Evaluation activity of bacteria *Bacillus thurengensis* and different concentrations of plant extracts on adult of house fly *Musca domestica*

Raghad Khalaf Al-Joboory¹* and Elaf Adnan¹

¹Department of Biology, Education College, Iraqia University, Baghdad, Iraq.

*E-mail: raghadaljoboory@yahoo.com

**Abstract.** This research included evaluating the efficacy of plant aqueous extracts for myrtle *Myrtus communis*, Nerium oleander, Eucalyptus spp. and *Bacillus thuringiensis* on adults of the house fly *Musca domestica* L. under laboratory conditions at a temperature of 2 ± 28 °C and with a humidity of 50-70 %. It was found that the highest rate of laying eggs was in the extract of eucalyptus leaves, where the number of eggs laid was 60 eggs on average and at a concentration of 5%, while in the concentration of 10% the average egg was 55 eggs then in the extract of the leaves of the oleander was at a concentration of 10% at a rate of 50 eggs and in control treatments the number of eggs reached 80 eggs and the results showed the highest death rate for adults before laying eggs was in the extract of the eucalyptus leaves at a concentration of 15% where the death of 6 adults and the lowest percentage of death by 5% was recorded One adult death and the highest rate priest for eggs, with a concentration of 10%, reached 94%, and the highest percentage of larval mortality reached 68.12%, with a concentration of 10% in the transactions of extract of oleander leaves, and in it also the highest rate of exclusion is 31.93%. The third larval stage with a concentration of 5% with a ratio of 3.67 days and the highest incidence rate in the third larval stage with a concentration of 2.5% at a rate of 26.70% and the lowest incidence rate of 6.70% with a concentration of 2.5% and 5% and 7.5% in the first and second stages respectively and the lowest dawning rate was recorded in the larval phase the first with a concentration of 2.5% at 16.66% and the highest deformation rate in the third larval stage with a concentration of 7.5% at 66.66%. As for its effect on the number of eggs was a rate of zero% of all concentrations (2.5%, 5% and 7.5%) compared to 80 control egg either its effect on the longevity of the adult was the highest rate of longevity amounting to a concentration of 7.5% by 5.33 day.

**Keywords.** House fly, *Musca domestica*, Myrtle, Oleander, Eucalyptus, *Bacillus thuringiensis*.

1. Introduction

The house fly (*Musca domestica*) is considered the most common among all other types of flies, accounting for 90% of all types of flies in human housing all over the world, as it is one of the most important insects spread widely around the world, especially in warm regions [1]. Where it lives in association with humans and poses a major threat to public health due to its ability to transmit intestinal parasites and other pathogenic microorganisms [2]. Adults can emergence from pupa in as
little as three and a half days at 35º C and five days under normal conditions, while there may be a need for several weeks under adverse conditions [3]. Despite the effectiveness of chemical control methods for flies, they have become resistant to many chemical groups, which prompted researchers to turn to modern control methods, like the use of microbes and plants control [4, 5]. The bacteria *Bacillus thuringiensis* is a widely used biocide against biological microbes in controlling harmful insects [6]. As it is natural, degradable and safe for pest control based on the insecticide activity of *Bacillus thuringiensis*, commercial biopesticides have been developed to control larvae of Diptera, Lepidoptera, and Coleoptera [7]. Several compounds and plant extracts were evaluated for their toxicity and effect and for reducing the density of different species of double winged flies, especially house flies, including petroleum-ether extracts of *Griffonia simplicifolia* and *Zanthoxylum xanthoxyloides* were evaluated as toxic to house flies [8]. The secondary metabolites in plants work with different mechanisms to affect the life of the insect. They may be contraindicated to nutrition, some of which affect the effectiveness of digestion, reduce the metabolism, or have a repellent effect for the feeding stages of the insect, or be toxic to the insect’s tissues, or they may affect the biosynthesis process of chitin thus preventing the phases shedding. The larva and its development, or the nymph, and finally it may affect the productivity of the adult insect, such as preventing egg laying, leading to sterility of adults, or preventing the meeting of the sexes for the same species [9]. The study was conducted with the aim of determining the biological effect between plant extracts and bacteria on house flies *Musca domestica*.

