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Effect of Alcoholic Extract of *Citrus aurantium* Leaves on Larvae and Pupa of House Flies, *Musca domestica* (Diptera:Muscidae)

Lahib S. Mahdi and Thaer M. Taha

Department of Biology, College of Education for Girls, University of Kufa, Iraq ***E-mail:** <u>lahibs.albusayr@uokufa.edu.iq</u>; <u>thairtaha_2008@yahoo.com</u>

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ABSTRACT

This study assessed the impact of an alcoholic extract of Citrus aurantium leaves on the mortality percentages in the larvae and pupae of house flies, Musca domistica, and was carried out in the Advanced Entomology Laboratory in the Department of Biology at the Girls College of Education at the University of Kufa in 2022. The results indicated an increase in the mortality percentages of larvae in the first and third stars of the house fly, Musca domistica with increasing concentrations used. In this study, the highest mortality percentages were recorded after three days of treatment with a concentration of 50 mg/ml (100 and 61%) in the first and third instar larvae, respectively. The effect of the alcoholic extract on some biological aspects when treating the third instar larvae of houseflies, as it led to a decrease in pupation percentages to 24% when treating pupae with a concentration of 50 mg/ml, and the pupal deformation percentages to 24% and a decrease in adult emergency percentages to 0% when treating pupae with the alcoholic extract of Citrus aurantium leaves at a concentration of 50 mg/ml, which increased the pupal deformation to 50% and decreased adult emergency percentages to 50%.

INTRODUCTION

Adult houseflies can be distinguished by the presence of four longitudinal dark lines on the dorsal side of the thorax (Sinthusiri and Soonwera, 2013). Houseflies transmit pathogens such as *Vibirio cholera, Salmonella typhi, Bacillus dysentery, Shigella sp., Staphylococcus aureus, Streptococcus, Bacillus anthraces, Leprosy tuberculosis* caused by *Mycobacterium tuberculosis*, and Bilgeways caused by Bacterium, *Treponema peetenue* (Farooq and Freed, 2016). The larvae of this insect cause myiasis, and this insect causes allergic reactions in some individuals as a result of the flying of parts of its body such as thorns and hairs, which may enter through the respiratory system or the eye (Seghal *et al.,* 2002). Adults also cause harassment and inconvenience to humans and animals, especially in poultry farming fields. It causes significant losses in the economy as a result of low productivity of meat and eggs (Acevedo *et al.,* 2009). Flies are carriers of viral pathogens such as hepatitis A virus, Hepatitis, trachoma and poliovirus (Moon, 2009). Houseflies also carry cysts of primary parasites such as *Entaemoeba coli* and *Giardia lambilia,* as well as eggs of Taenia and pinworms *Enterobious vermicularis* (Shono and Scott, 2003). Flies also transmit some fungi that cause fungal diseases in humans, such as *Trichophyton* mentagrophytes and Microsporum gypseum (Zarrin et al., 2007). In order to reduce the problems arising from the use of chemical pesticides, attention has been directed in recent research to find alternative methods for the use of chemical pesticides from natural sources, including the use of plant extracts as successful and effective natural control methods against insect pests. citron leaves are also an important source of biologically active compounds, including flavonoids. Ascorbic acid and its components are known as natural antioxidants (Khettal et al., 2017), also many studies were conducted by (Gholivand et al., 2013) on bitter orange leaves and found that they contain different types of essential oils including limonene, linalool, α -terpineol, linalyl acetate. Periyanayagam et al. (2013) also conducted a chemical analysis of C. aurantium leaves and found many compounds such as flavonoids, phytosterols, carbohydrates, saponins, volatile oil, tannins, terpenoids, proteins, and 35 compounds were diagnosed using GC-MS analysis through distillation Aqueous of *Citrus citrus* leaves. The main essential oils identified are eucalyptol (43%), sabinene (17%), β -pinene (1%), Ω -terpineol (8%), α -pinene (1.3%), β -myrcene (1.2%), 4-terpineol (1.1%), β -pinene (1%), D-limonene (1%), 0-cymene (1%)

MATERIALS AND METHODS

Collection and Breeding of The House Fly, M.domestica:

