J. Agric. Sci. Mansoura Univ., 29(5): 2907 - 2911, 2004

FUNGICIDAL ACTIVITY OF *METARHIZIUM ANISOPLIAE* AGAINST SOME LAND SNAIL SPECIES UNDER LABORATORY CONDITIONS.

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ABSTRACT

The fungicidal activity of *M. anisopliae* was assessed against *M. cartusiana* and *E.vermiculata* land snails in compared with the carbamate insecticide, Methomyl. Results revealed that *M. anisopliae* at concentrations of 0.5 $\times 10^8$, 1 $\times 10^8$, 2$\times 10^8$ and 4 $\times 10^8$ spore / ml induced (32.14, 53.57, 75 and 92.86 %) and (28.75, 50 , 67.86 and 85.71 %) mortality against *M.cartusiana* and *E.vermiculata* land snails after 7 days exposure period, with LC50 values of (0.873 $\times 10^8$ and 1.071 $\times 10^8$ spore / ml) respectively. In contrast Methomyl at concentrations of 0.5, 1, 2 and 4 % as poison bait, exhibited (26.66, 53.33, 66.66 and 86.66 %) and (40, 76.66, 90 and 96.66 %) mortality after 7 days against the two tested snails species, with LC50 values of (0.819 and 0.475 %), respectively.

INTRODUCTION

The use of microbial control is a potentially valuable alternative to the high cost, possible pest resurgence, development of resistance and environmental contamination associated with chemical insecticides. Thus, as a first step toward the development of a biocontrol program, an investigation was begun to find natural enemies of these pests in field-collected individuals (Sosa-Gomez *et al.* 1994)

The control program of Schistosomiasis includes snail biological methods. Fungi, protozoa and bacteria have all been reported, mostly without precise identification, to produce adverse effects in species of Bulinus and Biomphalaria snails (Madsen, 1990).

Ragab and Ismail (2001) reported that the fungal strain, *Trichoderma viride* was most potent against *Biomphalaria alexandrina* snails.

In concern of land snails Godan (1983) mentioned that fungi attack mainly the eggs of gastropods, for example those of *Deroceras reticulatum* slugs and fungal infections are important when rearing molluscs in the laboratory, since they may destroy the whole stock.

Lack information in literature are available about the role of fungi in land snail biological control programs. Therefore our effort were oriented to gain more information in this respect during the present study.

MATERIALS AND METHODS

Tested agents

1- Micro-organism

The *Metarhizium anisopliae* fungi was obtained from Prof.Dr. Ahmed, R. Hamed, biological control Dep. Plant Protection Institute. The strain was maintained on potato dextrose agar medium, grown at 25-28 °C for 48 – 72 hrs, then stored at 4 °C.

2- Methomyl (Neomyl, S.L 20 %)
- Primary use : Insecticide
- Secondary use : Molluscicide

Fungal preparation

The entomopathogenic fungi *M.anisopliae* were cultured on an autoclaved potato dextrose medium to obtain the condiospores. Spores were harvested from two weeks old cultures grown at 25 °C by rising with sterilized distilled water. Collected spores were filtrated through cheese cloth. The number of spores in one ml was adjusted to 1×10^8 spores.

Media used:

Potato dextrose agar medium (P.D.A.), consists of extraction of 200 gm potatoes, 20 gm glucose, 20 gm agar and PH 5.5. the previous components was appreciated as gm / l . the medium was sterilized in autoclave at 121°C for 20 minutes. (Difco manual, 1984).

Tested snails

Individuals of the land snails *Monacha cartusiana* and *Eobania vermiculata* were collected from infested clover fields and ornamental plants near Mansoura city, Dakahlia Governorate and kept in laboratory under 20 ± 2 °C and 80 ± 3 % R.H for further investigation.

