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## Fungi as Control Bioagents Against the Terrestrial Snail, *Eobania vermiculata*

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### ABSTRACT

The molluscicidal potencies of the recommended fungal isolates; *Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Trichoderma harzianum* and *Verticillium albo-atrum* against juveniles (two and four months old) age and adults of *Eobania vermiculata* (Müller, 1774) (Gastropoda, Stylommatophora, Helicidae) snail was investigated. Three tested concentrations of  $2 \times 10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  spores/ml from each fungal isolate were tested against the snail ages under the laboratory conditions. The two most effective fungal isolates that achieved the highest molluscicidal effect against adult snails in the laboratory were selected to be applied against the adult individuals in the field. The results indicated that *V. albo-atrum* and *T. harzianum* were the most potent fungi against all ages of snails in the laboratory. They recorded 90 & 83.33% and 86.66 & 76.66% mortality for juveniles two and four months old at the highest concentration of  $6 \times 10^5$  spores/ml after one month of the infection, respectively. After this period and at the tested concentration, both fungal isolates caused 63.33 and 60% mortality in adult individuals, respectively. On the contrary, *P. lilacinus* had the lowest molluscicidal activity against all stages of the snail. Under the field conditions, the molluscicidal influence of *V. albo-atrum* against snails was greater than that of *T. harzianum* as they achieved 58.06 and 55.10% reduction of snails at the highest concentration of  $6 \times 10^5$  spores/ml after a month of application, respectively. These results indicate that the fungal isolates in this study especially *V. albo-atrum* and *T. harzianum* have a high potential molluscicidal impact on all developmental stages of *E. vermiculata* snails making them promising fungi that can be commercially manufactured and used as future safe biopesticides for control this harmful snail.

### INTRODUCTION

Land snails have increased rapidly causing great damage in the different crops. They attack plants at their different stages of growth, causing a reduction in their yields (Khidr *et al.*, 2011). Moreover, they leave an unpleasant taste and smell due to the secretion of mucus during their movement on crops, which decreases crop quality and causes animals and humans to aversion to feeding on these crops (Enzo *et al.*, 2019). The chocolate band snail, *E. vermiculata* was the most harmful snail species which infested many important crops in Egypt (Gabr *et al.*, 2006). Many investigations evaluated several controls means against land snails under laboratory and field conditions to obtain the most suitable molluscicide for the control of this pest (Farang, 2012; Ismail *et al.*, 2015). The extensive use of chemical

pesticides to control land snails is not environmentally friendly and has adverse side effects on the non-target organisms. Moreover, it is difficult to control terrestrial snails by chemical methods (Godan, 1983). Thus, biological control is the most suitable way to minimize these problems. Natural enemies such as nematodes, Sciomyzid flies and red ants can be used for the control of land snails (Glen *et al.*, 2002). On the other hand, fungi represent the best microbial agent used for control land snails. Due to it being cheap in cost, easy to use and applied as spray or dust. In addition, it can produce toxins and other antimicrobial agents to control plant diseases (Geasa *et al.*, 2013). Biozed is a commercial product of *Trichoderma album* fungus, it showed significant potent activity in the control of *Monacha cartusiana* terrestrial snail (Abd El-Atti *et al.*, 2020). Moreover, the fungal species *Metarhizium anisopliae*, *Paecilomyces* spp., *Trichoderma* spp. and *Verticillium* spp. were found to be the most effective fungi against the land snail, *E. vermiculata* (Hend, 2013).

This study was conducted to evaluate the effect of some recommended fungal isolates against juveniles (two and four months) old and adults of *E. vermiculata* snails under laboratory conditions. The molluscicidal potency of the most effective fungal isolates in the laboratory was also investigated against adult snails under field conditions.