Numbers of house fly insects were collected during the month of May of the year 2022, and the insect was bred in the advanced insect laboratory of the Department of Biology / College of Education for Girls / University of Kufa, and the adults were placed in breeding cages designed in the form of a cuboid with dimensions (40 x 40 x 35) cm Its base is wooden. As for the four side faces and the upper surface, it was covered with tulle, and a circular hole with a diameter of 10 cm was made in one of its side sides, allowing the hand to enter and deal with the insect. Adults were fed using a mixture of 100 gm milk powder + 100 gm caster sugar + 2 gm dry yeast according to Keiding and Arevad's (1964) method, and 5 cm thick pieces of cotton were placed in a petri dish.al). Pieces of cotton 5 cm thick were placed in a petri dish, and moistened with a solution consisting of 80 g of sugar, 250 ml of distilled water and milk powder for the purpose of feeding adults and laying eggs on them. The insect was reared under laboratory temperature conditions (Martiradonna et al., 2009). Eggs were collected with a soft brush and transferred to plastic boxes with a capacity of 500 ml for the purpose of developing larvae, which consisted of strips of tissue paper with a thickness of 5 cm, which were wetted with a solution consisting of distilled water, yeast and milk powder. After laying the eggs on the surface of the tissues, they were covered with a layer of dry tissue paper, while providing conditions of 24 hours in complete darkness by covering the cans with a black cloth up to the pupal stage and then transferring them to the adult breeding cages, and so the farm was purified for three generations before experimenting on it (Martiradonna et al., 2009). **Collecting and Classification of Plant Samples:**

Collecting *Citrus aurantium* samples and classifying them. The leaves of *C*.

aurantium were collected from the trees of the home garden in the province of Najaf during the month of June 2022, then they were transferred to the laboratory and the leaves were cleaned of impurities, then washed and left to dry for 7 days and spread on filter papers in the shade with good ventilation in the laboratory, taking into account Continuous stirring to prevent infection with fungus until complete drying. Then the leaves were ground with an electric mill to obtain a fine powder. The plant powder was stored in clean and sterilized glass bottles, taking into account the registration of the name of the plant sample and the dried plant part on those bottles. It was preserved until used in the extraction process.

Preparation of Methanol Alcohol Extract:

20 gm of the powder of the dry substance of the leaves of the bitter orange plant was taken and placed in the thimble of extraction, then put in the Soxhlet apparatus extraction device, then 200 ml of methanol alcohol at a concentration of 100% for a period of 24 hours. A glass of known weight and placed in an electric oven at a temperature of 45 o C, to obtain the dry extract of the plant sample, and then the sample was kept in the refrigerator until use (Ladd et al, 1978). take 5 g of the dry matter. It was dissolved in 100 ml / distilled water, and thus the concentration of the resulting solution became 5%, or the equivalent of 50 mg/ml, and from it, the concentrations (10, 20, 30, 40) mg/ml were prepared using the law of dilution or volumes: V1xC1=V2xC2

Whereas V1 = first volume C1 = first concentration V2 = second volume C2 = second concentration

Larvae Treated with *Citrus aurantium* Extracts:

The contact effect of methanol alcohol extract of Citrus aurantium leaves on the first and third instar larvae of *Musca domistica*. The larvae of the first larval instar were collected immediately after the eggs hatched using forceps, and their lengths ranged between (1.5-3) mm. They were placed in a clean and sterilized Petri dish, then the larvae were sprayed with a hand sprayer at a height of 25 cm, which represented one replication of three replications for each concentration of the alcohol extract. Methanol of bitter orange leaves, with concentrations of (10, 20, 30, 40, and 50) mg/ml separately, while the comparison treatment was sprayed with distilled water only, then a quantity of food was added to feed the larvae, and each dish was covered with a piece of tulle cloth and tied with A rubber ring was used, and information related to the larval stage, the extract and the date of the experiment were recorded for each replicate, and the dishes were placed in the incubator at a temperature of 2 ± 30 and a humidity of $5 \pm 65\%$ (Al-Lahibi, 2015). Seven days for the purpose of recording mortality percentages, deformation percentages of pupae, and adult emergence percentages. In the same way, the larvae of the third instar were treated, which were identified through their lengths of (6-9) mm.

The Contact Effect of Methanol Alcohol Extract on Pupae of Musca domistica:

The last larval stage, which is about to transform into pupae, was isolated in Petri dishes for the purpose of obtaining one-day-old pupae. (10) one-day-old pupae were transferred with (3) replicates in Petri dishes, and the date of transformation into pupae was written on the plate, and 10 pupae at the age of one day were sprayed. One day by hand spraying of methanol alcohol extract of Citrus aurantium leaves separately, at concentrations (10, 20, 30, 40, 50) mg/ml. Each 10 pupae represent one replication out of 3 replications for each concentration used from the concentrations of methanol alcohol Citrus aurantium leaves extract, in addition to 10 pupates representing the control group, with three replications placed in the incubator for the purpose of following up on the deformation percentages and the adults emergency percentages (Al-Luhaibi, 2015).

