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Influence of Supernemic on Growth and Sporulation of Entomopathogenic Fungi *Meterhiziun anisopliae* (Deuteromucotina:Hypomucetes)

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Abstract: ***This study was carried out to evaluate the influence of some concentrations of botanical insecticide (supernemic) on mycelial growth and sporulation of two isolates of Metarhiziumanisopliae (a local isolate, the other was imported) . Results showed that the dried weight of the vegetative growth was not affected significantly by the insecticidal concentrations that tested (0.3, 0.6 and 1 ml/L), dried weight of the vegetative growth and mean of spores number of the local and imported isolates of M.anisopliae were 0.069 g/20ml, 7.5×10^6 spore/ml and 0.0439/20ml, 3×10^{6}spore/ml respectively when treated with 1ml/L of supernemic.. Supernemic was significantly compatible and safe to conidial sporulation which makes it promising in integrated pest management.***

Keywords: Growth, Sporulation, Neem oil, *Metarhiziumanisopliae*, Supernemic

1. Introduction

The genus Metarhizium is one of the important soil borne imperfect fungi widely distributed in the world, and regarded as one of the important entomopathogenic fungi that causes green Mascardine disease(1). This fungus used before 120 years as bio control agent against insect and it was recorded that it can infect more than 220 insect species (2).

The variant *M.anisopliae var anisopliae* is widely distributed and it can infect many insect families belonging to many orders such as Coleoptera , Diptera , Hemiptera and Hymenoptera (2-6).

This fungus is regarded as an environment friendly and have no harmful effect against birds , fishes and mammals (7-9). Among the important reasons for the use of the entomopathogenic Fungi as biopesticide are their harmless effect on plant and other non-target organisms, host specificity as compared by chemical insecticides.

The utilization of many compatible control methods can play important key factor for effective controlling of any pest specially when insect pest can develop resistance for chemical pesticides.

The application of entomopathogenic fungi in combination with compatible botanical insecticides can increase efficiency of pest control and reduce the harmful effect on environment and general health.

The aim of this study is to evaluate the effect of botanical insecticide supernemic on growth and sporulation of two isolates of *Metarhiziumanisopliae* (local and introduced)

2. Material and Methods.

1) Isolation of *Metarhizium anisopliae* from soil

Trap method was used for isolation *M. anisopliae* from soil using larvae of wax moth (*Galleria mellonella*) as bait which obtained from directrate of agricultural research/Ministry of Science and Technology (10). Three soil samples (1kgm/sample) were collected from different locations in Baghdad: Al-Graate , Al-Madaen and Al-Atifia . Soil surface was removed and the soil was collected at depth of 2-5 cm , in plastic bags; transferred to laboratory and subjected to an isolation technique (11).

2) Fungal identification and cultivation

Infected wax worms were observed by disectiny microscope and fungal growth was purified on PDA plates supplemented with 100 ppm Chloramphenicol to prevent bacterial contamination. *Metarhizium* culture was identified according to recommended keys and the identification was confirmed by chief scientific researchers Dr.Hadi M.Aboud /head of biotechnology center directorate of agricultural research/Ministry of Science and Technology. The isolate named as local isolate while introduced isolate was used as a control and obtained from the agricultural research center /Egypt.

3) Toxic effects of supernemic on growth and sporulation of *M.anisopliae*

Food posion technique was used to determine the inhibitory effect of three concentrations of(0.3,0.6,1ml/L) of supernemic (Azaderachtine). On growth and sporulation of the two tested isolates of *M. anisopliae* (local and introduced). Potato sucrose Broth (200 gm potato extract and 10 gm sucrose in 1000 ml water).the pH was adjusted to be 5 using Hcl solution. The medium autoclave sterilized at 121 C and pressure 1 bar for 20 minutes and supplemented with 100 ppm chloramphenicol before poured in 50 ml flasks (20 ml / flask) and the insecticide was added to obtain the tested concentrations, the treatment triplicated . The flasks inoculated with 0.5 cm of fungal culture (7 days old on PDA), flasks incubated at 27 +_ 2C for two weeks. Then the biomass separated by filtration throughWhatman (2) filter paper, dried in a hood for three days at room temperature.

Weight of dried biomass was calculated according to the following equation : Biomass=weight of biomass with filter paper – weight of dried filter paper).

To count mean number of spores, the dried biomass resuspended in 100 ml DL using magnetic stirrer for five minutes and the number of spores/ ml was accounted using Haemocytometer (12),data analysis was done by using SPSS program copy 20.