## MATERIALS AND METHODS

### 1. Collection and Rearing of Snails:

Adult individuals of *E. vermiculata* snail were collected from navel orange trees at Mit Assas village, Sammannoud district, Gharbia Governorate, Egypt in November 2022. The collected snails were transferred to the laboratory and maintained in a glass container containing moist clay soil and provided daily with fresh cabbage leaves. The egg laying was observed daily and all deposited eggs were carefully collected and followed until their hatching for obtaining the juveniles (two and four months) age for experiments.

### 2. Tested Fungi and Preparation of Fungal Inocula:

Four fungal isolates (*Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Trichoderma harzianum* and *Verticillium albo-atrum*) were used in this study; these isolates were obtained from the Fungi Research Department, Plant Diseases Research Institute, Agricultural Research Center. These fungal species are recommended for pest control and are safe for non-target organisms, plants and people (Butt and Copping, 2000; Samojlov *et al.*, 2010). To prepare the fungal inocula, firstly each isolate was grown on Potato – dextrose agar (PDA) slants of PDA medium (Potato extract 250 ml, glucose 20 g and water 730 ml) for 7 days at 27°C. After that the spores were taken by rinsing in 10 ml sterilized distilled water and then it was filtered using cheese cloth. The number of spores in the suspension was determined using a haemocytometer slide and adjusted to the three concentrations;  $2 \times 10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  spores/ml by adding the suitable amount of sterilized distilled water to the fungal spores (Hend, 2007).

### 3. Efficiency of Fungal Isolates against *E. vermiculata* Snail at Different Ages Under Laboratory Conditions:

The molluscicidal potency of the fungal isolates at the three tested concentrations of  $2 \times 10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  spores/ml was evaluated separately against the two and four months old juveniles and adults of *E. vermiculata* snail. Plastic boxes with a capacity (3/4 kg) were used in this experiment. About 1/2 kg of sterilized clay soil was placed into each box, this clay soil is just saturated with water. Ten individuals of snails for each age and ten discs of fresh cabbage leaves were put on the surface of the soil. The fungal spores were sprayed directly on the soil and cabbage discs. Three replicates were prepared for each concentration of each fungus for each age of the *E. vermiculata* snail. The other three replicates were also prepared in the same way for each snail age but without fungal treatment

as control. All boxes were covered with muslin cloth and secured with rubber bands to prevent snails from escaping. Mortality percentages of treatments and control were recorded every three days for one month and corrected by Abbott's formula (1925).

#### **4. Field Application of Fungal Isolates Against Adults of *E. vermiculata* snail:**

The most two effective fungal isolates against adult snails in the laboratory were selected to evaluate their influence against the adult individuals under field conditions. This field experiment was conducted in March 2023 in a navel orange field heavily infested with *E. vermiculata* snails at Mit Assas village, Samannoud district, Gharbia Governorate, Egypt. The field was irrigated one day before the treatment. Three infested trees were selected for spraying with each concentration of the two isolates. The trees of each fungus concentration were separated from the others by the same number of trees to avoid overlapping treatments. Before the treatment, live snails were counted in an area of  $25 \times 25$  cm under each of the tested trees, at 1m. of a tree trunk and on 5 branches of the tree. Control trees were sprayed with distilled water only without any fungal spores. Numbers of living snails were recorded in the same areas under each tree for three days successively for one month. The reduction percentages of snails were calculated according to Henderson and Tilton (1955).

#### **5. Statistical Analysis:**

Data obtained in both laboratory and field experiments were statistically analyzed by T-test using Costat Program software Version 6.311, (Costat 2005).

## **RESULTS AND DISCUSSION**

### **1. Molluscicidal Potency of Fungal Isolates Against Juvenile Snails Under Laboratory Conditions:**

Results of the molluscicidal potency of fungal species at  $2 \times 10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  spores/ml against *E. vermiculata* juveniles two months old were presented in Table 1. indicated that increasing the concentration of all tested fungi and the experimental period, increased mortality rates. The fungus *V. albo-atrum* achieved the highest mortality of 90% of juveniles at the highest concentration of  $6 \times 10^5$  spores/ml after 21 days from initial treatment and this rate remained constant until one month. It was followed by *T. harzianum*, *M. anisopliae* and *P. lilacinus* which recorded 83.33, 76.66 and 70% mortality at the same concentration of  $6 \times 10^5$  spores/ml after one month from initial treatment, respectively. During, the same period, the fungus *P. lilacinus* recorded the lowest mortality 40% at the lowest concentration  $2 \times 10^5$  spores/ml. All untreated snails in the control were still alive until the end of the experimental period.

