking insects. It is a good vector for many pathogens that cause humans with very serious diseases in many countries of the world. Mosquitoes transmit pathogens such as malaria, yellow fever, filariasis and zika virus (Benelli, 2015). There are more than 23 species of mosquitoes that transmit epidemics and diseases to humans (Turell, 2012). The Culicidae family that the mosquitoes follow are *C. pipiens*. One of the most widespread families worldwide (kovendan *et al*., 2012). Because insecticides are part of the vector control strategy, and there is an urgent need to develop alternative insecticides to control mosquitoes, which are safer and more effective than conventional pesticides (WHO, 2005), as many health and environmental damages have resulted from the use of chlorine, phosphorous, and organic pyrethroids in mosquito control. They are especially toxic to the aquatic organisms and animals that depend on them (Lundberg, 2002).

So attention was drawn to plants as a source of new chemical compounds, especially since the use of plants as pesticides did no harm to the environment (Lundberg, 2002).

That is why this study was designed to keep pace with recent trends towards the necessity of rationalizing the use of traditional chemical pesticides in mosquito control by assessing the effectiveness of some non-traditional pesticides against the most prevalent *C. pipiens* larvae in the Kingdom of Saudi Arabia with follow-up to the late effects of delayed effects of these pesticides on some biological biological properties aspects of adult mosquitoes.

Scientists have found that plants are an inexhaustible source of substances with various structural structures that are biologically effective, and it has been recorded that more than 1,800 species of plants possess insecticide properties. The intricate association between the behavioral and physiological effect of these plants makes it difficult for insects to build resistance against them (Rice, 1993; Charleston *et al*., 2005)

(Salam, 1995) proved that the aqueous extract of the seeds of *M. azedarach* had a stimulating activity that was promoted compared to the extract of the leaves of the same plant against the larvae of the fourth life of *C. pipiens*. The value of LC_{50} of the seed and leaves extract was 9000 and 1500 ppm respectively, as he emphasized that all tested concentrations of seed extract showed a destructive effect of eggs up to a concentration of 5000 ppm and on egg laying in gravid females bearing eggs up to 10,000 ppm.

Kulkarni *et al*. (2013) studied the effect of both *Lavandula gibsoni* oil, *Plectranthus mollis* oil and the estonian extract from both, and compared their effect with the effect of the estonian extract of both lavender and mint oil against the three species of mosquitoes *Aedes aegypti*, *Anopheles stephensi* and *Culex quin* LC_{50} for lavender oil 48.3, 62.8 and 54.7 mg / L and 118.5, 137.2 and 128.1 mg / L for acetone extract respectively and the value of LC_{50} for peppermint oil 25.4, 33.5 and 29.5 mg / L and 195.0, 213.8 and 209.0 mg / L for acetone extract extracted from mint.

Govindarajan and Sivakumar (2014) study the ovicidal, larvicidal and adulticidal activities of crude hexane, ethyl acetate, benzene, chloroform and methanol extracts of root of *Asparagus racemosus* were assayed for their toxicity against three important vector mosquitoes, viz., *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). The mean percent hatchability of the eggs was observed after 48 h post-treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in methanol extract of root of *A. racemosus* against the larvae of *C. quinquefasciatus*, *A.aegypti* and *A. stephensi* with the LC_{50} and LC_{90} values were 115.13, 97.71 and 90.97 ppm and 210.96, 179.92, and 168.82 ppm, respectively. The highest adulticidal activity was observed in methanol extract against *A. stephensi* followed by *A. aegypti* and *C. quinquefasciatus* with the LD_{50} and LD_{90} values were 120.44, 135.60, and 157.71 ppm and 214.65, 248.35, and 290.95 ppm, respectively. No mortality was recorded in the control. The finding of the present investigation revealed that the root extract of *A. racemosus* possess remarkable ovicidal, larvicidal and adulticidal activity against medically important vector mosquitoes and this is the low cost and ideal eco-friendly approach for the control of mosquitoes.

Bilal *et al*. (2017) studied the effectiveness of plant oils of two types of lemon seeds against the larvae of A. aegypti, where it proved that lemon seed oil *C. ambhiri* recorded the lowest value of LC_{50} equal to 200.8 ppm while lemon seed oil of *C. sinensis* had The highest value of LC_{50} was recorded at about 457.3 ppm, after 24 hours of exposing the larvae to the effective concentrations of these plant oils.

Awosolu *et al*. (2018) evaluated the efficacy of aqueous extracts of *A. indica* and *Codiaeum vasriegatum* leaves against the fourth age larvae of *Culex* spp. Under laboratory conditions, the results confirmed that aqueous croton extract was more effective against mosquito larvae than neem aqueous extract, on the basis of LC_{50} values, which recorded 5.9 gm / ml (4.5-7.5%) in the case of treatment with croton extract, while it recorded 57.3 gm / ml (24.7-89.9)% when treated with neem leaf extract.

Kala *et al*. (2019) Stated that when using neem oil tablets that were soluble in water against the third stage of Anopheles mosquitoes, the death rate was 80% when using the concentration of 40mg / L with the pesticide effect remaining for up to 6 weeks and the effect of neem was also observed on reproductive efficiency. So that the rate of egg laying decreased by 89%, and it was not observed to affect the non-target fish.

Culex quinquefasciatus Say (Diptera: Culicidae), an arboviral and filarial vector, is one of the most widespread mosquitoes in the world. The effect of continuous exposure to crude extracts of *Argemone mexicana*, the Mexican poppy, on the development and growth stages of second-instar larvae of the mosquito was studied, Inhibition, mortality, and larval and pupal duration phases were assessed. Second-instar mosquito larvae were exposed to crude ethanol extracts of flowers, stems, and seeds. Flower extract exhibited the strongest larvicidal activity with LC_{50} and LC_{90} values after 24 h of exposure of 18.61 and 39.86 ppm, respectively, and 9.47 and 21.76 ppm after 48 h. Extracts from stem and seeds were significantly less effective. The flower extract registered a Growth Inhibition Index of 0.01 at 25 ppm, with stems and seeds registering 0.05 and 0.08, respectively, at 100 ppm (control group 1.02) (Granados-Echegoyen *et al*., 2019).