2. Materials and Methods

2.1. Insects breeding

The larvae of house flies were collected from north of Baghdad city were transferred to the insect laboratory. They were divided and placed in 500 gm plastic containers containing larvae feeding and colony maintenance media consisting of 100 gm of floating fish meal consisting of phosphories 1.8%, Ash 10-15%, Crude Fiber 6-8%, Crude fat 8-10%, Crude protein 30 ± 2%) produced by company from Iran and obtained from the local market after being milled and sterilized with an Autoclave at a temperature of 121 degrees 1 centigrade and pressure 1 atmosphere for 25 minutes, 200 ml sterile distilled water and 10 g of dry yeast were added to it, and the center of the feeding was divided on plastic pots, which were transferred to wooden crates with dimensions (30*30*30 cm) made of wood and covered with muslin cloth (with Small pores prevent the exit of the insect.) After the larvae reached the third stage, sawdust were placed on the edges of the pots in order for the larva to dwell and transformed into a pupa, and after 6-7 days of excuses, the virgins began to emerge, and in order to feed the budding adolescents and maintain the group. A clove of a container containing 50 grams of sugar powder and powdered milk (1: 1) (weight: weight) to feed the whole grains and a bottle filled with water and the nozzle of it covered with cotton 12 light: 12 dark hours.

2.2. Preparing plant extracts

Collected plant leaves (myrtle, oleander, eucalyptus) the leaves were washed in running water well several times and left to dry completely in a dark place, at room temperature, taking into account constant stirring to prevent rotting, then these dried leaves were ground using an electric grinder sterilized using sterile alcohol, and after grinding them well, they were placed in sterile cans. The plant extracts were prepared according to the method of [10], where 25 g of each dried plant was weighed and dissolved with 250 ml of distilled water and placed in a Soxhlet extractor for 7 hours at a temperature of 45 ° C and then filtered. Using filter paper, then dry the raw extract produced by a Rotary Evaporator until it becomes in a powder form, and is well preserved until use in the transactions.
2.3. Study of the effect of plant extract concentrations on whole house flies

Prepared 12 wooden crates (30*30*30 cm), in each box, a plate containing 30 grams of whole milk, consisting of (15 grams of milk powder + 15 grams of sugar) was placed and added to it. The three concentrations of each extract (myrtle, oleander, eucalyptus) (5%, 10%, 15%) for each one of them plus the comparison treatment. In another vessel, water was placed for her to drink, where the mouth of the vessel was covered with cotton, a pair of camels, a male and a female, were entered into each cage, and the observation and examination were carried out, and for each repeat a pair of whole house flies (1 male: 1 female) was entered at the age of two days, and the daily monitoring and recording of the effect of the extract on the pair of adults and their survival time and its effect on the laying of eggs and hatched larvae and their duration of survival and the resulting distortions.

2.4. Study of the effect of bacteria on adults house flies

In the first group, three previously described wooden crates (representing 3 replicates) were prepared. In each duplicate, a plate consisting of 30 gm of adult food was placed (15g milk powder + 15g sugar) and 0.075g of the biocide B.t.k. was added to it. (It represents a concentration of 2.5%), the materials were mixed well for a minute with each other and in another container put drinking water and the mouth of the vessel was covered with cotton. The second group included three cages representing three replicates, each one containing a food dish with 0.15 gm (5% concentration) added to a bacterial pesticide in addition to a bowl of water, while the third group included the same previous details with the addition of the bacterial pesticide at a concentration of 7.5% at 0.21 g per dish in addition to cages the comparison, which contained only food and water, included in each repeat a pair of whole house flies (1 male: 1 female) at the age of two days, and daily monitoring and recording of the effect of the biocide on the pair of adult and their survival time and its effect on laying eggs and hatched larvae and their duration of survival and deformities were carried out.