Bioassay

1- Serial concentrations of *M.anisopliae* spores suspension, *i.e* 0.5×10^8 , 1×10^8 , 2×10^8 and 4×10^8 Spore / ml in 10 ml disteld water, were prepared. Similar pieces of fresh lettuce leaves were dipped for 5 second in the tested fungi solution, then left to dry before being offered to the tested snails. The leaves in control treatment were dipped in water. Ten adult individuals were exposed to each treated leaf in disposable plastic cup for 48 hours. The cups were covered with muslin cloth held by rubber bands to prevent snails from escaping. Each treatment was replicated 3 times in addition to control. After 48 h, the treated leaves were changed with another untreated leaves, and mortality percentages were estimated for 7 days and corrected for natural mortality according to Abbott's formula (1925). Then subjected to probit analysis by Finney's method (1952).

2- Methomyl SL 20 % was tested against snails as poisonous bait at concentrations of 0.5, 1, 2 and 4 %. Mortality were recorded up to 7 days.

RESULTS AND DISCUSSION

Fungicidal activity of *Metarhizium anisopliae* fungi was evaluated against *M.cartusiana* and *E.vermiculata* land snails in comparison with the carbamate insecticide Methomyl. Data were tabulated in Tables from(1 to 3).

Data presented in Tables 1 and 2 revealed that *M.anisopliae* fungi, when tested against *M.cartusiana* and *E. vermiculata* land snails at concentrations of 0.5×10^8 , 1×10^8, 2×10^8 and 4×10^8 Spore / ml exhibited [(32.14 , 53.57, 75 and 92.86 %) and (28.75, 50, 67.86 and 85.71 %) mortality after 7 days, respectively. LC_{50} values were 0.873×10^8 and 1.071×10^8 spores / ml for the two tested snails, respectively.

Table (1) : Fungicidal activity of *Metarhizum anisopliae* against *M. Cartusiana* landsnail under laboratory conditions.

Concen. Spore / ml	Corrected Mortality %	LC_{50} Spore/ ml	C.L		LC_{90} Spore/ml	CL		Slope
			Lower	Upper		Lower	Upper	
0.5×10^8	32.14	0.873×10^6	0.633×10^6	1.205×10^6	3.506×10^6	2.541×10^6	4.838×10^6	2.12
1×10^6	53.57							
2×10^6	75							
4×10^6	92.86							

Table (2) : Fungicidal activity of *Metarhizum anisopliae* against *E.vermiculata* landsnail under laboratory conditions.

Concen. Spore / ml	Corrected Mortality %	LC_{50} Spore/ ml	C.L		LC_{90} Spore/ml	CL		Slope
			Lower	Upper		Lower	Upper	
0.5×10^8	28.75	1.071×10^6	0.741×10^6	1.548×10^6	5.529×10^6	3.826×10^6	7.989×10^6	1.741
1×10^6	50							
2×10^6	67.86							
4×10^6	85.71							

In contrast, Methomyl exhibited (26.66, 53.33, 66.66 and 86.66 %) mortality, after 7 days at concentrations of 0.5, 1, 2 and 4 % against the two tested snails, respectively. LC_{50} values were 0.819 and 0.475, Table (3).

The obtained results are in agreement with those of Godan (1983) who reported that fungi attack mainly the eggs of gastropods such as *Deroceras reticulatum* slugs.

Table (3) : Percent mortality of certain land snails treated with different concentration of Methomyl and the corresponding LC_{50} values as poisonous bait method.

Tested snails	Concentrations%				LC_{50} %	C.L		LC_{90} %	CL		Slope
	0.5	1	2	4		Lower	Upper		Lower	Upper	
M.cartusiana	26.66	53.33	86.66	86.66	0.819	0.52	1.289	5.072	3.220	7.988	1.873
E.vermiculata	40	76.66	90	96.66	0.475	0.309	0.731	1.449	0.942	2.230	2.643

Ragab and Ismail (2001) found that the fungal strain, *Trichoderma viride* was most potent against *Biomphalaria alexandrina* snails. Monchan and Domhon (2004) tested two concentrations of *Paecilomyces lilacinus* fungi (Bcc6121) in the wattable powder formulation in two trials against egg stage of golden apple snail *Pomacea canaliculata* in greenhouse.they found that the percent infected eggs were 69.78 and 87.78 & 61.21 and 84.16 in the first and the second trials at concentrations of 1×10^6 and 1×10^7 cfu / ml respectively. Also they tested two isolates of the genus Metarhizium and eight isolates of the genus Paecilomyces at concentration of 1×10^8 cfu / ml. On the 1 day old snail's eggs, results showed that *Paecilomyces* sp. Was superior to *Metarhizium* sp. In controlling eggs.

