Effect of different concentrations of extract of Urtica dioica and Cladosporium cladosporiodes on Tribolium castaneum or: Coleoptera after 24-48 hours of exposure in Samarra City/Iraq

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Abstract
A current study is conducted to test the efficacy of the effect of different concentrations of the filtrate of the fungus Cladosporium. cladosporiodes and the alcoholic extract of the Nettle plant (Urtica dioica) (25%, 50%, 75%, 100%) on adults of the red rusty flour beetle (Tribolium castaneum) and the study revealed the following: The superiority of U. dioica extract, The it is in the killing rate, and this is due to the cumulative effect of the toxic substances in the extract at 100% concentrations. The lowest percentage killed after 24 hours of exposure is (1.33) at a concentration of 25% in 24 hours and the lowest killing rate of the filtrate of the fungus C. cladosporiodes is (6.67) in 24 hours. The use of the fungus C.cladosporium in terms of the killing percentage, is slightly lower, around (8.67), at 100% concentrations in 48 hours, while the lowest percentage killed after 24 hours of exposure the filtrate of the fungus C. cladosporiodes is (7.67) at a concentration of 25% in 24 hours. The C. cladosporiodes infiltrate achieved the highest death rate for the whole, which is 8.67 insects at a concentration of 100%, while when using the alcoholic Nettle plant extract, the death rate is 8 insects at a concentration of 100%, and when using the aqueous plant extract, the death rate is 4.67 insects at 100% concentration.
Keywords: The red rusty flour beetle (Tribolium castaneum) Nettle plant (Urtica dioica)

1. Introduction

Pests that contribute to humans in their food are influential, especially in third world countries, which threatens to cause a serious food crisis if the loss in agricultural crop production reaches more than 50% as a result of the increase in insect and plant diseases, as well as the increase in the number of the population and what this rise demands of foods that are only available by addressing problems Factors affecting the increase in nutritional value, foremost of which is the increase in the surface area of agricultural land and to improve the quality of plant protection and pest control methods [1].

One of the most common pests that destroy stored crops and other food products is *Tribolium castaneum* (Herbst) belonging to the family Tenebrionidae of the order Coleoptera. It is a major pest of warehouses, as it lives in its harmful, larval and adult stages on infected grains and the infected flour shows an unpleasant odor as a result of the gaseous secretions of the pest and causes a decrease in the viscosity and low degree of rubberyness .Therefore, many studies have been conducted recently on the use of plant parts that act as inhibitors for feeding insects, repellents or growth regulators [2]

The World Health Organization (WHO) indicated that about 27-30 million people are infected with pesticide toxins annually, and that approximately 20 thousand people die annually, so it is necessary to develop safe, non-toxic alternatives to humans and animals that represent one of its biological control methods and is intended to reduce the population numbers of the pest or its harmful species to The percentage that does not constitute serious harm to humans or animals or their activities by developing other biological types of parasitic insects, worms, plant extracts, bacteria, fungi, or others. Being cheap, it is characterized by its high ability to eliminate limited pests. [3]

2. Materials and Methods

Materials used:

Flour, petri dishes, sieve, plastic tubes for breeding insects, medical gauze
- Sensitive scale, distilled water, cork piercing.

Methods

The red flour beetle (*T. castaneum*) is taken from a farm that has been bred in the entomology laboratory, Department of Biological Sciences, Faculty of Education, University of Samarra.

Breeding of the rusty flour beetle:

The *T. castaneum* is taken from the insect breeding fields and placed in plastic bottles containing flour, and then covered with a perforated piece to prevent

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it from coming out and in order to breathe.
Dilutions are prepared in varying proportions (25%, 50%, 75%, 100%) for *U.dioica* Nettle extract. The above concentrations are used in conducting experiments. The plant extract concentrations are placed in a spray bottle of 100 ml capacity, and the whole red rusty flour beetle is exposed to the concentrations of the extract above in the Laboratory. .

Evaluation of the toxicological efficiency of several different concentrations of aqueous extract of Nettle U.dioica on red flour beetle adults in Lab.

Ten samples are taken for the whole rusty red flour beetle for concentrations (25% 50% 75% 100%), 1.5 ml of each concentration is added, then the number of adults of the insects killed for each concentration is calculated, then the rates are calculated for the percentage of killing.

Preparation of aqueous plant extract

The plant samples are collected and cleaned. Their aqueous extract is prepared as follows: 40 g of each plant and its flowers are weighed, then 160 mm of water is added to it and placed in a glass beaker, taking into account that the closure is tight, as all experiments are carried out in a sterile room in the laboratory, the mixture is left in a refrigerator for a period ranging from Approximately 24 hours to soak and then filter through several layers of perforated cloth and then filter again using filter paper (whatman, no.5) to get rid of the non-powdered parts of the plant, the extract is poured into sterilized glass dishes and placed in an electric oven at a temperature of 40°C Until use, the extract is placed after drying in glass or plastic bottles with a tight lid and kept in a refrigerator until the time of use.[4].