Statistical Analysis:

The statistical system SPSS version 26 was used, using chi-square, to show the significant differences in mortality percentages, pupation percentages, deformation percentages, and adult emergence percentages in any of the concentrations used.

RESULTS AND DISCUSSION

Effect of Methanol Alcohol Extract of *Citrus aurantium* Leaves on The First Instar Larvae of The House Fly, *M. domestica*:

The results of Table (1) indicated an increase in the mortality percentages of the first instar larvae with the increase in the concentrations used in the experiment during the

first three days of following up the mortality. As the highest mortality percentage was recorded 100% on the first day at a concentration of 50 mg/ml, and the larval mortality percentage (100) % on the third day at the two concentrations (40 and 50) mg/ml, respectively and the mortality percentages increased with the follow-up time. From the results of Table (1), it was found that there were significant differences in the mortality percentages and that they were concentrated in the concentration of 50 mg/ml after three days of treatment, as well as the concentration of 30 and 40 mg/ml after three days of treatment, as the chi-square arithmetic value of the mortality of larvae was (4.3, 5.8, 6.7) on the first, second, and third days, respectively, and it was greater than the tabulated chi-square value of 1.15.

Perhaps the reason for the increase in the mortality of the first instar with the increase in the concentration of the extract is due to the increase in the concentration of active compounds that affect the digestive system of the larva and prevent it from feeding, and thus the death of the larvae.

The results of the current study agree with the results of Al-Lahibi (2014), who found that the mortality percentages of the first instar larvae of the house fly insect, M. domestica, increased with the increase in the concentration of the ethyl alcohol extract of the fruits of the rosary and bitter melon, as the highest mortality percentages were recorded (83.3, 100%), respectively, at a concentration of 40 mg. / ml.

The results of the current study agree with the findings of Murugasan (2014), who studied the effect of methanol alcohol extract of orange peels, Citrus sinensis, on the larval stages of the house fly M. domestica, and found that the first-stage larvae are more sensitive than the second and third instars larvae. The results of the current study agree with what Singh and Gwarjo (2017) concluded when treating first-instar larvae of M. domestica with alcoholic extract of orange peels (*C. sinensis*) at different concentrations (1.5, 1, 0.5, 2) mg/l. The death rate of the first instar larvae increased with the increase of the concentration used and the follow-up time, as the lowest death rate of 0% was recorded after one hour of treatment, while the highest death rate of 100% was recorded after 24 hours of treatment and at a concentration of 2 mg / L.

Anaya-Gil *et al.*, (2021) found that when the second instar larvae of *Drosophila melanogaster* were treated with *C. sinensis* orange peel extract modified by electric pulses and at different concentrations, the lowest mortality rate was recorded at 28.23% at a concentration of 100 ppm, while the highest mortality rate was recorded at 58.33% at concentration 10000 ppm.

Conc. Mg /ml	Mortality percentages after	Mortality percentages after	Mortality percentages after
	One day	two days	three days
Control	0	0	0
10	53	83	93
20	63	86	96
30	70	90	100*
40	80	96	100*
50	100*	100*	100*
chi-square arithmetic	4.3	5.8	6.7
Tabular chi-square P < 0.05	1.15	1.15	1.15

Table 1: The effect of the interaction of different concentrations of alcoholic extract leaves of *Citrus aurantium* extracts on the mortality percentages of first instar larvae.

Effect of Methanol Alcohol Extract and *Citrus aurantium* Leaves Extracts on The Third Larval Instar of The House Fly, *M. domestica*:

