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Effect of Alternaria alternata Fungus Filterates on Some Biological Aspects of House Flies, Musca domestica

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### **ABSTRACT**

The goal of this study, which was carried out in 2022 in the Advanced Entomology Laboratory of the Department of Biology, Girls College of Education, University of Kufa, was to determine how well the fungus filtrates of Alternaria alternata affected the mortality percentages of house fly larvae and pupae, Musca domistica. The results indicated an increase in the mortality percentages of larvae in the first and third instar of the house fly, Musca domistica with increasing concentrations used. In the study, the highest mortality percentages were recorded after three days of treatment with a concentration of 10% (100 and 85%) in the first and third instar larvae, respectively. The effect of the fungus filtrates on some biological aspects when treating the third instar larvae of houseflies, as it led to a decrease in pupation percentages to 15% when treating pupae with a concentration of 10%, and the pupal deformation percentages to 10% and a decrease in adult emergency percentages to 0%. when treating pupae with the fungus filtrates of Alternaria alternata at a concentration of 10 %, which increased the pupal deformation to 80% and decreased adult emergency percentages to 20%.

## **INTRODUCTION**

House flies are found in large numbers near animal breeding fields, which has led to loss and weakness in animal production, in addition to entering homes and other public places, causing a nuisance (Abobakr *et al.*,2022). Houseflies also transmit the cysts of primary parasites such as *Entaemoeba histolytica*, *Entaemoeba coli*, and *Giardia lambilia*. They also transmit the eggs of the tapeworm Taenia and the pinworm, *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). Fungi are also considered highly efficient pathogens in biological control, which infect many insect pests. These pathogens are characterized by causing fatal infections and can regulate the natural community of insects, as there are about 1,800 cases of association between fungi and different types of insects. Fungi also differ in the way they infect insects from other pathogens such as viruses, as they can penetrate the insect's body through thin areas such as the respiratory openings, sides, and the area between the abdominal rings (Jankevica, 2004). The Alternaria alternata fungus is considered one of the most common and widespread species among the species of the genus Alternaria. The

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fungus colony is flat, velvety, and olive green to black in color. Conidial spores are dark in color and pear-shaped, conical, straight, slightly curved, or rectangular in shape. Conidial spores are pale, semi-golden, or olive-brown in color, and smooth, and their length ranges between 150-300 cm. Micron (Ateeq, 2007). The A. alternate fungus secretes many types of well-known toxins, including Alternariol (AOH), ATX II, Alteratoxin II, and Alternariol monomethyl ether (AME), which were diagnosed using high-performance liquid chromatography (HPLC) and GC-MS (Al-Fakhr Al-din, 2017).

### **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. 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).

### **Cultivation Media Used in Growing Fungi:**

## **Medium Potato Dextrose Agar (P.D.A):**

This medium was used to grow the fungi Alternaria alternate. I dissolved 39 grams of the prepared medium into a liter of distilled water in a glass beaker and added the antibiotic chloramphenicol at an amount of 250 mg/liter. I closed the mouth of the beaker with a cotton tampon and wrapped it with a strip of aluminum foil. I sterilized it with a device. Annealed at a temperature of 121°C and a pressure of 15 lb/in2 for 20 minutes. After the end of the sterilization period, the flasks containing the medium were left to cool to a temperature of 50°C, then the nutrient medium was poured into Petri dishes with a diameter of 9 cm, and placed in the refrigerator until use. The dishes were inoculated with the above fungi using a cork drill with a diameter of 1 cm, and the dishes were incubated in an incubator at its temperature. 25±2 for 7 days.

## **Preparing Potato Dextrose Broth (P.D.B):**

Prepare the medium by dissolving 24 grams of the prepared medium into a liter of distilled water in a glass beaker and adding the antibiotic chloramphenicol to it in an amount of 250 mg/liter. Then, it was distributed in glass beakers with a capacity of 250 ml. The mouth of the beaker was closed with a cotton tampon and wrapped with a strip of aluminum foil. Sterilized with an autoclave. A temperature of 121 °C and a pressure of 15 lb/in2 for 20 minutes. After the end of the sterilization period, I left the flasks containing

the medium to cool to a temperature of 50 °C.

## Fungu Filtrate of Alternaria alternata:

The filtrate of this fungus was prepared by inoculating glass bottles containing P.D.B medium with discs with a diameter of 0.5 cm for each bottle from a 7-day-old fungus culture grown on P.D.A medium. The inoculated bottles were incubated in a shaking incubator for 28 days at a temperature of  $(25\pm2)$  °C. The extract was filtered using filter paper, and then passed the filtrate through the Milipore microfilter, which has a hole diameter of 0.45 mm, to obtain the raw filtrate from which the following concentrations (2, 4, 6, 8, 10) % were prepared for use in laboratory experiments.

