

## **Study the effect of different dilutions from filtrates of two fungi *Alternaria alternata* and *Aspergillus flavus* on the ground Beetles adults (carabidae: Coleoptera) *Harpalus rufipes***

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The present study was conducted to test the efficiency and effectiveness of different dilutions of filtrate innate aftran *A. flavus*, *Alternaria alternata* (25%, 50%, 75%, 100%) on the adult insect ground beetle, results showed no significant effect dilutions filtrate innate Aftran *A. flavus*, *Alternaria alternata* in the decimation adults ground beetle *Harpalus rufipes* and 100% concentration may outweigh the rest of the other dilutions to give a higher proportion of the loss amounted to (80%, 100%) at 24.48 hours of treatment for a leaky mildew fungus *A. flavus* and (70% 0.90%) at 24 hours, 48 hours of treatment for a leaky mushroom fungus *Alternaria alternata* As for dilutions other has given dilution of 25%, 50%, 75% the proportion of the loss amounted to ( 0.0 % , 10 % , 20%) and , respectively, after 24 hours of treatment, the proportion of the loss (10% , 20% , 50%) and respectively after 48 hours of labor for a leaky mildew *A. flavus* As for the other dilutions leaky mildew fungus *Alternaria alternata* ( 25 % , 50 % , 75% ) was given to the proportion of the loss ( 0.0 % , 10 % , 30 % ) after 24 hours of treatment , respectively (10% .20 % .60 % ) after 48 hours of treatment , respectively.

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**Keywords:** Fungi; Concentration; Analysis.

### **1. INTRODUCTION**

Ground beetles Carabidae are the most important insects of economic interest. They are belonged to a large family of coleoptera insects and they are one of the largest animal kingdom their known types of coleoptera estimated (350,000) types and about of these known insects are beetles vary significantly and ecologically and vary in size from one to a very large size of

3 cm [1]. The ground beetles are harmful agricultural pests and the blight is attributed to the larvae and imagophases [2]. Many of the beetles prey to other insects (adeophage) and there are other harmful species to the plants as the piercers of buds, fruit stems and leaves. They feed on dry materials, stocks as flour, cereals and clothes. Some of them are considered carnivorous [3].

The type of this family are common and many, including the genus *Harpalus rufipes* that can be seen on the ground running quickly or hidden beneath the rock, all these beetles are predatory, eating all animal products such as leather, wool silk, feathers, hair, dried meat, dead insects and dried plant materials. In addition to being a wool carpet biting, it is called carpet beetle [4]. The *Harpalus rufipes* has a nocturnal activity and its larvae are also found on the ground where they are moving effectively and are severe predatory. The imago is characterized by a nematoform and consisted of (11) articles and the wrist is form (5) articles. This insect passed the winter in the soil cavities or under the rocks or between the plant wastes or any hidden holes and went out in spring and put its egg in the soil [5;6].

The eggs hatched out the active and predatory small larvae and after a number of moultings the larvae is ripen in one or two weeks or three weeks and entered the soil to be a virgin taking a cycle of eggs to the full imago in five or six weeks, but the imago remained in the soil and sometimes lived for some years and laid eggs every year. This family is considered from the useful insects and it plays a great role in the biological control in fields and farms where it preys many agricultural pests [7;8]. The study objective: Due to the economic damage caused by this insect *Harpalus rufipes*. The abstracts of *Alternaria alternata* and *Aspergillus flavus* are used as a means of biological fighting to decrease the damage caused by this insect. As these insects contain a higher ratio of toxins called Aflatoxins AB having a higher capacity of killing the insect and breaking down its bodies [9].

## 2. METHOD OF ANALYSIS

It is following the next section.

## 3. EXPERIMENTAL PROCEDURE

### 3.1. Collecting of an insect Method

The 500 ground beetles carabidae were gathered from genus *Harpalus rufipes* in July. This process was continuous to one week from different sites in Samarra where they took from various holes in the soil, houses and some farms by hands and put them in to the sterilized plastic cans [1].

### 3.2. The cultura Media used for growing fungi

Prepare the broth by melting (39) gram of PDA mixture in a distilled water bath in a conical flask of (2) litres and put it in water bath and add to it (250)mg of chloramphenicol. Put the medium in an autoclave in 121°C and pressure of 15 pound /inch for 20 minutes. This is used for isolating and purifying the fungi to use in the next experiments [10].