2. Materials and Methods

1- Mosquito strain used:

The current study was conducted on a laboratory strain of *Culex pipiens* obtained from a research station of Dengue mosquitoes in the Department of Biology - College of Science - King Abdulaziz University - Jeddah Governorate, Saudi Arabia. The mosquito breed was raised in the prepared breeding room at temperatures (27.1 ± 1)°C, relative humidity around (70 ± 5)%, 14-hour lighting periods, and 10-hour darkness.

2- ***Culex pipiens* life Sycle:**

After a blood meal that feeds about (50-500) eggs, a female mosquito lays an egg within two to three days. In *C. pipiens*, the eggs are shaped like a boat so that the eggs stick together by a colloidal substance and take the white color first as it comes out, then it becomes pigmented and turns into a color Dark and the development of embryos in mosquitoes begins immediately after laying eggs depending on the temperature, as it takes (2-7) days or more. As for *C. pipiens* at a temperature of 30 ° C, the eggs hatch after one day of laying and at (10 -20) ° C takes 3-10 days. After the eggs hatch, the larvae come out, and the larval body is divided into three parts: the head, chest, and abdomen. The larva passes through four stages of cleavage before entering the stage of exclusion. The larvae feed on the microorganisms, protozoa, invertebrates, and algae. Larvae grow at a temperature of (10-30) ° C, how many days. Then it occurs after that. The viruses are mosquitoes and are watery and are characterized as moving and fast unlike the rest of the insects. Also at the temperature and at the end of the virgin phase floating on the surface of the water and trumpets are broken after the virgin exit to the adult phase, then the adults increase the blood pressure, which leads to the expansion of the legs and wings and then immediately extracts drops from the liquid to empty the gut and with the emptying of the air the adults are able to fly, it needs two or a half days to control metabolism. *C. pipiens* larvae were placed in Pottery bowls porcelain plates, white, oblong (20 x 30) cm, and 6 cm deep and filled halfway with tap water.

In this study, mosquito larvae were fed on a mixture of rusk powder, dry skim milk and dry yeast, in equal proportions (Al-zahrani *et al.*, 2019). During breeding, it was taken into consideration that the number of larvae in a single dish should not exceed 300 larvae in order to avoid the problems associated with breeding mosquitoes larvae such as overcrowding and starvation. Once the larvae have turned into virgins, the virgins are collected daily by a 15 cm (pipeline) Plastic pipette and transferred to small white plastic cups with tap water and are inserted into mosquito adult cages.

3- Mosquito adults rearing:

Pupae have been placed inside cages to breed full insects. The cube breeding cage is composed of metal frames of equal dimensions (30 x 30 x 30) cm and is covered on all sides by a white cloth except the back side as it is a transparent muslin cloth for easy viewing of mosquitoes inside, and the front face contains a long open sleeve of fabric The cloth sleeve is about 20 cm long and no less than 12 cm in diameter, allowing cups containing virgins to be inserted into the cage.

Male and female mosquitoes were fed a 10% sugar solution. To maintain its vitality and activity as a small cotton swab is immersed in the diabetic solution and then placed in a small plastic cover and inserted into the breeding cage where this adult mosquito was considered to be the Parental strain that will be generated by the following generations for tests on the biological evaluation of the compounds tested in the current study.

4- Egg production:

Adult female mosquitoes were fed about 5 days after hatching on a blood meal and for one hour a living pigeon was placed on the top surface cloth of the breeding cage. The legs and wings of the pigeon were tied with a linen rope after the feathers were removed from the chest area for easy feeding on the blood. The breeding cage was provided with a small plastic cup with water until the middle of it, to receive the egg blocks Egg rafts, the eggs were laid after 5-4 days passed From the time of feeding on the blood where the egg masses were collected and placed in the larval rearing dishes (3-4 blocks / plate) which contain tap water, where the eggs hatch and the larvae exit and complete the life cycle.

5- Oils tested:

The oils used in the biological evaluation tests were obtained from the local market, which is of high quality and is as follows:

A- Plant oils

1-Peppermint oil (30 ml, 100% pure)
Scientific name: *Mentha piperita*
Order: Lamiates
Family: Lamiaceae

2-Lavender oil (30 ml, 100% pure)
Scientific name: *Lavandula angustifolia*
Order: Lamiates
Family: Lamiaceae

3-Lemon oil (30 ml, 100% pure)
Scientific name: *Citrus limon*
Order: Sapindales
Family: Rutaceae

4-Neem oil (30 ml, 100% pure)
Scientific name: *Azadirachta indica*
Order: Sapindales
Family: Meliaceae

B- Diesel motor oil

The diesel oil used in the biological evaluation tests was obtained from a petrol station in Jeddah.

6- **Preparing standard solutions:**

The standard solution for the tested oils was prepared by taking 0.1 ml of the oil to be tested and dissolving in 100 ml distilled water in a standard 100 ml beaker containing 0.5% Triton X-100 as an Emulsifier to ensure complete solubility of the oil in the water.

7- Susceptibility test:

The WHO standard method (WHO, 2005) was used to estimate the sensitivity of *C. pipiens* mosquito larvae to the tested oils. Mosquito larvae were exposed at the beginning of the fourth age of a series of different concentrations for each compound. The tests were carried out in small white plastic plates (diameter 11 cm, depth 4 cm) containing 100 ml of water with five replicates / concentration, where each refined contains 20 larvae, in addition to five iterations of the control, and providing the larvae with food during the test.