2.5. Statistical analysis

Completely Randomize Design (CRD) was followed in the implementation of the experiments, and the percentages of larval decay were corrected based on the [11] equation which states:

\[
\text{Loss\%} = \frac{\text{Perishing in comparison} - \text{Perishing in the treatment}}{100 - \text{Perditation in comparison}} \times 100
\]

3. Results and Discussion

3.1. Study of some biological aspects of the house fly by adding different concentrations of plant extracts to the diet of adults

It is evident from the results of the table (1) that the highest rate of egg laying was in the eucalyptus leaf extract, where the average number of eggs laid was 60 eggs at a concentration of 5%, where at a concentration of 10%, the rate of eggs laid was 55 eggs, where in the extract of the leaves of the oleander it was at a concentration of 10 An average of 50 eggs, and in the control factors, the number of eggs was 80 eggs, the results of the statistical analysis showed that there are high significant differences between plant extracts and the reason for the low female productivity is that the chemical compounds contained in the plant extracts inhibit the insect's nutrition while it is in the larval stage, which led to the inhibition of egg formation later because this process depends on the active substances that were stored during Larval feeding, whereas the toxic effect of myrtle plant is due to monoterpenes. He confirmed that these compounds affect the female reproductive system of insects and
reduce the number of eggs produced from each ovary [12]. The results of the statistical analysis showed that there were significant differences between the death rates of adult females before laying eggs, as some of the adults did not lay eggs because of their infertility, no eggs were laid, and some adult deaths were recorded before laying eggs in eucalyptus extract at a concentration of 5%, and some adult deaths were recorded before laying eggs in eucalyptus and oleander extract at a concentration of 10%. In the control treatments, the adult mortality rate was 0% in the myrtle and babble treatments, the reason for the superiority of myrtle leaf extract over eucalyptus in reducing the life expectancy of male and female adults is due to the fact that myrtle leaf extract contains compounds that are more toxic than the compounds in eucalyptus leaf extract, which led to the death of adult females in a faster time compared to eucalyptus extract, whose effects were slow and not. Shows clear adult toxicity when food is treated.

Table 1. The biological aspects of the *Musca domestica* house fly by adding different concentrations of plant extracts to the adult death.

| CON. | Number of eggs | Mortality | Longevity of females | Longevity of males |
|------|----------------|-----------|----------------------|--------------------|
|      | 5% | 10% | 15% | control | 5% | 10% | 15% | control | 5% | 10% | 15% | control |
| Myrtle | 0 0 0 80 | 6 6 6 0 | 2.67 ± 4 2.67 ± 3.67 | 3.33 ± 4 3.33 ± 4.33 |
|       | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± |
| Oleander | 0 0 0 577 | 6 6 6 0 | 3.33 ± 3.33 ± 3.67 | 3.33 ± 4 ± 5 4.67 |
|       | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± |
| Eucalyptus | 55 0 80 | 2 2 6 2 | 4 3 4 3.33 | 4.67 3.33 5.33 4 |
|       | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± | ± ± ± ± ± ± ± ± |
| LSD | *0.69 | * | N.S | N.S |

NS means that there are no significant differences, and * means that there are significant differences between some treatments between the probability level (0.05 ≤ p) according to the LSD test.

It is agreed with [13] that the extract of crude terpenes for leaves and fruits of *C.spinosa* had a significant effect on some life performance parameters of the house fly, as the productivity of females treated with leaf and fruit extract decreased from 1147 eggs / female in control factors to 498 and 555. Egg / female, respectively, at a concentration of 20 mg / ml.

3.2. Study the biological aspects of the house fly by adding different concentrations of plant extracts in the death of adults to the laid eggs