Sterilization of plant extracts and preparation of the concentrations used in the research:

One gram of dry extract is taken and dissolved in 10 mm of sterile distilled water, to form a stock solution with a concentration of 100 mg / ml. 35 It is prepared from 25, 50, 75 and 100% concentrations.

Preparation of the alcoholic plant extract:

The plant samples are collected and cleaned, their alcoholic extract is prepared as follows: 40 gm of the entire plant and its flowers are weighed, then 160 ml of 80% methanol alcohol is added to it and placed in a glass beaker, taking into account the provisions of the closure. All experiments were carried out in the sterile room in the laboratory. The mixture is left in the refrigerator for 24 hours for the purpose of soaking and then filtered through several layers of perforated cloth and then filtered again using filter papers (whatman, no.5) to get rid of the non-powdered plant parts. The extract is poured into sterilized glass dishes and placed in an electric oven at a temperature of 40°C until use. Then the extract is placed after drying in glass bottles with a tight lid and kept in a refrigerator until use.[5].

Sterilization of extracts and preparation of the concentrations used in the research:

One gram of dry extract is taken and dissolved in 10 mm of sterile distilled water, to form a stock solution with a concentration of 100 mg/ml. 35 It is prepared from 25, 50, 75 and 100% concentrations.

Preparation of C. cladosporiodes filtrate:

After preparing the liquid potato medium as mentioned in paragraph 3.4.2, take beakers containing 250 ml of the cooled liquid medium and then inoculate each of these beakers by adding a 4 mm diameter disc of the agar culture medium on which the mushrooms are at the age of 7 days For all the fungi tested in the experiment, the flasks are incubated at a temperature of 1≠ 25°C for a period of four weeks, (Dewan, 1989) The fungal filtrate is filtered by two filter papers of type whatman No.1 and using a sterile funnel, the filtration process is repeated twice under sterile conditions, then the filtrate is filtered once Others, using a Milipor filter with a diameter of o.22 micrometers. The filters are kept in the refrigerator until use, at a temperature of 4 °C [6].

The Method

Concentrations are used (25%, 50%, 75% 100%) with the use of two replicates for each concentration and each concentration is sprayed on insects placed in plastic bottles and placed for 24-48 hours after exposure at 28 °C.

Preparation of fungus extractin potato lakes. Potato Agar PDA:

The medium is prepared by dissolving 39g of the PDA mixture in 1 liter of distilled water in a 2 liter glass beaker placed in a water bath. The mechanism of 250 mg of Chloramphenicol was added. The medium was sterilized with the target at 121 ° C at 15 lb./ng and for 20 minutes in sticky experiments [7].

Preparation of potato and dextrose medium

The medium was prepared by boiling 200 grams of peeled and cut potatoes into small pieces with 500 ml of distilled water for 2 minutes in a glass baker. I filtered the cooked potatoes with a piece of sterile gauze. Take the filter and add 20 g of dextrose to the mechanism and complete the volume to 1000 ml in addition to distilled water. The filtrate was planted in a glass beaker. Capacity of 250 ml, at a rate of 150 ml per beaker, the media was sterilized in an autoclave at 121 ° C per inch for 20 minutes using *C. cladosporiodes* [8] .

Preparation of isolated fungal filtrates

The liquid nutrient PDB is prepared and distributed into 250 mL flasks of 150 mL / beaker. Then the antibiotic, chloromphenicol, was added at 250 mg / mL. Each beaker is inoculated with three 5 mm

diameter tablets with the cork hole from the edge of the fungal colonies. Purified in the culture medium (PDA) and extracted at the age of 7 days for *C. cladosporiodes* [10]. The flasks were incubated at a temperature of 25 ±1 m, taking into account the shaking of the beaker every 3-4 days to distribute the fungal growth after 28 days. The inoculum is filtered using Filter paper, vacuum airless device, and re-filtered using a micro-filter. Concentrations of (25%, 50%, 75% and 100%) concentrations of the fungus *C. cladosporiodes* are prepared and the filtrate concentrations were used in subsequent experiments [9].

3. Statistical analysis

The data are statistically analyzed using the ANOVA Analysis one way variation test by performing the statistical program to choose Duncans Multiple Rangest with significant level P70.05. [10].

4. Results and Discussion

Effect of *U. dioica* extract in comparison with *C. cladosporiodes* on the whole rusty flour beetle *T. castatneum* after 24 hours of exposure.

