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Effect of Some Aromatic Plants on The Life of Mosquito Larvae and Virgins and Their Possible Use In Biological Control

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Abstract. *Contemporary control tactics emphasize the biological management of immature mosquito stages. It was observed that a seedling weighing 10 g in 100 cm of water containing Origanum vulgare and 30 larvae of Culex pipiens molestus accelerated their life cycle duration from 14 days in the control group to 11 days in the experimental group, alongside an 80% apparent reduction in larvae of C. pipiens molestus. The efficacy diminished to 50% and 30% at 200 cm and 300 cm, respectively, with stable plant weight. At start, there was a distinct distortion of the complement; Mint of the species Mentha pulegium has a lethal impact of 50% on mosquito larvae with individual seedlings. The constant weight in 100 cm of water was reduced to 35%, while an increase of 20% in water amount was seen at 200 cm and 300 cm, respectively, with stable plant weight. Additionally, this resulted in deformities in the remaining buds (inability to fly) by 18% ± 5%. The mint plant had no impact on the life cycle of mosquitoes.*

Keywords: *Origanum Vulgare, Culex Pipiens, Ghazwan Khadir, Mentha Pulegium, Bugs*.

INTRODUCTION

Mosquitoes are the most blood-sucking arthropods and there are about 3500 species of mosquitoes, but a few of them have been recorded as transmitters of pathogens, in addition to malaria, mosquitoes of different species transmit other serious diseases such as Rift Valley fever, yellow fever and dengue fever. With the growth of environmental awareness and recognition of the dangers of pesticides, and because many pesticides have lost their effectiveness and most insect pests have acquired resistance, the search for other ways to control pests and not exterminate them was the correct environmental thinking, and this can be achieved using many biological means, including biological control. Recently, the use of environmentally friendly strategies to combat mosquitoes has increased, and one of these possible options is the use of various predators, including insects and plant extracts [1]. The World Health Organization has recommended the creation of natural enemies for mosquitoes, and Plants that contain in their composition components (flowers, fruits and leaves) chemical substances or volatile oils play a major role in biological control [2]. Aromatic plants have significant effects on the life of insects and their immature stages, which may ultimately lead to their control without harming the environment. Some plant leaves, such as marjoram (*Origanum majorana*) and mint, contain strong aromatic odors and penetrating oils, and their composition includes (tannins, phenols, flavonoids, saponins, carbohydrates and alkaloids), in addition to types of vitamins and resinous materials that enter into the composition of these plants [3]. There are more than 17 types of chemical compounds that enter into the composition of the essential oils of the marjoram plant, which were used in the manufacture of natural pesticides for Anopheles stephens and Culex quinquefasciatus mosquitoes, extracted from the leaves of this plant [4].

Marjoram from the genus Origanum vulgare and mint may lead to physiological changes in the tissues of living organisms, especially in the glandular tissues of these organisms. There are nine types of aromatic plants, including wild mint *Mentha pulegium* L., which is ten times more effective in combating mosquitoes than other chemical pesticides [5] due to its pungent smell that keeps adults away from their aquatic breeding areas. Many aromatic plants have the ability to live in shallow and semi-flowing aquatic environments, which are the most suitable aquatic environments for the reproduction of immature stages of mosquitoes. They can be used and cultivated in these environments to be innovative and invasive natural enemies in the biological control of mosquitoes.

Objectives of the study:

1. Using aromatic plants in the biological control of mosquitoes.
2. Inventing natural enemies for immature stages of mosquitoes.
3. Finding suitable and effective alternatives to chemical control or complementary to it.

MATERIALS AND METHODS

a. Collection and rearing of mosquitoes

Larvae and pupae of mosquitoes Culxe pipiens molestus Forskal were collected from some rainwater drainage channels at the University of Mosul, specifically near the Deanship of the College of Engineering, and the samples were transferred to the laboratories of the Research Unit of the Department of Life Sciences at the College of Education at the University of Mosul in April 2018 AD, and the tested mosquito strain was classified in the Natural History Museum - University of Baghdad.