The effectiveness of diesel and vegetable oils of mint, lavender, lemon and neem was evaluated on the basis of calculating the cumulative mortality of the larvae recorded daily 24 hours after the start of the treatment, where the number of dead larvae, as well as the moribund larvae, was included in the percentage of larval death. Dead larvae are those that are unable to move when touched with the dissection needle in the Siphon or cervix neck region. dying larvae are unable to climb to the surface of the water or dive when touching or shaking water.

As for the living pupae, they were transferred daily to other clean cups with a quantity of water to record any observations such as the occurrence of death or changes in the apparent shape of each of the virgins or complete insects resulting from the transactions, so this was taken into account when the final evaluation of the effectiveness of these compounds against Mosquito larvae by calculating the percentage of inhibition of complete insects resulting from these larval parameters.

8- Statistical analysis:

The Abbott equation (1925) was used to correct the percentage of death in transactions according to those in the control group if it exceeded 5% death to 20%.

- IC-p lines of toxicity curves were drawn that illustrate the relationship between the concentrations used from the compound and the percentage of inhibition of complete insect hatching caused by larval factors.
- Statistical parameters have been extracted from toxicity curves such as the Chi square (X2) values, Slope function (S), IC_{50} values, Fiducial limits, Slope and so on using the probit software statistical package.

Tests of the delayed effects of the tested oils on some biological aspects of the mosquito adults:

After drawing the toxicity curves for diesel oil and vegetable oil for mint, lavender, lemon and neem, the IC_{50} values (the concentration needed to inhibit the exit of 50% of adult insects) were extracted for each of these tested oils. Concentrations corresponding to the previous values were prepared separately for each compound.

Mosquito larvae were exposed at the beginning of the fourth age of the half-lethal concentration of the tested oil corresponding to its IC_{50} value, using 20 replicates per compound of 20 larvae / repeated. The virgins resulting from these larval treatments for each compound were collected and inserted into new breeding cages, especially for each compound, until the adult insects were released with the cages supplied with a 10% sugar solution. Whole insects resulting from these larval treatments were used to carry out the following studies and experiments:

A - Study the longevity of adult insects:

1- Adult longevity:

Use the hand extractor to pull the newly hatched adult insects resulting from the larval treatment from inside the special breeding cages for each type of tested oil. Each male mosquito was isolated with a female (not fed to the blood) in a small plastic cup with water and covered from above With a piece of transparent cloth from muslin and tight with a rubber band, and provided from above with a small piece of cotton saturated with 10% sugar solution for feeding. So that the cotton swab is constantly changed every 48 hours to avoid the growth of fungi on it. Use 20 reps for each type of oil as well as the control group. Every day, deaths of complete insects, whether male or female, were recorded in each of the treatments and the control group. At the end of the test, the average longevity of both sexes was calculated and analyzed statistically.

2- Reproductive potential:

The resulting female mosquitoes were fed to a blood meal. Then, each female fed with blood was isolated with a male in a small plastic cup filled to the middle with tap water and covered from the top with a tight transparent cloth with a rubber strap, and provided from the top with a small piece of cotton saturated with a 10% sugar solution. The test consists of 20 repetitions of the treatment and the same for the witness. The total number of eggs (Egg production) that were laid by the female mosquitos resulting from the larval treatments, as well as the female control group. The average number of eggs / female was calculated, eggs were hatched and the number of hatching larvae was calculated, from which the hatchability percentage was calculated to estimate the reproductive efficiency of *C. pipiens* (Saleh and Wright, 1990) and statistically analyzed results.

3. Results

Susceptibility of *Culex pipiens* mosquito larvae to some plant oils and diesel oil.

In the present study, the sensitivity levels of the fourth-year larvae of the *C. pipiens* were assessed against four plant oils (*Citrus limon* oil, *Mentha piperita* oil, *Lavandula angustifolia* oil, *Azadirachta indica* oil) and diesel oil. Sensitivity tests were performed according to the World Health Organization (WHO, 2005) standard methods, and Toxicity lines were drawn for the tested oils with the calculation of Statistical parameters for the biological evaluation tests.

The results of the tests showed that the effective range of effective concentrations used from *C. limon* oil against *C. pipiens* larvae ranged between (2-40) ppm causing (11-71)% mortality of the larvae (Table 1), while the effective range of concentrations *M. piperita* oil against larvae of the fourth age of the mosquito ranged between (10-50) ppm, causing (10-82)% mortality in the larvae (Table 2). The results of the sensitivity tests obtained when using *L. angustifolia* oil (Table 3) confirmed that the effective concentrations ranged between (15-55) ppm giving (10-75)% death in mosquito larvae while using the effective concentrations (10-100) ppm of *A. indica* oil has caused (6-66)% death in mosquito larvae (Table 4). The results of the biological evaluation of diesel oil also showed that the compound gave a death rate in the larvae ranging between (10-85)% when using the effective concentrations range (20-180) ppm (Table 5).

Generally the obtained results confirmed that the effective concentrations used from plant oils of *C. limon*, *M. piperita*, *L. angustifolia* and *A. indica* had caused varying proportions of the cumulative death of *C. pipiens* larvae.

Table (1): Biological effects of different concentrations of *Citrus limon* oil on different growth stages in *C. pipies* and adults emergence.

Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	Adult hatched (%)	Adult emergence inhibition[b] (%)
2	11	89	83	17
5	18	82	68	32
15	41	59	28	72
25	52	48	28	72
40	71	29	10	90

[a] Five replicates, 20 larvae each= 0 - 3%, [b] Inhibition of adult emergence in control= 0 - 3%

$(Chi)^2$ Calculated from the data= 6.6878, $(Chi)^2$ Tabulated at 0.05 probability level= 7.81

IC_{50} (ppm)= 9.6686, Fiducial limit of IC_{50}= 8.02 - 11.54, IC_{90} (ppm)= 63.2072, Fiducial limit of IC_{90}= 46.61 - 95.40, Slope= 1.5717

Table (2): Biological effects of different concentrations of *Mentha piperita* oil on different growth stages in *C. pipiens* and adults emergence.

Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	Adult hatched (%)	Adult emergence inhibition[b] (%)
10	10	90	86	14
20	35	65	54	46
30	51	49	34	66
40	71	29	20	80
50	82	18	6	94

[a] Five replicates, 20 larvae each= 2 - 4%, [b] Inhibition of adult emergence in control= 2 - 4%

$(Chi)^2$ Calculated from the data= 3.0707, $(Chi)^2$ Tabulated at 0.05 probability level= 7.81

IC_{50} (ppm)= 21.38, Fiducial limit of IC_{50}= 19.43 - 23.29, IC_{90} (ppm)= 50.2346, Fiducial limit of IC_{90}= 44.42 - 58.92 Slope= 3.4545

Table (3): Biological effects of different concentrations of *Lavandula angustifolia* oil on different growth stages in *C. pipiens* and adults emergence.

Concentrations (ppm)	Larval mortality [a] (%)	Pupae produced (%)	Adult hatched (%)	Adult emergence inhibition [b] (%)
15	10	90	87	13
20	18	82	73	27
30	39	61	54	46
40	58	42	33	67
55	75	25	12	88

[a] Five replicates, 20 larvae each= 1 - 3%, [b] Inhibition of adult emergence in control= 1 - 3%
$(Chi)^2$ Calculated from the data= 1.9563, $(Chi)^2$ Tabulated at 0.05 probability level= 7.81
IC_{50} (ppm)= 29.8179, Fiducial limit of IC_{50}= 27.71 - 32.107, IC_{90} (ppm)= 63.655, Fiducial limit of IC_{90}= 55.88 - 75.85
Slope= 3.8912

Table (4): Biological effects of different concentrations of *Azadirachta indica* oil on different growth stages in *C. pipiens* and adults emergence.

Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	Adult hatched (%)	Adult emergence inhibition[b] (%)
10	6	94	86	14
30	13	87	70	30
50	36	64	46	54
70	57	43	22	78
100	66	34	11	89

[a] Five replicates, 20 larvae each= 1 - 3%, [b] Inhibition of adult emergence in control= 1 - 3%
$(Chi)^2$ Calculated from the data= 13.3987, $(Chi)^2$ Tabulated at 0.05 probability level= 7.81
IC_{50} (ppm)= 38.0579, Fiducial limit of IC_{50}= 20.92 - 58.95, IC_{90} (ppm)= 133.1769, Fiducial limit of IC_{90}= 121.63 - 502.96, Slope= 2.356

Table (5): Biological effects of different concentrations of diesel oil on different growth stages in *C. pipiens* and adults emergence.

Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	Adult hatched (%)	Adult emergence inhibition [b] (%)
20	10	91	88	12
50	28	73	69	31
80	40	60	56	44
120	55	45	42	58
180	85	18	13	87

[a] Five replicates, 20 larvae each= 0 - 4%, [b] Inhibition of adult emergence in control= 0 - 4%
$(Chi)^2$ Calculated from the data= 9.3179, $(Chi)^2$ Tabulated at 0.05 probability level= 7.81
IC_{50} (ppm)= 80.8945, Fiducial limit of IC_{50}= 53.79 - 124.96, IC_{90} (ppm)= 304.525, Fiducial limit of IC_{90}= 256.84 - 1073.57
Slope= 2.2261

The results obtained in Table (1) showed that treatment with *C. limon* oil at the above-mentioned effective concentrations against *C. pipiens* larvae caused (17-90)% inhibition of complete insect outage resulting from the treatments, and the effective concentrations of *M. piperita* oil gave inhibition in the exit of adult insects by (14-94)% (Table 2), while the effective concentrations of *L. angustifolia* oil (13-88)% recorded an inhibition of the adult insect emergence (Table 3), and it was also found that the treatment of mosquito larvae with effective concentrations of *A. indica* oil had the reason (14-89)% inhibition of adult insects emergence (Table 4), while the larval treatment with diesel oil using effective concentrations caused about (12-87)% inhibition of complete insect emergence resulting from these transactions (Table 5. (

In the present study, IC_{50} values, the concentration needed to inhibit the exit of 50% of insects from larval parameters, were taken as a indicator to compare the efficacy of the tested oils in terms of their biological effect on *C. pipiens*. The results (Table 1) showed that the value of IC_{50} for *C. limon* oil was 9.67 (8.02-11.54) ppm, while it was equal to 21.38 (19.43-23.29) ppm in the case of *M. piperita* oil, as recorded 29.82 (27.71-32.12) ppm for *L. angustifolia* oil, as the value of IC_{50} for *A. indica* oil was 38.06 ppm, minimum and higher respectively about 20.92 and 58.95 ppm, while IC_{50} value was equal to 80.89 (53.79-124.96) ppm when treating mosquito larvae with effective concentrations of diesel oil. These results confirm that *C. limon* oil was more effective against *C. pipiens* larvae, whose late effect extends to inhibit the exit of whole insects, followed by *M. piperita* oil, *L. angustifolia* oil, *A. indica* oil, and diesel oil by about 2.2, 3.1, 3.9, and 8.4 times, respectively (Fig. 1).

Table (6): Comparison of Larvicidal activity between different plant oils and Diesel oil against *C. pipiens* larvae based on IC_{50} values.

