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# THE EFFECT OF DIFFERENT CONCENTRATIONS OF METARHIZIUM ANISOPLIAE (METCHNIK OFF) SOROKIN IN THE FOURTH LARVAE INSTAR OF TROGODERMAGRANARIUM EVERTS(COLEOPTERA: DERMESTIDAE)

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## ABSTRACT

The present study was conducted to determine the effects of *Metarhizium anisopliae*fugus(Metchnikoff)as biocontrol agent against the fourth larvae instar of *Trogodermagranarium*(Everts) under laboratory conditions different concentrations $4x10^7$,$4x10^5$,$4x10^3$ conidia/ml. Each concentration treated 10 larvae and replicated 3 times.

The results showmortality rate was elevated with conidial concentration. The killing rate was 70%,60%and 56.5% at the different concentrations $4x10^7$,$4x10^5$,$4x10^3$ conidia/ml, respectively.

The effect of fungi clearly appears in the total number of eggs laid by emergedadultsfrom treatmentlarvae, it highly decreased at $4x10^7$ conidia/ml about 10 eggs which is a significant difference compared with control 100 eggs.

**KEYWORDS**: Metarhizium anisopliae, Trogodermagranarium, biocontrol

## I. INTRODUCTION

*Trogodermagranarium*Everts (Coleoptera: Dermestidae) is an important pest of stored product [1].The presence of stages of theof*T.granarium* insect indicated the existence of the pest on stored products. Only larvaewas feedingstage. The larvae was covered with numerous long hairs[1]**.**The larvae can eat a wide range of store products andhas the ability to survive under extreme conditions such as high and low temperatures, starvation and low humidity [2].Random useof insecticides resultedin resistanceof the insects to the insecticides In addition, they are toxic to human and beneficial insects [3].Therefore,microorganisms such as fungi and bacteria are used because they are not toxic to beneficial animals and humans [4].

*Metarhizium anisopliae* (Metschinkoff) Sorokin (Ascomycota:Hypocreales) isentomopathogenic fungi and grows naturally in soil [5].

This study aims to study the effect of *M. anisopliae* in the fourth larvae instar of *Trogodermagranarium.*

## II. MATERIAL AND METHODS

**Insect culture**

*Triticumaestivum*was cleaned of impurities and put in a frozen (-20ºC) for 72 hours to be sure that they are free from any other pests.the culture consists of 5 g of yeast and 500g of wheat.

The collected adults (male and female) from other culture were moved out to one liter Plastic jars (10 cm indiameters), wrapped in Aorquenza,and set in place by aelastic band which allowed proper

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respiration, and prevent the exit or entry of insects. The culture was incubated at27±1°C and 70±11%RHproportional humidity[6].

**Fungal isolate**

*M. anisopliae* were gained and diagnosed by Dr. Hussam Elldeen A. Mohammad/ College of Agriculture / Baghdad University. Conidial suspensions were prepared by growning fungi on Potato Dextrose Agar (PDA) in dishes ofPetri with 10 cm diameter and brood under specific conditions at 27°C for two weeks for entire sporulation. Conidia of fungal were collected by scrubbing of conidial layer using sterilized lancet. A mixture of conidia was reaped by adding a sterile distilled water to the Petri dishes and agitating with sterilized glass rod [7].

**Fourth larval instar treatment**

10 larvae were used for each concentration and replicated 3 times. The fourth larvae instar was obtained by isolating the eggs and follow-up stages of development until reaching the fourth larvalinstar, which is recognized by larvae size and exuviae skin. They were treated by fungal suspended concentrations$4x10^7$,$4x10^5$,$4x10^3$conidia/ml and placed in sterile petri dishes with diameter is of 5 cm, larvae per dish which contains one kg of crushed wheat grains and five grams of yeast.

The concentrations $4x10^7$,$4x10^5$,$4x10^3$ conidia/ml were prepared by adding 5 ml of distilled distilled water to the Petri dish Containing the fungus colony at the age of 7 days, The spores were collected by the L-shaped harvester,

The contents of the dish were filtered with a piece of filter paper No.0.33 mm and installed on a sterile glass funnel on a 20 mL conical glass flask ,After adding another 5 ml of distilled water on the sides of the filter paper to ensure the release of all spores, this obtained the suspension of stock fungal. attended the fungal concentrations using sterile test tubes of 1-8 and placed in each tube 9 ml of distilled distilledwater,Pull 1 mL of the pre-prepared base fungal suspension mediated by a sterile pipette and added to tube 2 containing 9 ml of distilled water.

Thus, dilution is $4x10^7$conidia/ml and 1 ml of tube 2 is removed and added to tube 3 containing 9 ml of distilled distilled water, thus diluting $4x10^6$.

The remaining tubes continued to reach the concentration of $4x10^3$, thus obtaining the required concentrations of the experiments.

The Neubauer counting chamber (Hemocytometer) was used to calculate the number of spores. Place a drop of the stock suspension on the slide and place the slide cover and calculate the number of spores in the five squares within the slide with the light microscope at 40 x.

$$\text{Number of Spores} = \frac{N}{80} \times 10^6 \times 10$$

N = number of blackboards calculated in the five squares
80 = the sum of the squares in the five count squares
$10^6$ = dilution correction factor
10 = Volume Correction Factor

The number of blackboards counted in the Neubauer counting chamber (Hemocytometer)
$4 \times 10^8$ spore / ml.(8).
The control treatments were prepared by spraying the larvae with three ml of sterile distilled water, then recording the following data:

Mortality percentage ofthe larvae, the average duration of larval stage starting from the fourth larval, the mortality percentage of pupa, the average duration of pupa stage, the proportions of the emergence of normal

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and abnormal adults, the average age of mated adults and the total number of eggs produced by adults emerging from larval treatment .