### 3.3. potato dextrose broth (PDB)

Prepare the medium from cooking (200)gram potato of potato cutting into small pieces with (500)ml of distilled water for (2) minutes in a beaker. The cooked potato filtered with a piece of sterile gauze and add a 20 grams of dextrose and complete the volume to a litre with distilled water and implant the supernatant in glass flasks of (250)ml and a rate of (150)ml per flask. The media were sterilized by an autoclave at 121°C, and pressure of 15 pound /inch for 20

minutes. Use the medium to prepare the fungal supernatant of the fungus *Aspergillus flavus* and *Alternaria alternata* [10].

### 3.4. potato dextrose broth (PDB)

Prepare the PDB and put it in a conical flask of 250ml with amount of 15ml /flask and then add chloramphenicol of 25 mg /ml and incubate each flask three tablets each diameter of 5mm with a cork borer from the edge of the fungal colonies purified on the middle of PDA and extract after 7 days for *A. flavus* and *A. alternata*. The flasks are incubated at  $\pm 25^{\circ}\text{C}$  considering shaking the flasks each (3-4) days to distribute the fungal growth and after (28) days; the vaccine was filtered by using filtration papers and by the air discharge. The refiltration was done by the accurate filterer. The concentrations (25%, 50%, 75%, 100%) are prepared from the fungal supernatant *A. flavus* and *A. alternata* and the concentrations of the supernatant are used in the following experiments [11].

### 3.5. Valuation of the toxin efficacy of different dilutions taken from the fungal supernatant for *A. flavus* and *A. alternata* isolated in labo on growing and developing the ground beetles adults genus *Harpalus rufipes*

1- The effect of the fungal supernatant for *A. flavus* and *A. alternata* on the ground beetles adults genus *Harpalus rufipes* under ( $\pm 27^{\circ}\text{C}$ ) and relative humidity 5%. The 25 insects are taken for each concentration of the fungal supernatant for two fungi (25%, 50%, 75%, 100%) at a rate 5ml by using a half liter plastic sprayer. The insects that were put in sterile plastic cans were sprayed at a distance (5cm). The number of dead adults is calculated in each concentration and for each fungal supernatant respectively.

### 3.6. The statistical Analysis

Data are analyzed statistically by (Anova) analysis on way variation Test and by applying the statistical program (Minitab) and the arithmetic averages of the coefficients were compared with the use of Duncan's multiple range test at probability level 0.05 [12].

## 4. RESULTS AND DISCUSSION

The results in table (1) showed the ratio of killing is directly proportional to the concentration, the higher the concentration, the higher the killing rate. The rate of killing concentration (25%) is zero within 24 hours of treatment, while the killing rate was 90% at 100% concentration within 24 hours of treatment, but in 48 hours of treatments, the killing rate was 100% at 100 concentration in comparison with the control sample that did not have a killing rate within 24 and 48 hours of treatment. Thus, through these results, it is clear that there is a significant effect on the fungal supernatant dilutions of *A. flavus* (25%, 50%, 75%, 100%) in the ground beetles adults genus *Harpalus rufipes*. The concentration (100%) could be higher than the rest, which is different in the effect on the death of the ground beetles adults, giving the highest ratio of death at 95%. It is significantly different from the rates of death in the other dilutions, which differ from each other in impact. As for the effect of periods in death ratios after the treatments. The highest death rate in the ground beetles adults was after 48 hours of treatments 52.5% and it is significantly different from the proportions of the loss after 24 hours of treatments, which amounted to 35%. The interference between the dilutions and periods showed that the highest rate of death occurred after 48 hours after treatments at 100% concentration, the lowest rate of death occurred after 48 hours of treatments at the 25% dilution, which reached 10%. The mechanism of lethal effect of the fungal supernatant for *A. flavus* on the ground beetles adults was due to several causes. One of the most important causes is the effect of this toxin on the gas exchange between the insect and the environment.

Thus lead to the death of insect or some of the toxins are combined in this fungus with cytoplasm of the insect body wall and thus lead to poisoning it and then its death. The results of our study conform to the results of many researchers such as [13], which explained the toxic efficacy of the fungal supernatant for *A. flavus* in killing and analyzing the various tissues of the body and stopping the work of all the cells and tissues of different life phases of the *Culex pipiens* of. The study Ali [12], which explained the efficacy of *Beauveria* for the most important effects observed during treating the ground beetles adults, genus *Harpalus rufipes* with the fungal supernatant of *A. flavus* [14]. It has resulted in very high killing and deformation ratios due to the overlapping of toxic compounds of the fungal supernatant with vital system of the gastro intestinal cells with some of the main components within the gastro intestinal tract such as lipid resulting in the decompositions of the digestive tract tissue as well as fracturing, twisting and blocking the bronchi and obstructing respiratory openings as a result of gathering toxic substances (Systematic toxins) because they damaged and analyzed all parts of body organ and that is what has been proved in our study [15].