Oils	IC_{50} (ppm)	Fiducial limits		RR*
		Lower L. (ppm)	Upper L. (ppm)	
Citrus limon	9.67	8.02	11.55	1
Mentha piperita	21.38	19.43	23.29	2.2
Lavandula angustifolia	29.81	27.72	32.11	3.1
Azadirachta indica	38.06	20.93	58.96	3.9
Diesel	80.89	53.79	124.96	8.4

Resistance ratio RR= LC_{50} of the test oil /LC_{50} of *Citrus limon* oil

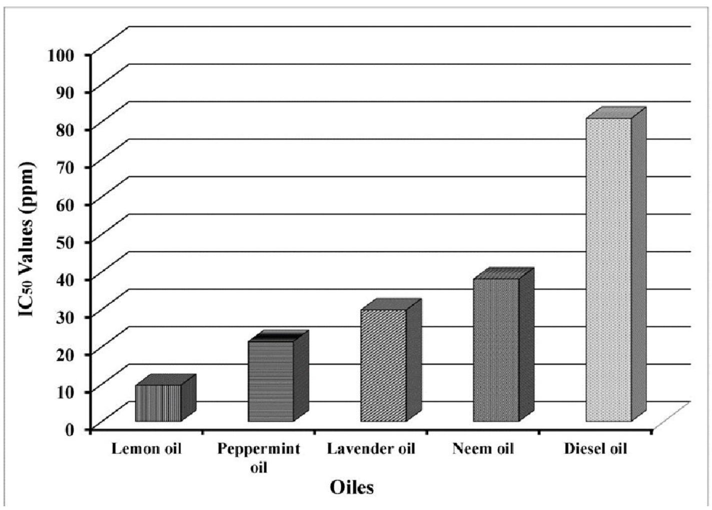

Figure (1): Comparison between the tested oils (Lemon oil, Peppermint oil, lavender oil, neem oil, Diesel oil) in terms of efficacy against *C. pipiens* based on IC_{50} values.

The delayed effects of larval treatments with plant oils and diesel oil on some biological aspects of *C. pipiens* adults.

A- The effect on egg production:

Table (7) and (Fig. 2) show the late effect of treatment of *C. pipiens* larvae in concentrations corresponding to the IC_{50} values for plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) as well as diesel oil on egg production in adult female mosquitoes that lived after larval coefficients. The results confirmed that there was a marked decrease in the number of eggs laid by female mosquitoes resulting from the treatment of larvae compared to the witness in the first feeding and spawning 1st gonotrophic cycle, where the average number of eggs / female was recorded in the treatments with the four plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) and diesel oil respectively 97.4, 102.5, 127.1, 107.2, 124.4 eggs compared to the average number of eggs / female in the control, which recorded 141.6 eggs. These results confirm that the treatment of larvae of *C. pipiens* with *C. limon* oil has caused 31.2% a decrease in eggs production in females who lived from the treatments followed by *M. piperita* oil 24.6%, then *A. indica* oil 24.3% and *L. angustifolia* oil 10.2%, while it recorded diesel oil 12.1 % decrease in egg production. Generally, the statistical analysis showed that the decrease in the average number of eggs in the larval treatments with the tested oils compared to the control group was significant in the case of treatment with *C. limon* oil, *M. piperita* oil, *A. indica* oil and not significant in the case of treatment with *L. angustifolia* oil and diesel oil.

B-The effect on hatchability of eggs:

The results in Table (8) and Fig. (3) showed the effect of treating *C. pipiens* mosquito larvae with plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) as well as diesel oil on the eggs hatching rates of female mosquitoes that lived after those larval treatments. The percentage of eggs hatching (84.2, 82.1, 91.2, 81.5, 89.1) was recorded in the oils *C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) and diesel oil, respectively, compared to 96.2% in the control. These results confirm that the larval treatments with the tested plant oils and diesel oil led to a decrease in the levels of egg hatching in the treatments by about (12.5, 14.7, 5.2, 15.3 and 7.4)%, respectively, compared to the control.

Table (7): Effect of treatment of *C. pipiens* larvae with plant oils (*Citrus limon*, *Mentha piperita*, *Lavandula angustifolia*, *Azadirachta indica*) and Diesel oil on egg production in adult female mosquitoes that lived through larval treatments.

Oils	IC_{50}* (ppm)	Total No. of eggs	No. of eggs/ female		Decrease in egg production (%)
			Range	Mean** ± SE	
Citrus limon	9.67	1948	86 - 136	97.4^{b} ± 10.3	31.2
Mentha piperita	21.38	2050	75 - 147	102.5^{b} ± 1.6	24.6
Lavandula angustifolia	29.81	2541	101- 154	127.1^{a} ± 3.1	10.2
Azadirachta indica	38.06	2143	78 - 126	107.2^{b} ± 0.9	24.3
Diesel	80.89	2489	89 - 141	124.4^{a} ± 12.5	12.1
Control		2832	111 - 161	141.6^{a} ± 12.2	

* Obtained from toxicity lines (IC - p lines).
** Mean of 20 engorged mosquito females; means followed by the same letter are not significantly different ($P \leq 0.05$).