The results of Table (2) indicated that there was a direct relationship between the concentrations of the alcoholic extract of the leaves and mortality percentages of the third instar larvae treated with each of them, as the highest mortality percentage was recorded 31% on the first day at a concentration of 50 mg/ml, and the larval mortality percentage (60, 76) % on the third day at the two concentrations (40 and 50) mg/ml, respectively while the lowest percentage of death (10, 20)% was recorded at the concentration of 10 mg/ml on the first and third days, respectively. From the results of the same Table (2) above, it was found that there were significant differences in the mortality percentages, and they were concentrated in the concentration of 50 mg/ml after the first day of treatment, as well as the concentration of 30 and 40 mg/ml after three days of treatment, as the chi-square arithmetic value of larval mortality was (3.7, 4.6, 5.8) on the first, second, and third days, respectively, and it was greater than the tabulated chi-square value of 1.15. the effectiveness of the indicated AL-Kafaji and AL-Zubaidi, (2014) alkaloid extract of the plant Amaranthus gracilis in the third instar larvae of the house fly M. domestica, with a mortality percentage of (43.3-66.6) % at a concentration of (5,10) mg/ml, respectively. Compared with control treatments, the results of the current study agree with the results of Murugasan (2014), who found that the mortality percentage of the third instar larvae of the house fly, M. domestica increased with the increase in the concentration of the alcoholic extract of C. sinensis peels, as the highest mortality percentage was 100% at a concentration of 100 parts. in the million. The results of the current study agree with the results of El-Khyat et al. (2017), which indicated a high mortality percentage of fourthinstar larvae of the date moth, Ephestia cautella, when exposed to a different concentration of C. aurantium leaf extract, as the lowest mortality percentage reached 58.67% at a concentration of 62.5. mg/L and the highest mortality percentage was 98.67% at a concentration of 1000 mg/L after 7 days of treatment, meaning that the mortality percentages of larvae are directly proportional to the increase in concentrations and the treatment period. Sanei- Dehkordi et al. (2016), found that when treating the fourth instar larvae of Anopheles stephensi with extracts of the peels of C. aurantiumand and Citrus paradisi. The larval mortality percentages increased by 100% at a concentration of 80 ppm and for both extracts. Baranitharan et al. (2020) studied the effect of methanol alcohol extract of Citrus limetta leaves on the third instar larvae in three genera of mosquitoes Aedes albopictus, Anopheles maculatus, Culex mimulus, and the larval mortality percentage of C. Mimulus was higher than the rest of the genera, as the GC-MS analysis showed that the plant contains Contains six plant compounds, includingCorynan-17-01,18,19-didehydro-10-methoxy-,acelate (ester) (C22H28N2O3), which could be the element responsible for the fatality and toxicity of larvae. Rani et al., (2022) the effect of Citrus sinensis plant extract on the third instar larvae of the house fly M. domestica, where the treated larvae appeared shrunken and black in color, while the microscope showed the presence of bubbles as well as damaged to the intestinal cells.

Conc.	Mortality	Mortality	Mortality
Mg /ml	percentages after	percentages after	percentages after
	One day	two days	three days
Control	0	0	0
10	10	15	20
20	15	25	30
30	20	36	56
40	26	46	60
50	*31	*61	*76
chi-square arithmetic	3.7	4.6	5.8
Tabular chi-square P < 0.05	1.15	1.15	1.15

Table 2: The effect of the interaction of different concentrations of alcoholic extract of *Citrus aurantium* leaves extracts on the mortality of the third larval instar.

The Effect of Methanol Alcohol Extract of *Citrus aurantium* Leaves Extracts on Death Rates, Rates of Inactivity, Deformation Percentages, and Exit Rates of Adults Resulting from The Third Instar Larvae of The House Fly, *M. domestica*:

The results of Table (3) indicated that there was a direct relationship between the percentage of total larval mortality and the concentrations of the alcoholic extract and Citrus aurantium leaves extracts in the third instar larvae of house flies, and the highest mortality percentage was recorded at 76% at a concentration of 50 mg/ml. and the effect of the alcoholic extract on the pupation percentages, where the lowest pupation percentages were recorded. 24% of pupae developed from larvae treated with alcoholic extract at a concentration of 50 mg/ml, while the highest mortality percentage of immobility was 80% at a concentration of 10 mg/ml, and deformities occurred in the treated pupae, which are third larval instar with alcoholic extract and Citrus aurantium leaves extracts, and the highest deformation percentages were 24% at the concentration is 50 mg/ml. As for the adult emergency percentages, no healthy adult emerged from pupae treated as larvae with alcoholic extract and Citrus aurantium leaves extracts at a concentration of 50 mg / ml. and the statistical analysis using the chi-square showed that there were statistically significant differences in the mortality percentages of the third larval instar at a concentration of 50 mg / ml compared to other concentrations, and the arithmetic chisquare value was 3.6, while the tabular chi-square value was 1.15, and significant differences appeared in the pupation percentages, especially at a concentration of 10 mg / ml compared to other concentrations, where the arithmetic chi-square value was 4.2, while the tabular chi-square value was 1.15, and the chi-square values indicated that there were significant differences in the deformation percentages at concentration 50 mg / ml compared to other concentrations, and the arithmetic chi-square value was 3.2 and the chisquare value was 3.2 Tabular 1.15 Finally, statistically significant differences appeared in the adult emergency percentages, as clear significant differences were recorded at the concentration of 10 mg / ml compared to other concentrations. The arithmetic chi-square value was 5.1 and the tabular chi-square value was 1.15.