## III. STATISTICAL ANALYSIS

The Statistical Analysis System- [9]program was utilized to determineof variation factors in study parameters . Least significant difference –LSD test was utilizedto significant contrast between averages in this research.

## IV. RESULTS

**The Fourth larval instar treatment**

Theresults show that the highest killing rate of fourth larvae stagewas 70% at concentration $4x10^7$which is a significant difference compared with other treatments whose killing rates were about 56.5% and 60%at $4x10^3$ and $4x10^5$conidia/ml respectively. Concerning control, it did not record any killing rate in the fourth larvae instar.

The average duration of larval stage at control had a significant difference compared with other treatments 10.2 days, and at $4x10^7$, $4x10^5$and $4x10^3$conidia/mlabout (4,10.2,13) days respectively.

On the other hand,the mortality percentage of pupa did not record any killing rate at control treatment and $4x10^5$ conidia/mlbut the highest percentage appear at $4x10^3$conidia/mlabout 23%. The average duration of pupa stage of the treatment did not show any significant difference.

As for The proportions of the emergence of normal male adults,Table (1) shows a significant difference between treatments $4x10^7$, $4x10^5$, $4x10^3$conidia/mland control (25.5,40,20.5,100%) respectively. In addition, the normal average of maleadults was (10, 6, 8, 19) days $4x10^7$, $4x10^5$, $4x10^3$conidia/mlandcontrol daysrespectively.Thenormal age offemale adultsin control was 20 day which is a significant difference compared with other treatments.

The effect of fungi clearly appears in the total number of eggs produced by adults emerging from larval treatment, it highly decreased at $4x10^7$conidia/ml about 10 eggs which is a significant difference compared with control 100 eggs. The fungus have high effects on percentage of eggs hatching; the lowest percentage was 10% at $4x10^7$ conidia/ml which is asignificant difference compared with $4x10^5$and $4x10^3$conidia/ml and control treatment 18,44.4 and90% respectively, Table(1).

***Table (1) Effect of Metarhizium anisopliae concentrations at $4x10^7$, $4x10^5$ and $4x10^3$ conidia/ml on fourth larval instar of Trogodermagranarium***

| treatments | | mortality percentage of the larvae% | average duration of larval stage (day) | mortality percentage of pupa % | average duration of pupa stage (day) | proportions emergence of a normal adults % | average age of adults (day) | | average of eggs produce | Percentage Of Hatched Eggs % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | male | female | | |
| Fungi conidia/ml | $10^7$ | 70a | 4b | 4.5 b | 3a | 25.5c | 10 b | 14b | 10d | 10c |
| | $10^5$ | 60 b | 10.2 a | 0b | 3a | b 40 | 6c | 8c | 25c | 18c |
| | $10^3$ | 56.5b | 13 a | 23.0a | 4.2a | 20.5b | 8bc | 22a | 90b | 44.4b |
| control | | 0 c | 10.2a | 0 b | 3.3a | 100 a | 19 a | 20 a | 100a | 90a |
| valueLSD | | *9.04 | 5.32 * | 5.62 * | 1.84 NS | * 9.06 | 2.09 * | 5.37 * | 9.742 * | 10.57 * |

*(p>0.05) significant difference
NS :non significant difference

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## V. DISCUSSION

Table (1) shows that there is a high killing rate with increasing conidia concentration.This agrees with [2]when he used $1x10^5$ to $1x10^9$ conidia/ml to control *T.granarium*about 3.33% ,20.83%, 70.83%, 80.5% and 83.9% respectively.

In addition, this is due to sensitivity of larvae due to having a thin cuticle wall that is easy to enter the fungi by using their secreted enzymes and then consuming nutrients in the cavity of the insect and fat bodies reasoned starvation and then death of the larvae affected the productivity and hatching of eggs produced , or ability of the fungus production of toxins like destruxin A,B which affectsATPase leading to its inhibition. It also affects $Ca^{++}$ and insects muscles and inhibition cellular immunity [10].

When conidia land on the surface of cuticle, they will germinate and spore forms appressonium infection structure, penetrates cell wall by enzymes such as chitinases and lipasesthen reach haemolymph thus killing the insects by secretion of enzymes and toxins into hymolymph, hyphae grow out through cuticle and produce new spores under adequate humidity.

Moreover, with good conditions the fungus will sporulate and liberation more spores [11].

[12]elaborate the efficacy of Iranian isolates of *M.anisopliae* against *Rhyzoperthadomonica* Fabricius (Coleoptera: Bostrichidae) in larvae stage.The fungi registered 79 and 75% death-rate of insect at the concentrationsof $4.6 \times 10^9$ conidia/ml respectively, and the same study allusion that *Sitophilus granaries* (Coleoptera: Corcolionidae) was sensitive to *M. anisopliae*.

They also demonstrated high killing rate when *Callosobruchusmaculatus*F. (Coleoptera: Bruchidae), and treated with *M. anisopliae*81% and 84% for$5.2 \times 10^{10}$ and $1 \times 10^{11}$ conidia/ml[13].

[14]tested efficacy *M.anisopliae* against Dermestesmaculatuslarvae (Coleoptera: Dermestidae) larvae on solid, leather, plastic, and wood surfaces. The results indicate that entomopathogenicare useful for monitoring of hide beetles.

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