The results of Table (2) showed that the highest rate of laying eggs was in eucalyptus extract, where the number of eggs laid was 60 eggs on average with a concentration of 5%, but at a concentration of 10%, the rate of eggs laid was 55 eggs, and then in the extract of leaves of oleander, it was at a concentration of 10% at a rate of 50 eggs compared to the treatments. Control where the number of eggs reached 80 eggs. The reason for the difference in the rate may be due to the different nature of the active substances present in the plants used with food and their effect on the performance of the reproductive system. It has been found that limonene, which is one of the volatile compounds found in plant extracts, which inhibits growth and reproduction and has a toxic effect on the nervous system of insects in several types of insects. a number of researchers have confirmed its presence in the eucalyptus and myrtle plants. The results of the statistical analysis showed that there were significant differences between the rates of egg hatching, where the highest percentage of eggs hatching was recorded in the oleander leaf extract at a concentration of 10%, reaching 94%, followed by the
eucalyptus extract at a concentration of 5%, at 86.67%, compared with the control treatment for eucalyptus 93.75% and the guava 96.25%, respectively. The cause of egg decay may be attributed to the effect of toxic substances in the extracts on the biological systems of the embryo, or to impede the gas exchange inside the egg, which leads to its destruction. The failure to hatch eggs in general may be due to the fact that the secondary compounds present in myrtle and eucalyptus were Toxic to the embryos and led to failure of hatching eggs [14], the results showed that there were high significant differences between the mortality rates of larvae after hatching, as the highest percentage of mortality was 100% in eucalyptus leaf extract, followed by the oleander leaf extract at a concentration of 10%, reaching 68.12% compared to the control factor, where it reached 0%. and there were no significant differences between the exclusion ratios for the larvae, as the highest percentage of exclusion was reached in the extract of oleander, at 31.93%. The results showed significant differences between the rates of eruption failure, as the highest rate of eruption failure was 100% in all concentrations of ace and eucalyptus and in oleander at a concentration of 5% and 15%, and the lowest rate of emergence in oleander extract at a concentration of 10% at a concentration of 33.30% compared to control, where it was 0%. The results of the statistical analysis showed significant differences between the deformation rates of the buds, as the lowest deformation percentage reached 0% in all concentrations of myrtle and eucalyptus, respectively, and the highest deformation percentage in the oleander extract at a concentration of 10% by 30%. Organic extracts produce pupae with deformed shapes, and adults who have successfully exited often suffer from wing reduction.

Table 2. The biological aspects of the house fly by adding different concentrations of plant extracts in the adult death to the laid eggs.

| Extract | Con. | The percentage of hatchability | The percentage of larvae mortality after hatching | Percentage pupa | Emerges failure | Distortion ratio |
|---------|-----|-------------------------------|-----------------------------------------------|-----------------|---------------|----------------|
|         |     |                               |                                               |                 |               |                |
| Myrtle  |     |                               |                                               |                 |               |                |
|         | 5%  | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 10% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 15% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | control | 0±0                       | 96.2±0                                       | 96.2±0          | 0±0           | 0±0            |
|         |     |                               |                                               |                 |               |                |
| Oleander|     |                               |                                               |                 |               |                |
|         | 5%  | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 10% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 15% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | control | 0±0                       | 100±0                                         | 100±0           | 0±0           | 0±0            |
|         |     |                               |                                               |                 |               |                |
| Eucalyptus |     |                               |                                               |                 |               |                |
|         | 5%  | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 10% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | 15% | 0±0                           | 0±0                                           | 0±0             | 0±0           | 0±0            |
|         | control | 0±0                       | 100±0                                         | 100±0           | 0±0           | 0±0            |
|         |     |                               |                                               |                 |               |                |
| LSD     | * 0.95 | *0.89                        | N.S                                           | *               | *             |                |

3.3. Study of the effect of adding concentrations of Bacillus thuringiensis to the whole food of house fly

The results of table (3) proved that no eggs were laid for adult domestic flies in all concentrations compared to the control factor, where the number of eggs was 80 eggs, as well as the results showed that there were no significant differences between the longevity of adult females, where the longest life span for adults was 5.33 days with a concentration of 7.5% and the lowest age for adults was 4.33 A day compared to the control was 5 days.
Table 3. Effect of addition of *Bacillus thuringiensis* bacteria to the death of *Musca domestica* whole flies.

| Bacterial concentration | The number of eggs for the adult | Adult longevity |
|-------------------------|----------------------------------|-----------------|
| 2.5%                    | 0.00 b                          | 4.33 a          |
| %5                      | 0.00 b                          | 4.67 a          |
| %7.5                    | 0.00 b                          | 5.33 a          |
| Control                 | 80.00 a                         | 5.00 a          |
| LSD                     | -                               | N.S             |