The results of the current study agreed with the findings of Rashad et al., (2019), as the treatment of the third instar larvae of the *Culex pipiens* insect with ethyl alcohol extract of *Moringa oleifera* seeds increased the larval mortality percentages with increasing time and concentration, as it recorded the highest mortality rate of 81.39% at a concentration of 100 ppm. The pupae resulting from the treated larvae showed different patterns of deformities compared to the control treatments, and the percentage of emergence of normal adults decreased by 3% at a concentration of 100 parts per million, compared to the control treatments, which amounted to 84%.

Conc. Mg /ml	Mortality percentages%	Pupation percentages%	Pupation deformation%	Adult emergency%
Control	0	100	0	100
10	20	80*	6	74*
20	30	70	10	60
30	56	43	13	30
40	60	40	20	20
50	76*	24	24*	0
Arithmetic chi-square	3.6	4.2	3.2	5.1
Tabular chi- square P < 0.05	1.15	1.15	1.15	1.15

 Table 3: The effect of the different concentrations of alcoholic extract and Citrus aurantium leaf extracts on some biological aspects of third larval instar

Effect of Methanol Alcohol Extract and *Citrus aurantium* Leaves Extracts on Pupae of House Fly, *M. domestica*:

The results of Table (4) indicated an increase in the deformation percentages of house fly pupae treated with concentrations of the alcoholic extract of Citrus aurantium leaves. The deformation percentages increased with the increase in the concentration used, and the highest deformation rate of 50% was recorded when the pupae were treated with a concentration of 50 mg/ml of the alcoholic extract of Citrus aurantium leaves. In contrast, the decreased Adults emerged from pupae treated with the alcoholic extract of Citrus aurantium leaves. The lowest rate of emergence of adults was 50% when pupae were treated with a concentration of 50 mg/ml. Statistical analysis using Chi-square showed that there were statistically significant differences in the deformation percentages of pupae treated with the aqueous extract at a concentration of 50 mg/ml, and the value was The arithmetic chi-square was 3.8, while the tabular chi-square value was 1.15. and there were clear significant differences in the adult emergency, especially at a concentration of 10 mg/ml, compared with the low emergence percentages of adults at the rest of the concentrations, The arithmetic chi-square was 6.7, while the tabular chi-square value was 1.15. The reason for the death of the virgins may be the inability of the adults to get out of the pupal bag due to the hardening of the ice and therefore they cannot get rid of it, or the active compounds such as alkaloids and terpenes present in the extract have affected the ice-breaking hormone 20-hydroxyecdyson (Klein, 2004). The results of the current study agree with the results of Murugasan (2014), who studied the effect of different concentrations (20, 40, 60, 80, 100) ppm of the methanolic extract of C. sinensis peels on the pupae of the house fly M. domestica, where the highest mortality percentages were recorded for the pupae. At a concentration of 100 ppm. The results of the current study agree with what was mentioned by ALKafajiand AL-Zubaidi, (2014) who studied the effect of the alkaloid extract of the plant Amaranthus gracilis in the pupae of the house fly M. domestica, where the mortality percentages reached 30% and at a concentration of 10 mg/ml, the pupal mortality could be due to the interaction of the chemical compounds of the extracts Vegetarianism with the hormonal systems of virgins, which delays their growth processes or the occurrence of morphological deformities that cause death. Al-Sultani (2015) also conducted a study on the effect of an extract of terpene compounds of

the *Chrozophora tinctoria* plant on the pupae of house flies M. domestica, as these compounds caused a mortality rate for house fly pupae, and the percentage reached 18.06% at a concentration of 1 mg/ml, compared with 0.40% in the control treatment. Majeed et al., (2018) showed the effect of ethanol alcohol extract of C. aurantium leaves on the second instar of *Drosicha mangiferae* nymphs, where the mortality rate of nymphs was 86.75% at LC50 = 135.50 after 24 hours of treatment.

Changbunjong *et al.*, (2022) found that when treated adults of the stable fly, *Stomoxys calcitrans* with *C. aurantium* peel extract, the mortality percentages of adults increased with increasing concentration and exposure time, as the lethal dose was 90% at a concentration of 499.25 μ g/fly in the contact effect.

Conc. mg /ml	Pupation deformation%	Adult emergency%
Control	0	100
10	0	100*
20	26	74
30	30	70
40	40	60
50	50*	50
Arithmetic chi-square	3.8	6.7
Tabular chi-square	1.15	1.15
P < 0.05		

Table 4: The effect of the interaction of different concentrations of alcoholic extract of *C*.

 aurantium leaves on the mortality percentages rates of pupal house flies.

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