The efficacy of fungal isolates against juveniles four months old is shown in Table 2. As shown in this table, *V. albo-atrum* was the most effective fungus against the juveniles. It causes the highest mortality 86.66% at  $6 \times 10^5$  spores/ml concentration after one month from initial treatment. At the same period, this fungus showed 80 and 73.33% mortality for its other tested concentrations  $4 \times 10^5$  and  $2 \times 10^5$  spores/ml, respectively. On the other hand, *P. lilacinus* achieved the same period's lowest mortality 33.33, 50 and 66.66% at  $2 \times 10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  spores/ml concentrations consecutively.

**Table 1.** Molluscicidal activity of fungal isolates against two-months old juveniles under laboratory conditions

Fungal isolates	Conc. (spores/ml)	Mortality % after periods in days				
		3	7	14	21	30
<i>M. anisopliae</i>	$2 \times 10^5$	36.66 <sup>de</sup>	40.00 <sup>ef</sup>	40.00 <sup>fg</sup>	46.66 <sup>e</sup>	53.33 <sup>fg</sup>
	$4 \times 10^5$	43.33 <sup>cde</sup>	46.66 <sup>de</sup>	50.00 <sup>ef</sup>	53.33 <sup>de</sup>	60.00 <sup>def</sup>
	$6 \times 10^5$	50.00 <sup>bcd</sup>	63.33 <sup>bc</sup>	66.66 <sup>bcd</sup>	70.00 <sup>bc</sup>	76.66 <sup>abc</sup>
<i>P. lilacinus</i>	$2 \times 10^5$	23.33 <sup>e</sup>	26.66 <sup>f</sup>	26.66 <sup>g</sup>	30.00 <sup>f</sup>	40.00 <sup>g</sup>
	$4 \times 10^5$	36.66 <sup>de</sup>	43.33 <sup>de</sup>	43.33 <sup>f</sup>	50.00 <sup>e</sup>	56.66 <sup>ef</sup>
	$6 \times 10^5$	43.33 <sup>cde</sup>	56.66 <sup>cd</sup>	63.33 <sup>cde</sup>	63.33 <sup>cd</sup>	70.00 <sup>cde</sup>
<i>T. harzianum</i>	$2 \times 10^5$	40.00 <sup>de</sup>	46.66 <sup>de</sup>	53.33 <sup>def</sup>	66.66 <sup>c</sup>	73.33 <sup>bcd</sup>
	$4 \times 10^5$	56.66 <sup>abcd</sup>	63.33 <sup>bc</sup>	70.00 <sup>abc</sup>	70.00 <sup>bc</sup>	76.66 <sup>abc</sup>
	$6 \times 10^5$	63.33 <sup>abc</sup>	76.66 <sup>ab</sup>	76.66 <sup>abc</sup>	80.00 <sup>ab</sup>	83.33 <sup>abc</sup>
<i>V. albo-atrum</i>	$2 \times 10^5$	46.66 <sup>cd</sup>	50.00 <sup>cde</sup>	66.66 <sup>bcd</sup>	73.33 <sup>bc</sup>	80.00 <sup>abc</sup>
	$4 \times 10^5$	70.00 <sup>ab</sup>	73.33 <sup>ab</sup>	80.00 <sup>ab</sup>	80.00 <sup>ab</sup>	86.66 <sup>ab</sup>
	$6 \times 10^5$	76.66 <sup>a</sup>	80.00 <sup>a</sup>	83.33 <sup>a</sup>	90.00 <sup>a</sup>	90.00 <sup>a</sup>
<b>Control</b>		0.00 <sup>f</sup>	0.00 <sup>g</sup>	0.00 <sup>h</sup>	0.00 <sup>g</sup>	0.00 <sup>h</sup>
<b>P</b>		.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>
<b>LSD<sub>0.05</sub></b>		2.02	1.47	1.44	1.20	1.34