The larvae were taken and placed in oval plastic containers made of melanin, filled with water left for 48 hours, with dimensions of 35 x 20 cm in the middle and a height of 10 cm. The rearing was done in suitable conditions of a temperature of 27° and a relative humidity of about 75%-80% [6]. The lighting was automatically regulated to obtain 16 hours of light and 8 hours of darkness. After 2-3 days of transferring the egg boats from the rearing cage to the previously prepared basins, the first-instar larvae began to appear and were fed using rabbit feed consisting of a paste consisting of yellow corn, flour, protein and dry milk. We put 1 gm in each basin.

b. Collection of aromatic plants

Six types of aromatic plants spread in the geographical area of Mosul city were collected and two plants were selected from them, namely marjoram Origanum vulgare and mint Mentha pulegium L., which were collected from a home garden in Al-Hadba area in Mosul city. The two plants are from the order Lamiaceae of the Lamiaceae family.

c. Design of biological control experiments for immature stages of mosquitoes using aromatic plants:

Preparing replicates and isolating larvae

1. The replicate: is a transparent plastic container in which water is placed and left for 24 hours and a seedling of the plant under study is placed in it, complete with roots, leaves and stems weighing 10 g and fixed in a way that allows breathing so that the largest part of it is inside the water with a part of it outside the water for the purpose of breathing.
2. Isolation of larvae: The newly hatched first-instar larvae are isolated from the previously prepared colony using a small circular metal clip, 5 cm in diameter, with very fine holes that allow water to pass through but do not allow the larvae to pass through. Then the larvae are placed in a Petri dish and counted with the naked eye or a microscope. 300 larvae were used and distributed equally on all replicates including the control group

Using the marjoram plant Origanum vulgare in biological control of mosquitoes

1. Three treatments were conducted and each treatment consisted of three replicates (in addition to one replicate which is the control group without a plant) and these treatments were conducted using transparent semi-cylindrical plastic pots of different sizes cm150, cm250 and cm350 respectively, and each replicate was as follows:
2. The semi-cylindrical plastic pot with a capacity of cm150 was placed in cm100 of water and left for 24 hours and the same is the case in the other sizes cm250 was placed in cm200 of water and cm350 was placed in cm300 of water left for 24 hours and then a complete seedling of the marjoram plant was placed in it containing leaves, stem and roots weighing 10 g in each replicate with the seedling fixed in a way that allows parts of the stem to emerge The leaves were outside the water for the purpose of breathing, then 30 newly hatched first-age larvae were placed in each of the nine replicates and the control group which was also without a plant. The replicates were followed up every day and the results were calculated until the fourteenth day. The evaporated water was completed every day to avoid calculation errors. The tests were conducted in laboratory conditions of 16 hours of light and 8 hours of darkness.

Figure 1. Origanum vulgare plant

Using the mint plant Minta pulegium L in biological control of mosquitoes

As is the case with the marjoram plant, three treatments were made, each treatment consisting of three replicates with the control group. The aforementioned plastic pots were used in three sizes from 100 cm to 300 cm with water left for 24 hours. In each replicate, the entire mint plant Mentha pulegium L (leaves, stem and roots) was placed with a constant weight in all replicates of 10 g, except for the control group, which was left without a plant. In each replicate, 30 newly hatched first-age larvae were placed. The results were recorded daily until the fourteenth day, and the evaporated water in the plastic pots was completed daily to avoid mathematical errors. The experiment was conducted in laboratory conditions .The percentage of death in the treatments was corrected using the Abbott equation.