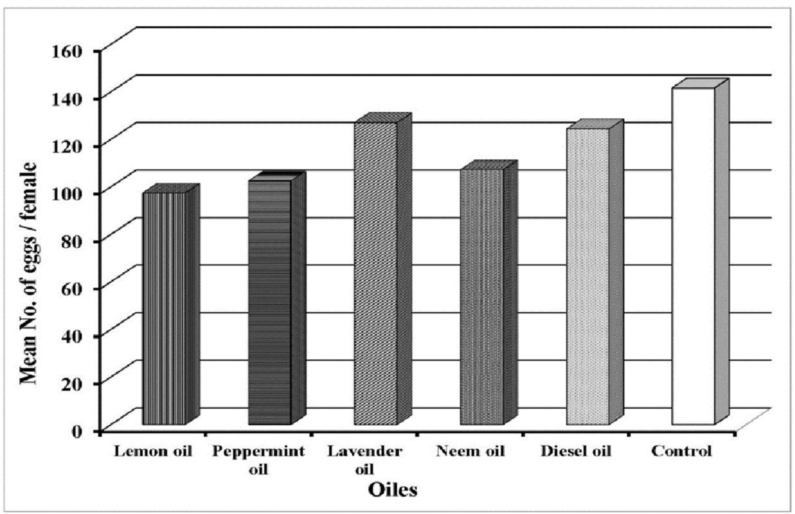

Figure (2): Average number of eggs produced by female females of *C. pipiens*, which lived after treating the fourth age larvae with concentrations corresponding to the IC_{50} values for the tested oils.

Table (8): The effect of treating *C. pipiens* mosquito larvae with plant (*Citrus limon*, *Mentha piperita*, *Lavandula angustifolia*, *Azadirachta indica*) and Diesel oil on the percentage of eggs hatching produced by adult female mosquitoes that survived from the larval treatments.

Oils	IC_{50}* (ppm)	Total No. of eggs**	Total No. of larvae hatched	Hatchability (%)	Decrease in hatchability (%)
Citrus limon	9.67	1948	1640	84.2	12.5
Mentha piperita	21.38	2050	1683	82.1	14.7
Lavandula angustifolia	29.81	2541	2371	91.2	5.2
Azadirachta indica	38.06	2143	1746	81.5	15.3
Diesel	80.89	2489	2217	89.1	7.4
Control		2832	2724	96.2	

* Obtained from toxicity lines (IC - p lines). ** Total of 20 engorged mosquito females.

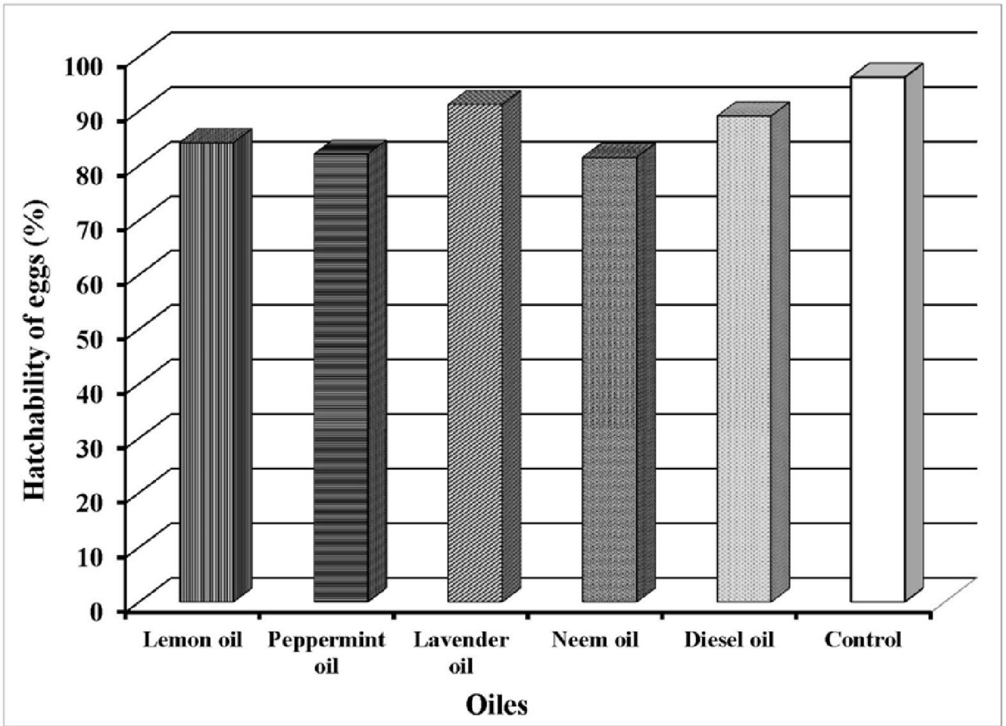

Figure (3): The percentage of eggs hatching laid by *C. pipiens* female mosquitoes that lived after treating the fourth age larvae with the concentrations corresponding to the IC_{50} values of the tested oils.

C-The effect on adult longevity:

Tables (9, 10) indicate the late effects of treating *C. pipiens* mosquito larvae with the concentration corresponding to the IC_{50} values of the following plant oils: (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) as well as diesel oil on the longevity of adult insects from male and female adult mosquitoes resulting from larval treatments, the results obtained showed that the average longevity of male mosquitoes resulting from treatment of mosquito larvae with previous oils was (12.9, 13.3, 13.9, 14.1, 12.6) days compared to male mosquitoes in the control group, which recorded 14.9 days (Fig. 4), while the average longevity of female mosquitoes resulting from the treatments was recorded respectively (18.9, 21.3, 20.2, 19.6, 19.9) days when compared to control mosquito females, which recorded 26.8 days (Fig. 5). These results confirm that the treatment of larvae *C. pipiens* with the tested oils had a negative effect on longevity in males by (13.4, 10.7, 6.7, 5.4 and 15.4)%, respectively, and in females by (29.5, 20.5, 24.6, 26.9 and 25.7)% on ranking in comparison to average longevity in male and female mosquitoes in the control groups. Generally, the results of the statistical analysis showed that the differences in the average longevity between males resulting from the treatments and those in the control were not significant, while the differences were significant when comparing the average longevity between the females resulting from the treatments and the females of the control group.

Table (9): The effect of treatment of *C. pipiens* mosquito larvae with plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) and diesel oil on longevity in adult male mosquitoes that lived after larval treatments.