**Table 2** The effect of various concentration of the fungal supernatant for *A. flavus* on the ground beetles adults *Harpalus rufipes* at  $\pm 38^\circ\text{C}$

Concentration/time	24hours	48 hours	Average of concentration effect	Average of supernatant TYPE effect
0	0	0	0	
25%	0.0g	10f	5c	38.8a
50%	10f	20e	15c	
75%	30d	60g	45b	
100%	80b	100a	90a	
Average of time effect	a(30.0)B	b(47.5)A		

The similar small letters in on column mean that no significant differences between them. It is observed from (3) there is a significant effect of the various dilutions for the fungal supernatant *A.alternata* on the ground beetles adults *H.rufipes*. The concentration 100% is the highest in giving the highest ratio of death amounted to 90%. It is significantly different from the ratios in other dilutions that are differed significantly from each other in tracing also AS for the effect of periods in death the ratios after the treatments the highest death rate was found in the ground beetles *H.rufipes* after 48 hours of treatment, which amounted to 47.5% and is significantly different from the rates of loss after 24 hours different of treatment either from the overlap between the dilutions and the period. It is obvious that the highest death rate was after 48 hours of treatment at 100% concentration, which reached 100%. The lowest death rate was 10% after 48 hours of treatment at 25% concentration. The mechanism of lethal effect of the different concentrations of the fungal supernatant *A.alternata* on the ground beetles adults *H.rufipes*. This is due to many causes; and the fact the toxins resulting from this fungus combined with cytoplasm of the insect body wall cells. thus lead to its poisoning as well as that some fungi including *A.alternata* have the ability to produce protease, lipase and chitinase enzymes which play a significant role in the destructions of the body wall [16].

It is also due to the entry of large amounts of toxins produced by fungi with nutrition, which causes them enter the digestive tract of insect leading to its poisoning [17;18]. This results in closure the respiratory openings and then it is death. From these findings, it is clear

that the rate of loss is directly proportional to the concentration of the fungal supernatant, and the higher the ratio of killing which is consistent with the study carried out by [19]. At treatment with house flies adults with different weights of fungi *A. alternata* because the death rates are increasing with weight of fungus. They amounted to 100% at weight 5g and 40% at weight 1g. This is consistent with the results of our study and ours with [11], and explained that the increase of spraying the nymphs and adults of white fly with supernatant of *Aspergillus*, which disrupted the activity of the tissues and thus affects their life performance leading to their death, as observed when treating the *H. rufipes* with various concentrations of the fungal supernatant *A. alternata* that led to blacken or patches on the wall of the insect's body. This is called the blackening. Also, the beetles that treated with the fungal supernatant *A. alternata*, they have been weakened or paralysed completely by the toxin transformed by fungus (Aflatoxins, one of the most dangerous toxins with high susceptibility in destructing tissues and cells of the organism's body). Their most important toxins are B1G1 [20], and these symbols have to do with the colour of any two compounds under the ultraviolet rays. [B] is for blue and [G] is for green. The results of our study referred to it [20;21], that the most important evidence adopted in the diagnosis of the fungal infections in insects is the change of the body's colour and instability to lay eggs, this is consistent with the results of our study [22].

**Table 3** The effect of different concentrations of the fungal supernatant *Alternaria alternata* on the ground beetles *H. rufipes* at  $38 \pm 2$  °C.

Concentration/time	24hours	48 hours	Average of concentration effect	Average of supernatant TYPE effect
0	0	0	0	
25%	0.0g	10f	5c	33.8b
50%	10f	20e	15c	
75%	20d	50c	35a	
100%	70b	90a	80a	
Average of time effect	B(25.0)B	B(42.5)A		

## 5. CONCLUSIONS

In experimental study, 1-The fungal supernatants are systematic toxin fungi on insects. 2- The fungal supernatants of *A.flavus* and *A. alternata* are effective in killing and analyzing various tissues and organs of *H. rufipes*.

4-It is noticeable that the different effect of dilutions of the used fungal supernatants is contrasted. They only concentration 100% is effective and affected more than others.

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