## larvae Treated with Alternaria alternata Fungus Filtrates:

## The Contact Effect of Fungus Filtrates of *Alternaria alternata* 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 fungus filtrates of *Alternaria alternata*, with concentrations of (2, 4, 6, 8, 10) % 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 filtrates and the date of the experiment were recorded for each replicate, and the dishes were placed in the incubator at a temperature of  $2 \pm 30$  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 E Fungus Filtrates of Alternaria alternata 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 by hand spraying of fungus filtrates of *Alternaria alternata* 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 fungus filtrates of Alternaria alternata, 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 *Alternaria alternata* Fungus Filtrates 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 at 90% on the first day at a concentration of 10%, and the larval mortality

percentage (100) % on the third day at the two concentrations (6, 8, and 10) %, 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 10% after the first day of treatment, as well as the concentration of 6 and 8 % after three days of treatment, as the chi-square arithmetic value of the mortality of larvae was (5.9, 6.2, 8.7) on the first, second, and third days, respectively, and it was greater than the tabulated chi-square value of 1.15. Aljumaily, (2017) showed that the mortality percentages of the first instar larvae of Culex quinquefaciatus mosquitoes reached 34% and 55.33%, respectively, 120 hours after treating them with the Bipolaris australiensis fungus at concentrations of 1 x 10<sup>4</sup> spores/ml and 1 x 10<sup>6</sup> spores/ml, respectively. He also attributed the reason for the increased larval mortality to an increased concentration, increasing the number of spores that attack the insect's body, thus weakening the immune system. The results of the current study agree with the results of Burhan and Annon, (2020), who treated the first instar larvae of the house fly M. domestica with different concentrations (25, 50, 75, 100)% of the fungal filtrate Paecilomyces lilacinus, as the lowest mortality percentage was recorded at 20% at the 25% concentration, while The highest mortality percentages 96.33% was recorded at 100% concentration compared to the 0% control. White et al., (2021) studied the effect of Beauveria bassiana against the first instar larvae of house flies, where the highest larval mortality percentages were recorded at the high concentration of 4 x 10<sup>11</sup> spores/ml. Farooq et al., (2020) studied the aim. to know the effect of three genera of fungi Verticillium lecanii, Beauveria bassiana, and Metarhizium anisopliae against the second instar larvae of the pink cotton bollworm, Pectinophora gossypiella, where the highest larval mortality percentage was recorded at 44.6%, 50.6%, and 53.6% at a concentration of 1 x 108 spores/ml after 12 days of treatment with the fungus V. lecanii., B. bassiana, M. anisopliae, respectively.

**Table 1:** The effect of the interaction of different concentrations of Alternaria alternata fungus filtrates on the mortality percentages of first larval instar.

| Conc<br>%                   | Mortality<br>percentages after<br>One day | Mortality<br>percentages after<br>two days | Mortality<br>percentages after<br>three days |
|-----------------------------|---|--|--|
| Control                     | 0   | 0  | 0  |
| 2                           | 50  | 70   | 90   |
| 4                           | 60  | 75   | 95   |
| 6                           | 70  | 80   | 100  |
| 8                           | 80  | 90   | 100  |
| 10                          | *90                                       | *95  | 100*   |
| chi-square arithmetic       | 5.9                                       | 6.2  | 8.7  |
| tabular chi-square P < 0.05 | 1.15                                      | 1.15                                       | 1.15   |

## Effect of *Alternaria alternata* Fungus Filtrates 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 fungus filtrates *A. alternata* and mortality percentages of the third instar larvae treated with each of them, as the highest mortality percentage was recorded 45% on the first day at a concentration of 10%, and the larval mortality percentage (75, 85) % on the third day at the two concentrations (8 and 10)%, respectively while the lowest percentage of death (16, 26, 40)% was recorded at the concentration of 2% on the first, second and third days, respectively. From the results of Table (2), it was found that there