Oils	IC_{50}* (ppm)	Longevity of adult males (in days)		
		Range	Mean ± S E**	Reduction
Citrus limon	9.67	6 – 16	$12.9^{a} \pm 4.2$	13.4
Mentha piperita	21.38	5 – 18	$13.3^{a} \pm 6.1$	10.7
Lavandula angustifolia	29.81	9 – 19	$13.9^{a} \pm 5.8$	6.7
Azadirachta indica	38.06	6 – 18	$14.1^{a} \pm 5.3$	5.4
Diesel	80.89	7 – 17	$12.6^{a} \pm 4.1$	15.4
Control		9 – 22	$14.9^{a} \pm 5.1$	

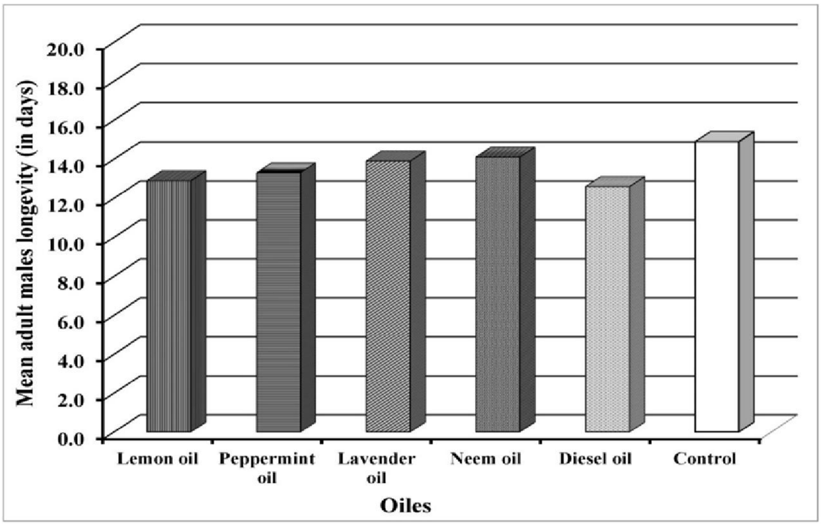

Figure (4): Average longevity of male *C. pipiens* mosquito, which lived after treating the fourth age larvae with the concentrations corresponding to the IC_{50} values of the tested oils.

Table (10): Effect of treatment of *C. pipiens* mosquito larvae with plant oils (*C. limon*, *M. piperita*, *L. angustifolia*, *A. indica*) and diesel oil on longevity in adult female mosquitoes that lived after larval treatments.

Oils	IC_{50}* (ppm)	Longevity of adult females (in days)		
		Range	Mean** ± S E	Reduction
Citrus limon	9.67	12 – 29	18.9^{b} ± 5.5	29.5
Mentha piperita	21.38	6 – 26	21.3^{b} ± 6.1	20.5
Lavandula angustifolia	29.81	9 – 31	20.2^{b} ± 4.8	24.6
Azadirachta indica	38.06	9 – 28	19.6^{b} ± 5.2	26.9
Diesel O.	80.89	10 – 24	19.9^{b} ± 6.3	25.7
Control		7 – 36	26.8^{a} ± 5.9	

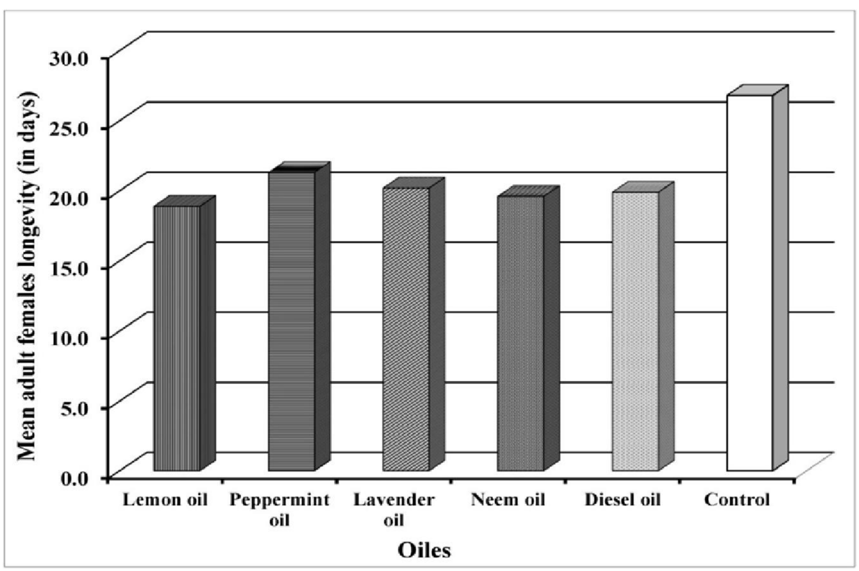

Figure (5): Average longevity of female *C. pipiens* mosquito, which lived after treating the fourth age larvae at concentrations corresponding to IC_{50} values of the tested oils.

4. Discussion

Numerous recent studies in the field of pest control have mentioned the role of plants and their products in controlling the Culex mosquitoes. This study is a contribution to the evaluation of the efficacy of a number of plant oils compared to diesel oil which is now widely used in our area to control the widespread *Culex pipiens* mosquitoes.

The use of various chemical pesticides with high toxicity and the dangers and damages they cause to the environment has led to the search for unconventional means in the control and this thesis aims to study the possibility of applying one of these methods of using compounds that have a toxic property on insects such as oils of some plants to combat a very dangerous pest in The environment and negatively affect human health, which is the *C. pipiens* mosquito. The following plant oils were used (*Citrus limon*, *Mentha piperita*, *Lavandula angustifolia*, *Azadirachta indica*) and diesel oil to compare the effect of the corresponding concentration of LC_{50} values and study the possibility of using plant oils as an alternative to oil diesel for control of *C. pipiens*.