Table (1) shows the effect of different concentrations of the aqueous and alcoholic extract of Nettle *U.dioica* in comparison with the fungus filtrate on the whole beetle after 24 hours of exposure at a concentration of (25, 50, 75, 100%). The results showed the effect of the fungal filtrate *C. cladosporiodes* used in the research. In causing a rate of killing in wholes, as well as a significant difference between Nettle extract and fungal filtrate. The results of Table (1) showed that the fungal filtrate *C. cladosporiodes* achieves the highest rate of killing of whole wholes of 677 at a concentration of 100 with a significant difference with the rate of killing of whole plants by the alcoholic plant extract after 24 hours, as it reached 6 insects at a concentration of 100% and the percentage of killing of wholes was By the aqueous plant extract after 24 hours 2 insects at 100% concentration, the lowest killing rate was by using *C. cladosporiodes* filter 6.67 insects at 25% concentration, the lowest killing rate was 1.33 insects for the aqueous plant extract at 25% concentration, and the lowest killing rate for the extract Alcoholic plant 2 insects at a concentration of 25%.

Table No. (1) The effect of different concentrations of U. dioica from aqueous extract and alcoholic plant extract after 24 hours, compared to the filtrate of C. cladosporiodes on the beetle adults after 24 hours of exposure.

concentrations	C. cladosporiodes on the T.castaneum adults after 24 hours of exposure	U.dioica plant from aqueous extract on the T.castaneum adults after 24 hours of exposure .	U.dioica plant from alcoholic plant extract on the T.castaneum adults after 24 hours of exposure .
25%	6.67BC	1.33a	2c
50%	6.67BC	1.67a	2.67bc
75%	7Bb	1.67a	4b
100%	7.67Ba	2a	6a

Table (2) showed the killing rate of U.dioica extract and alcoholic plant extract after 48 hours compared to the C. cladosporiodes, after 48 hours of exposure of T. castaneum beetle integuments at concentrations of 25-50-75-100%.

concentrations	C. cladosporiodes on the T.castaneum adults after 48 hours of exposure	U.dioica plant from aqueous extract on the beetle adults after 48 hours of exposure.	U.dioica plant from alcoholic plant extract on the T.castaneum adults after 48 hours of exposure .
25%	7.67Ac	1.67b	3.33c
50%	7.33Ab	2b	5bc
75%	8.67Aa	4ab	6.33ab
100%	8.67Aa	4.67a	8a

As the results showed in Table (2) shows the mortality rate of aqueous and alcoholic Nettle extract compared with the filtrate of the fungus *C. cladosporiodes* after 48 hours of exposure to red rusty flour beetle wholes at concentrations of (25, 50, 75, 100%).

The results of Table (2) showed that the *C. cladosporiodes* infiltrate achieved the highest death rate for the whole, which was 8.67 insects at a concentration of 100%, while when using the alcoholic plant extract, the death rate was 8 insects at a concentration of 100%, and when using the aqueous plant extract, the death rate was 4.67 insects at 100% concentration

Table (2) Effect of different concentrations of aqueous and alcoholic extracts of Nettle *U. dioica* in comparison with *C. cladosporiodes* filtrate on whole beetle after 48 hours of exposure.

The results in the two tables (1) , (2) show that the higher the concentration and the time period of exposure to both the aqueous and alcoholic extract *U.dioica* and the fungus *C. cladosporiodes* filtrate, the higher the mortality rate of the adults of the red rusty flour beetle T. *Castaneum*. The cumulative effect of the toxic substances present in the filter of the fungus *C. cladosporiodes* in the digestive system of the insect, leading to disturbances and damage to the enzymes of detoxification known as Mixed function oxidase (mfo), and the duration of exposure to the concentrations of the filter of the fungus *C. cladosporiodes* affected the generation of the insect. This is the result of mechanical damage to the parts of the alimentary canal, which is due to the fact that

the fungus *C. cladosporiodes* contains toxic substances. The fungal proved the effectiveness of global toxicity on the adults of the insect, as it observed its direct effect on the physiology of the nervous system, as well as it was observed during the exposure that confusion and irregularity in the path of the insect and cases of tremors occurred in the bodies of the insects under study, which indicates the effectiveness of the fungal filtrate *C. cladosporiodes*.

The results also indicate that the *C. cladosporiodes* infiltrate gave a higher significant mortality rate than the plant extract, as the duration of exposure to the active substance showed a greater effect than the dose used, as the greater the time period the insect was exposed to the concentration of the water and alcoholic extract of nettle and the fungus infiltrate *C. cladosporiodes*.

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