Figure 2. Mentha pulegium plant

RESULTS AND DISCUSSION

a. The effect of the plant Origanum vulgare

From Table (1), we note that the presence of the plant oregano in the medium in which the aquatic stages of mosquitoes live led to a disruption in the life of these stages. It is clear that the presence of 10 g of the plant in 100 cm led to a short life cycle of the aquatic stages. This was very clear in the larval stage and the pupal stage. This led to dwarfism or small size in the four larval stages and the pupal stage. It was very clear in the feeding stage, which was hyperactive. Figure (1). The reason may be due to a disruption in the secretion of the juvenile hormone and the ecdysteroid hormone. It is worth noting that the leaves of the plant oregano contain a large amount of compounds and aromatic oils such as tannins, phenols, flavonoids, and others [7]. This may be consistent with what was published by the researchers [8] using a group of Plant extracts, including marjoram extract, led to rapid growth in the third instar of the larval stage by affecting the anterior thoracic gland. It is also noted that this effect decreased when the amount of water increased with the plant weight remaining constant from 100 cm to 200 cm then 300 cm by gradual increase. The reason may be due to the low concentration of chemicals produced from the plant leaves by the transpiration process in the unit area, thus reducing their effect on the secretion of hormones and pheromones compared to their spread in 100 cm. This is consistent with what was stated by [9], as the toxic substances produced by *Origanum vulgare* L. had a significant physiological effect on the glands, and this effect decreased from LC50 to LC10 gradually. The presence of approximately 17 types of chemical compounds in the leaves and volatile oils of the marjoram plant led to an acceleration in the life cycle of the mosquito Culex pipiens molestus and consequently a deformity in the pupae, which led to the emergence of immature larvae (Figure 3) and unable to fly. It is clear from that the wings are relatively short and the body of the insect is generally small compared to the length of the legs of the insect.

The emergence of larvae or complete larvae also increased gradually with the increase in the amount of water with a constant weight of the plant. This was normal because the amount of chemical compounds is low in the unit area and it is logical that this percentage is close to the percentage of the larval stage and the pupal stage.

b. Statistical analysis

The results were statistically analyzed using a completely randomized design (CRD) factorial experiment using the SAS program, and the averages were compared using Duncan's multiple range test at a probability level of 5% [10].

Table 1. Effect of one seedling weighing 10 g of Origanum vulgare

The amount of water for one seedling weighing 10 grams of	% Adult emergence	Deformities and average numbers of emerging adults	Average duration of the parthenogenetic phase (in days)	Average duration of larval stage (days)

Origanum vulgare				
100 cm	37%	11 f	0.5 c	9.5 ed
200 cm	57%	17 d	0.75 b	9.5 ed
300 cm	73%	22 c	1 a	10.0 d
The Cantole Group	100%	a 30	a 1	a 13

Numbers with different letters differ significantly from each other in each column at the (5%) probability level.

c. The effect of the mint plant *Mentha pulegium* L

From Table (2) it is clear that the mint plant Mentha pulegium L (basil) has an effect on the life of the aquatic stages of mosquitoes in the event of its presence in the environment in which they live. In the event of the presence of (g10) of the complete plant in (cm100) of water and the presence of (30) larvae, this led to the death of 39% of the larval stage and 11% of the pupal stage compared to the control group, which was 0.0%. This may be due to the fact that the mint plant led to the formation of toxic compounds in the environment in which the aquatic stages of mosquitoes live. It is noted that the death rate of the larval stage is 4 times the pupal stage, and it is likely due to the exposure of the four larval ages to the components of the mint plant dissolved in water for a longer period than the pupal stage. This is due to the nature of the life of these stages in aquatic environments and the duration of their life cycle. The effect of Toxicity of aromatic plants on Culex pipiens molestus mosquitoes This effect is directly proportional to the presence of the aromatic extract of the plant in the aquatic medium of mosquito larvae, especially the larvae of the fourth instar. Increasing the volume of water from 100 cm to 200 cm and 300 cm, respectively, while maintaining the same weight of the mint plant resulted in a drop in the death rate of the combined aquatic stages of mosquitoes including larvae and pupae. The reason may be due to the low concentration of toxic compounds in the unit area. This may be similar to the study conducted by [11] where 11 types of aromatic oils extracted from plants were tested, including the mint plant *Mentha pulegium* L., but this did not lead to an effect on the duration of the life cycle for mosquitoes compared to the marjoram plant, but there was a clear congenital deformity in the fourth larval instar, especially skin deformities (Figure 2\3). This is certainly due to the nature of the chemical materials of the mint plant, which led to a change in the skin color of the larva. This effect was not limited to the ages. The larvae have passed to the larvae, affecting the emergence rate of the larvae by a percentage and the percentages of the effect on the larvae ranged between 18% - 5% Table (2) according to the concentration of the materials formed by the mint plant *M. pulegium* L. in the aquatic medium and the appearance of some congenital deformities in it and this was certainly due to the effect of the components of the mint plant M. pulegium L. dissolved in the medium in which these stages lived during their life cycle and this is consistent with the study presented by [12] where they proved that the components present in the mint plant led to the effect on the larval stages and inhibited the adults significantly and also had an effect on the reproduction and growth of the mosquito life in general Table 3.