Discussing the abovementioned results it is clear that the entomopathogenic fungi *M. anisopliae* exhibited the highest toxic action against *M.cartusiana* snail (LC_{50} = 0.873 $\times 10^8$ Spore / ml),than *E.vermiculata* snail (LC_{50} = 1.071 $\times 10^8$ Spore / ml). On the other side, Methomyl appeared to be more potent against *E.vermiculata* snail (LC_{50} = 0.475 %) than *M. cartusiana* (LC_{50} = 0.819 %).

In addition, the demecological factors such as age groups and individual size of snail species may be affected the sensitivity of snails under investigation to the tested materials as well as it's toxicity. Also, species-specific factors such as the sole size should be of considerable importance for the uptake of molluscicides (Godan, 1983). In this regard it might be assumed that larger animals, *i.e E.vermiculata* snails are more sensitive to treatments than smaller, *i.e. M.cartusiana* snails within the different species, not within the same species.

In contrast, while the pesticidal effect of the specific carbamate molluscicide, Methomyl is well known as nerve poison, the mode of action of the tested fungi could be attributed to the toxins related to *M.anisopliae* mycelium growth, which induced cellular changes such as cells granular content, irregular form and alteration in the cellular membrane, Very, *et al.* (1993), Dumas, *et al.* (1996) and Omayma Khamiss, *et al.* (2000) this explain the variation between snails sensitivity and the potency of the tested materials.

Finally it could be concluded that many hazards have reported due to the use of chemicals commonly used now in snail control. Chemical molluscicides apart from being expensive, they affect non target organisms. So it is quite necessary to search for some other safe and un-expensive means of control. The obtained results in the present investigation showed that fungi could be used in integrated pest control programs. Further investigations are needed in this respect to gain more information about the role of fungi in snail control.

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التأثير السام لفطر الميتاريزيم أنيسوبليا ضد بعض أنواع القواقع الأرضيـــة تحـــت الظروف المعملية.

حلمى على على زيدان

معهد بحوث وقاية النباتات – مركز البحوث الزراعية- ا لدقى الجيزة.

تم دراسة فاعلية فطر الميتاريزيم على القواقع الأرضية موناكا كارتيوسيانا & أوبانيـــا فيرميكيولاتا بالمقارنة بمبيد الميثوميل . أظهرت النتائج أنه عند استخدام الفطر تركـــيزات ٠,٥ × ١٠ ^ ٨ ، ١ × ١٠ ^ ٨ ، ٢ × ١٠ ^ ٨ ، ٤ × ١٠ ^ ٨ جرثومة / مل أحدثت نسبة مـــوت (٣٢,١٤ ، ٥٣,٥٧ ، ٧٥ ، ٩٢,٨٦ %) و (٢٨,٧٥ ، ٥٠ ، ٦٧,٨٦ ، ٨٥,٧١ %) لقوقعـــى الموناكـــا والأوبانيا بعد ٧ أيام على التوالى . وكانت قيم الجرعـــة النصفيـــة المميتـــة ٠,٨٧٣ × ١٠ ^ ٨ & ١,٠٧١ × ١٠ ^ ٨ جرثومة / مل على التوالى .

وفى المقابل، عند استخدام مبيد الميثوميل بتركيزات ٠,٥ ، ١ ، ٢ ، ٤ % كطعم ســـام أحـــــدث (٢٦,٦٦ ، ٥٣,٣٣ ، ٦٦.٦٦ & ٨٦,٦٦) و (٤٠ ، ٧٦,٦٦ ، ٩٠ ، ٩٦,٦٦ %) موت بعد ٧ أيام ضد كلا القوقعان على التوالى وكانت قيم الجرعة النصفيـــة ٠,٨١٩ & ٠,٤٧٥ %) على التوالى .