3. Results and Discussion

The results of isolation of *M. Anisopliae* using Bait technique revealed the isolation of one isolate from Al-graat location while no isolate was found from Al-Madaen and Al-Atafia locations. The fungus was identified based on taxonomic keys (13) as *M.anisopliae*. The occurrence of this fungus in Al-graat may be due to the presence of old citrus orchards and the avoidance of pesticide utilization in contrast to other tested locations.

This result was in a good agreement with Khudhair, *et.al*(2014)who studied the occurrence of entomopathogenic fungi in 300 soil samples collected from 42 locations in different Iraqi governorates and isolated 94 isolate through this study , with *M.anisopliae* occurrence percentage was 18.1% and they suggested that soil kind and host plant play an important role in fungal distribution and occurrence of citrus and date Palme trees were suitable for their occurrence in compared to other crop plants .

The results of the effect of three concentrations of botanical insecticide (0.3,0.6 and 1 ml/L) on growth and sporulation of local and introduced isolates of *M.anisopliae* was found to be insecticide concentration and fungal isolate depended(Table 1&2). The local isolate exhibited a compatible effect on dry weight of biomass with all the tested concentrations which recorded 0.069,0.070 and 0.069 gm/20 ml medium for 0.3,0.6 and 1ml/L respectively compared with 0.068 gm/20 ml medium for the control treatment, table (1).The same response recorded by the concentration 1ml /L regarding sporulation which recorded 7.5 X 10^6 spore/ml with no significant difference with the control treatment (9X10^6 spore/ml) while the concentrations 0.3 and 0.6 ml/L showed a promoting effect on sporulation and recorded 20 X 10^6 and 15 X 10^6 spore/ml respectively. The results also showed that all the tested concentrations of supernemic inhibited the growth and sporulation of introduced isolate which recorded 0.082 , 0.065 and 0.043 gm / 20 ml of the growth media and 5 X 10^6 ,3.2 X 10^6 spore/ml and compared to 0.195 gm/ 20 ml and 21 X 10^6 spore /ml in the control treatment.

The low toxicity of supernemic to *M. anisopliae* maybe due to the absence of metabolic pathway influenced by this insecticide in the fungus to degradation or overcome the toxic effect of the active ingredient of the insecticide. The results are in a good agreement with that of Hirose, *et. al.* (2001) who found that neem oil had a moderate toxicity to *M. anisopliae* and with pesticide Neemgold was compatible with *M. anisopliae*. Castiglioni ,*et. al*.(2003), recorded that the sporulation of *M. anisopliae* didn't significantly inhibited with 0.2 and 1% of Nimkol_ pesticide, while the concentration of 5% was significantly inhibited the growth and sporulation. Rodrigues *et.al*.(1997) , found that there was no toxic effect of neem oil on growth and sporulation of entomopathogeic fungi at a concentration less than 2.5%, so a neem pesticide was used in combination with bio pesticide to control insect pests. Finally this study demonstrates the compatibility of entomopathogenic fungus *M.anisopliae* with the commercial botanical pesticide neem oil (supernemic), this agrochemical can be used with *M.anisopliae* for controlling the pests.

Table 1: Influence of three concentrations of botanical insecticide (supernemic) on mycelial growth and sporulation of (*M.anisopliae*) local isolate

Average number of spores/ml$^{+}_{-}$ standered error	Average weight of living mass gm/20ml of calture media+ standered error	Concentration ml/l.
a1.15±10^6×21	a*0.026±0.195	Control
b0.57±10^6×5	a 0.003±0.082	0.3
c10^6×3.2 ±0.14	a0.007±0.065	0.6
c0.60±10^6×3	a0.004±0.043	1.0

* The similar letters in each column mean that there are no significant differences according to the Dunkin multilevel test and the level of significance($P \leq 0.05$)

Table 2: influence of three concentrations of botanical insecticide(supernemic) on mycelial growth and sporulation of (*M.anisopliae*) introduced isolate

Average number of spores/ml$^{+}_{-}$ standered error	Average weight of living mass gm/20ml of calture media+_standered error	Concentration ml/l.
c0.57±10^6×9	a*0.004±0.068	Control
a1.73±10^6×20	a 0.009±0.069	0.3
b10^6×15 ±1.15	a0.001±0.070	0.6
c1.14±10^6×7.5	a0.004±0.069	1.0

* The similar letters in each column mean that there are no significant differences according to the Dunkin multilevel test and the level of significance ($P \leq 0.05$)

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