Table 2. Shows the average lethal effect of the mint plant (basil) at a concentration of (g10) for each replicate with (30) larvae of the immature stages of mosquitoes.

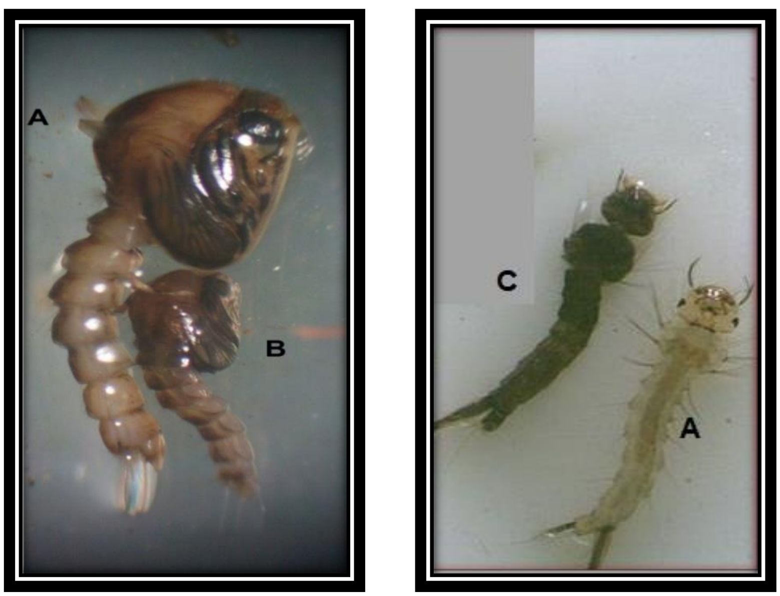
Larva per replicate 30 mosquito stages	malformation of the bulges		pupal stage		Larval stage	
g10 plant concentration per replicate	%	inability to fly	%	death	%	death
Cm100	18%	4.2 a	11%	3.5 a	39%	11.4 a
Cm200	9%	2.1 b	8%	1.8 cd	24%	7.2 de
Cm300	5%	1.2 bc	5%	1.2 d	20%	4.8 e
Control group without plant	0.0%	0.0 e		0.0 e		0.0 f

Table 3. The type of effect of the plants under study in general on the life of mosquitoes in water under laboratory conditions

Plant type	Type of direct and indirect impact					
	Attractive	repellent	killing	Development	congenital malformation	Adult emergence
Origanum vulgare	_	_	_	+	+	+
Mentha pulegium	_	+	+	_	+	+
Control group	+	_	_	_	_	_

Where the sign (+) indicates an effect and the sign (-) indicates no effect.

Figure 3. Deformities of the aquatic stages of Culex pipiens molestus mosquitoes



Where:

1. Dwarfism and deformity of the pupal stage of the mosquito with marjoram plant, where A is the control and B is a pupae in an aquatic medium containing marjoram plant.

2. Skin deformities in the fourth larval stage of the mosquito with mint plant, where A is the control and C is a larva in an aquatic medium containing mint plant. Magnification power (15x).

Figure 4. Deformity of Culex pipiens molestus and its inability to fly.

CONCLUSION

It is very clear from this study that aromatic plants such as Mentha pulegium and Origanum vulgare, which can grow in shallow water, secrete chemical compounds that can affect the life of mosquitoes, leading to a disruption in all stages of mosquitoes, whether larvae, pupa or adults.

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