It is evident from the results of our study that the plant oils used in this study had a delayed erosive effect, and it appeared in the incomplete stages, and the pernicious effect did not occur as quickly as it does in the case of using traditional chemical pesticides (Singhi and Hit, 2013).

And it was found that the difference between the tested plant oils and diesel oil in their effectiveness against *C. pipiens* may be due to the different mode of action, and the difference in the level of sensitivity of mosquito larvae to the tested plant oils may be due to the different level of effective concentrations used, as well as natural and specific. Active ingredients included in the components of plant oil, and this may need in subsequent studies to be separated and isolated to identify their chemical composition and method of action (Ghosh *et al*., 2012; Thongwat *et al*., 2018).

In general, there are many scientific studies related to the evaluation of the larval erosive effect of many plant extracts and oils against different types of mosquitoes, which are consistent with many of the results obtained in the current study, where the sensitivity tests conducted by Mwaiko (1992) on the larvae of *C. quinquefasciatus* mosquitoes showed that using oils Lemon, *C. limon* and orange *C. sinensis*, exhibit a high degree of eternal effect against the larvae.

- The effect on the biological aspects.

From the results of our study it appears that there is an imbalance and disturbance in the growth of the treated larvae. Our results are consistent with what was mentioned by (Jacobson, 1990), who mentioned that treating mosquito larvae with the active compounds extracted from the neem plant leads to hormonal disorders that impede their growth and reproduction. And our results agreed with Pushpalatha and Muthukrishnan (1995) who also reported the larval peridomonas activity of *Nerium oleander* leaf extract against different ages of *A. stephensi* and *C. quinquefasciatus*, where it was mentioned that low concentrations of this extract prolonged the life of the larvae and pupae, which is consistent with the results of the current study. He also confirmed that the first and second larval ages are more sensitive to the extract and that the difference in the level of sensitivity of mosquito larvae to the oleander leaf extract may be due to the difference of the two types of laboratory mosquitoes. Murugan *et al*., (1996) also studied the effect of neem oil and neem seed extract against *Anopheles. stephensi*, at a concentration of 5% each, as the study proved that neem oil is more effective against mosquito larvae than the seed kernel extract, but both extracts induced inhibition in the hatching of adult mosquitoes, confirming the presence of similar effects to the action of IGRs, causing distortions in the appearance. Morphogentic effects for different growth stages of mosquitoes.

Traboulsi *et al*., (2002) confirmed the erosive activity of plant oils extracted from the leaves of a number of aromatic plants belonging to the Lamiaceae family, which contain lavender, mint and basil, when treating the fourth-year larvae of *C. pipiens* mosquito, as these plant oils have proven their effective effect against the larvae. Treatment and the values for LC_{50} were 36, 70 and 89 mg / l, respectively.

Our results agree with Amusan *et al*., (2005) who found that the use of methanolic extract of *Citrus sinensis* peels against *Aedes aegypti* larvae causing yellow fever and observed that increasing concentrations of the extract caused an increase in the mortality percentage of larvae. On the other hand (El-Banoby, 2005) found that the crude alkaloids of *Sulanum nigrum* had genocidal activity against larvae of *C. pipiens* where the effective concentrations ranged between (200-900) ppm and gave (28-90)% inhibition in the adult emergence.

The analysis of our results shows that there is a decrease in the longevity of the adult stage, and these results are consistent with what was mentioned (Ascher, 2005), where he noted that when using a neem scales extract *A. indica*, there is a shortage in the life span of the adult phase for many insects.

(Jayakumar *et al*., 2016) has also studied the erosive activity of a number of plant oils such as lavender oil and lemon oil against the larvae of *C. quinquefaciatus* mosquitoes, the tests have proven the effectiveness of these oils against larvae, and the effect extended to include pupae and adult insects, which is also consistent with the results of the current study.

Jayarama and Pushpalatha (2008) also indicated that raising the larvae of mosquito species belonging to the genera Culex, Aedes, and Anopheles in water treated with plant leaf seed extract *Samadera indica* & *Solanum suratense* resulted in decreased fertility in females resulting from the treatments by (62.4-87.4)% The levels of egg hatching were also affected, which is consistent with our results and also consistent with the results (Devi and Bora 2017) that the phenolic extract of *Ziziphus jujube* had adverse effects on the growth rate when used against the larvae of the dengue mosquito *A. aegypti* and also reduced fertility rate and hatchability rate.

The reduction in longevity of adult insects resulting from the treatment of *C. pipiens* mosquito larvae with tested plant oils and petroleum oil may be due to the effect of these treatments that may result in weakening action of the physiological state in the treated larvae, which in turn affects later on longevity in adult insects. Resulting from it (Saleh *et al*., 2003). On the other hand, the results obtained showed that the rate of decrease in the longevity of adult insects resulting from the larval treatments with the tested oils was more evident in the females than the males and this may be due to the fact that the mosquito larvae that will later turn into adult females usually live a longer life in water reproduction, which extends the period of contact with the pesticide for those larvae that will later transform into adult males (Sawby *et al*., 1992; Belinato *et al*., 2009).

In general, the importance of these current research studies appears, especially in the field of mosquito control, as the decrease in the longevity of adult insects such as male mosquitoes may reduce the chance of mating, and the decrease in the longevity of female mosquitoes will reduce the number of feeding cycles and gonotrophic cycles and thus the numbers of offspring will decrease. Progeny in the environment. Also, from an epidemiological point of view, the decrease in the longevity of female mosquitoes as a result of these larval treatments with the tested compounds will reduce the number of blood meals that mosquitoes obtain from the hosts, and this in turn reduces the chance of transmitting pathogens (Ueno, 1994; Silva *et al*., 2009; Devi and Bora, 2017).

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