were significant differences in the mortality percentages, and they were concentrated in the concentration of 10% after the first day of treatment, as well as the concentration of 6 and 8 % after three days of treatment, as the chi-square arithmetic value of larval mortality was (4.6, 5.8, 7.4) on the first, second, and third days, respectively, and it was greater than the tabulated chi-square value of 1.15. The results of the study by Aljumaily, (2017) showed the effect of the Bipolaris australiensis fungus on the third instar larvae of the Culex quinquefaciatus insect, as it reached 33% and 45.33% at concentrations of 1 x 10<sup>4</sup> spores/ml and 1 x 10<sup>6</sup> spores/ml, respectively, compared to 0% in the control treatment.... The results of a study by El-Husseini, (2019) showed the effect of the spore suspension of the fungus Metarhizium anisopliae on the third instar larvae of the cotton leafworm S. littoralis, as it reached 44% and 90% at a concentration of 1 x10<sup>7</sup> spores/ml after 4 and 10 days, respectively, compared to 0% in Control treatment. The results of the current study agree with the results of Burhan and Annon, (2020), who treated the third instar larvae of the house fly M. domestica with different concentrations of the fungal filtrate Paecilomyces lilacinus, as the mortality percentages ranged between (13.33% and 53.33%) at the 25% concentration after (10 and 28) days respectively, while the highest mortality percentages of 46.66% and 96.33% was recorded at the concentration of 100% after 10 and 28 days, respectively, compared to the 0% control. The reason for the increase in the mortality percentages of larvae with increasing concentration was attributed to the increase in mycotoxins that accumulate in the cells of the insect's body. Which leads to their explosion and the death of the larvae. The results of a study by Deves et al., (2022) showed the effect of the fungus B. bassiana in the third instar larvae of the insect Chrysomya megacephala, reaching 14% and 36% at concentrations of 1 and 4%, respectively, compared to 0% in the control treatment. The histological results also showed the presence of conidia pigmentation in the intestines, and the fungus B. bassiana caused histological changes in the epithelial cells. The middle intestine interferes with the absorption of nutrients and the balance of the digestive system, causing the death of the larvae. The results of the study by Lana et al., (2023) showed the effect of different concentrations of spore suspension of the fungus B. bassiana in the third instar larvae of the cotton leafworm, S. littoralis, through contact and digestive effects, as the mortality percentages ranged between (43% and 50%) at the concentration of 10<sup>10</sup> spores/ml in the contact effect, while the mortality percentages ranged between (77% and 85%) at the concentration of  $10^7$ spores/ml in digestive effect.

**Table 2:** The effect of the interaction of different concentrations of *Alternaria alternata* fungus filtrates on the mortality percentages of the third larval instar.

| Conc<br>%                   | Mortality percentages after | Mortality percentages after | Mortality percentages after |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | One day                     | two days                    | three days                  |
| Control                     | 0                           | 0                           | 0                           |
| 2                           | 16                          | 26                          | 40                          |
| 4                           | 23                          | 30                          | 45                          |
| 6                           | 32                          | 44                          | 60                          |
| 8                           | 39                          | 55                          | 75                          |
| 10                          | *45                         | *75                         | 85*                         |
| chi-square arithmetic       | 4.6                         | 5.8                         | 7.4                         |
| tabular chi-square P < 0.05 | 1.15                        | 1.15                        | 1.15                        |

The Effect of *Alternaria alternata* Fungus Filtrates 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 A. alternata fungus filtrates in the third instar larvae of house flies, and the highest mortality percentage was recorded at 85% at a concentration of 10 %. 15% of pupae developed from larvae treated with A. alternata fungus filtrate at a concentration of 10%, while the highest mortality percentage of immobility was 60% at a concentration of 2%, and deformities occurred in the treated pupae, which are third larval instar with fungus filtrates of A. alternata, and the highest deformation percentages were 25% at The concentration is (2,4,6) %. As for the adult emergency percentages, no healthy adult emerged from pupae treated as larvae with A. alternata fungus filtrates at a concentration of 10%. 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 10% compared to other concentrations, and the arithmetic chi-square value was 4.7, while the tabular chi-square value was 1.15, and significant differences appeared in the pupation percentages, especially at a concentration of 2% compared to other concentrations, where the arithmetic chi-square value was 8.8, 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 2% compared to other concentrations, and the arithmetic chi-square value was 3.3 while the tabular chi-square value was 1.15, statistically significant differences appeared in the adult emergency percentages, as clear significant differences were recorded at the concentration of 2% compared to other concentrations. The arithmetic chi-square value was 8.9 and the tabular chi-square value was 1.15. Abdel-Raheem and Eldafrawy, (2016) indicated the effect of the fungus Paecilomyces fumosoroseus against the second instar larvae of the house fly M. domestica, where the highest larval mortality percentage was recorded, 75%, at a concentration of 4%, after 72 hours of treatment. The pupation percentages also decreased, as the lowest rate of adult emergence percentages was recorded at 38.5. % concentration 4% compared to control 90%. As White et al., (2021) pointed out, they studied the effect of B. bassiana against third-instar larvae of house flies, where the highest larval mortality percentage was recorded at 29.9% at the concentration (4 x  $10^{11}$ ) spores/ml. The study by Omar et al., (2021) also aimed to know the effect of the M. anisopliae fungus against the fourth instar larvae of the pink cotton bollworm P. gossypiella, as the highest larval mortality percentage was 57.5% at the concentration ( $10^{12}$ spores/ml), while the lowest pupation percentages and adults emergence percentages were 42.5% and 20%, respectively, at the concentration ( $10^{12}$  spores/ml).