Multiple comparisons cannot be calculated as its standard errors are zero

The cause of death of adult adults is that the microscopic crystals that they eat with food turn into toxic protein particles that destroy the stomach walls, so the insect stops feeding within hours after exposure to the bacteria and dies within 2 to 5 days [15]. This is in agreement with [16], who proved that females treated with *B.t.k* bacteria lay the lowest rate of eggs, ranging between 24 - 42 eggs as a maximum, compared to normal treatments that lay eggs at a rate of 33-59 eggs.

References

[1] Deakpe TE, Manyi MM and Utume LN 2018 Pathogenic parasites and bacteria associated with the housefly (*Musca domestica*) in Makurdi; a fly-infested area in central Nigeria *Nigerian J. Parasitol.* 39 111.

[2] Onyenwe E, Okore OO, Ubiaru PC and Abel C 2016 Housefly-borne helminth parasites of Mouau and its public health implication for the university community *Anim. Res. Int.* 13 2352.

[3] Tian Y 2017 *Toxicity and repellency of essential oils to the house fly (Musca domestica)* Thesis Auburn University 1.

[4] Khan HA A, Shad SA and Akram W 2013 Resistance to new chemical insecticides in the house fly, *Musca domestica* L., from dairies in Punjab, Pakistan *Parasitol. Res.* 112 2049.

[5] Gronvold J, Henriksen SA, Larsen M, Nansen P and Wolstrup J 1996 Biological control aspects of biological control with special reference to arthropods, protozoans and helminths of domesticated animals *Vet. Parasitol.* 64 47.

[6] Brar SK, Verma M, Tyagi RD and Valé ro JR 2006 Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides *Process Biochem.* 41 323.

[7] Glare TR and O’Callaghan M 2000 *Bacillus thuringiensis* biology, ecology and safety Wiley, Chichester; New York, No;632.

[8] Ogbalu OK, Umeozor OC and Ebere N 2005 Oviposition deterrent effect of extracts of neem against *Atherigona orientalis* (Schiner) on pepper (*Capsicum annum*) and tomato (*Lycopersicon esculentum*) fruits in Nigeria *Indian J. Agric. Res.* 39 18.

[9] Kelany IM 2001 *Plant extracts and utilization of their products for safe agricultural protection and for reducing environmental pollution. proceeding of the workshop on practice oriented result on use of plant extract and pheromones in integrated biological pest control Plant protection* Dept., Faculty of Agriculture Zagazig University Egypt,10.

[10] Rios JL, Recio MC and Villar A 1987 Antimicrobial activity of selected plants employed in the Spanish Mediterranean area *J. Ethnopharmacol.* 21 139.

[11] Schneider-Orelli O 1947 *Entomologisches praktikum verlag sauerlander* Aarau: 237.

[12] Zayzafoon G, Odeh A and Allaf WA 2011 The use of photo chemiluminescenc essay for the measurement of integral antioxidant capacity in Syrian *Myrtus communis* L. Leaves *Herba Polonica* In press.
[13] Al-Zubaidi FS, Al-Rubaie HMA and Al-Okaily L 2005 Terpenoids crude extract of Caparis spinosa affecting some biological aspects of house fly, Musca domestica L.(Diptera: Muscidae) J. Al-Nahrain Univ. 8 28.

[14] Don-Pedro KN 1989 Mode of action of fixed oils against eggs of Callosobruchus maculatus (F.) Pestic. Sci. 26 107.

[15] Sante Canada 2013 Fiche technique sur le Bti–Bacillus thuringiensis variété israelensis Canada, 6 http://www.hc-sc.gc.ca/cpsspc/pubs/pest/_factfiche/bti/index-fra.php.

[16] Saliha B, Wafa H and Laid OM 2017 Effect of Bacillus thuringiensis var krustaki on the mortality and development of Culex pipiens (Diptera; Culicidae) Int. J. Mosquito Res. 4 20.