| Coce<br>%                       | Motality percentages% | Pupation percentages% | Puopation deformation% | Adult emergency% |
|---------------------------------|-----------------------|-----------------------|------------------------|------------------|
| Control                         | 0                     | 100                   | 0                      | 100              |
| 2                               | 40                    | 60*                   | 25                     | 35*              |
| 4                               | 45                    | 55                    | 25                     | 30               |
| 6                               | 60                    | 40                    | 25                     | 15               |
| 8                               | 75                    | 25                    | 15                     | 10               |
| 10                              | 85*                   | 15                    | 15*                    | *0               |
| Arithmetic chi-square           | 4.7                   | 8.8                   | 3.3                    | 8.9              |
| Tabular chi-<br>square P < 0.05 | 1.15                  | 1.15                  | 1.15                   | 1.15             |

**Table 3:** The effect of the different concentrations of *Alternaria alternata* fungus filtrates on some biological aspects of third larval instar.

### Effect of Alternaria alternata Fungus Filtrates 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 filtrates A. alternata fungus filtrates. The deformation percentages increased with the increase in the concentration used, and the highest deformation rate of 80% was recorded when the pupae were treated with a concentration of 10 % of the A. alternata fungus filtrates. In contrast, the deformation rates decreased. Adults emerged from pupae treated with the A. alternata fungus filtrates. The lowest rate of emergence of adults was 20% when pupae were treated with a concentration of 10%. Statistical analysis using Chi-square showed that there were statistically significant differences in the deformation percentages of pupae treated with the A. alternata fungus filtrates of 8%, 10%, and the value was the arithmetic chi-square was 4.7, while the tabular chi-square value was 1.15. and there were clear significant differences in the adult emergency, especially at a concentration of 2%, compared with the low emergence percentages of adults at the rest of the concentrations, and the value was The arithmetic chi-square was 7.9 while the tabular chi-square value was 1.15. The results of the current study agreed with the study of Gharib and Abdel Ali, (2016) who studied the effect of B. bassiana, against the pupae of the house fly M. domestica, at different concentrations, as the percentage of pupal mortality percentages ranged between (40%, 47.5%, and 70%) at concentrations of  $2 \times 10^6$ ,  $2 \times 10^8$ , and  $2 \times 10^{11}$  spores/ml, respectively, after 96 hours of treatment, Compared with the 0% at control. The results of the current study agreed with the findings of Aljumaily, (2017), as treating mosquito pupae of the Culex quinquefaciatus insect with the B. australiensis fungus at different concentrations led to an increase in the death rate of mosquito pupae with increasing time and concentration, as the highest death rate of 49% was recorded at the concentration of 1 x  $10^6$ spores/ml after 72 hours. of the transaction. The results of the current study agreed with Omar et al., (2021), who studied the effect of the fungus *M. anisopliae* against pupae of the pink cotton bollworm P.gossypiella at different concentrations, as the pupal mortality percentages ranged between (17.5% and 72.5%) at concentrations of  $10^8$  and  $10^{12}$ spores/ml, respectively. The percentages of adult emergency percentages ranged between (82.5% and 27.5%), respectively, at the concentration ( $10^8$  and  $10^{12}$ ) spores/ml, respectively. Nasir and Rashid, (2023) also conducted a study on the effect of Penicillium commune fungus filtrate on nymphs and adults of the Bemisia tabaci insect, The results of the study showed that the fungus filtrate has an effect on nymphs and adults, as nymphal

mortality percentages were recorded at (46.85 and 89.8)% at concentrations of 25% and 75%, respectively, after 72 hours, while it reached The adults' mortality percentages of is 41.8% and 78.5% at concentrations of 25% and 75%, respectively, after 72 hours of treatment. The reason for the increase in mortality with increasing concentration and time period was attributed to the efficiency of mycotoxins that affect vital activities that disrupt the functioning of some tissues and thus affect the growth and development of the insect.

**Table 4:** The effect of the interaction of different concentrations of *Alternaria alternata* fungus filtrates on the rate of mortality rates of pupal house flies.

| Conc %                | Pupation deformation% | Adult emergency% |
|-----------------------|-----------------------|------------------|
| Control               | 0                     | 100              |
| 2                     | 25                    | 75               |
| 4                     | 30                    | 70               |
| 6                     | 45                    | 55               |
| 8                     | 65                    | 35               |
| 10                    | 80*                   | 20*              |
| Arithmetic chi-square | 4.7                   | 7.9              |
| Tabular chi-square    | 1.15                  | 1.15             |
| P < 0.05              |                       |                  |

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