**Table 2.** Molluscicidal activity of fungal isolates against four-months old juveniles under laboratory conditions

Fungal isolates	Conc. (spores/ml)	Mortality % after periods in days				
		3	7	14	21	30
<i>M. anisopliae</i>	$2 \times 10^5$	30.00 <sup>cd</sup>	36.66 <sup>ef</sup>	43.33 <sup>d</sup>	43.33 <sup>de</sup>	46.66 <sup>ef</sup>
	$4 \times 10^5$	36.66 <sup>cd</sup>	43.33 <sup>de</sup>	50.00 <sup>cd</sup>	53.33 <sup>cd</sup>	53.33 <sup>de</sup>
	$6 \times 10^5$	43.33 <sup>bc</sup>	56.66 <sup>bcd</sup>	63.33 <sup>bc</sup>	70.00 <sup>b</sup>	70.00 <sup>bc</sup>
<i>P. lilacinus</i>	$2 \times 10^5$	20.00 <sup>d</sup>	20.00 <sup>f</sup>	23.33 <sup>e</sup>	30.00 <sup>e</sup>	33.33 <sup>f</sup>
	$4 \times 10^5$	30.00 <sup>cd</sup>	36.66 <sup>ef</sup>	46.66 <sup>d</sup>	46.66 <sup>d</sup>	50.00 <sup>e</sup>
	$6 \times 10^5$	36.66 <sup>cd</sup>	50.00 <sup>cde</sup>	63.33 <sup>bc</sup>	66.66 <sup>bc</sup>	66.66 <sup>bcd</sup>
<i>T. harzianum</i>	$2 \times 10^5$	33.33 <sup>cd</sup>	40.00 <sup>de</sup>	43.33 <sup>d</sup>	50.00 <sup>d</sup>	60.00 <sup>cde</sup>
	$4 \times 10^5$	43.33 <sup>bc</sup>	56.66 <sup>bcd</sup>	63.33 <sup>bc</sup>	70.00 <sup>b</sup>	70.00 <sup>bc</sup>
	$6 \times 10^5$	56.66 <sup>ab</sup>	63.33 <sup>abc</sup>	70.00 <sup>ab</sup>	73.33 <sup>ab</sup>	76.66 <sup>ab</sup>
<i>V. albo-atrum</i>	$2 \times 10^5$	40.00 <sup>bc</sup>	46.66 <sup>cde</sup>	53.33 <sup>cd</sup>	66.66 <sup>bc</sup>	73.33 <sup>abc</sup>
	$4 \times 10^5$	66.66 <sup>a</sup>	70.00 <sup>ab</sup>	76.66 <sup>ab</sup>	76.66 <sup>ab</sup>	80.00 <sup>ab</sup>
	$6 \times 10^5$	73.33 <sup>a</sup>	76.66 <sup>a</sup>	83.33 <sup>a</sup>	86.66 <sup>a</sup>	86.66 <sup>a</sup>
<b>Control</b>		0.00 <sup>e</sup>	0.00 <sup>g</sup>	0.00 <sup>f</sup>	0.00 <sup>f</sup>	0.00 <sup>g</sup>
<b>P</b>		.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>
<b>LSD<sub>0.05</sub></b>		1.78	1.78	1.63	1.58	1.49

For all fungi, the mortality rate increased with increasing concentration and also with increasing the experimental period. Concurrently, no mortality occurred in the control until the end of the experiment. Moreover, a highly significant difference was recorded between the concentrations of tested fungi in comparison with the control at the different experimental periods. The obtained results in Tables 1 and 2 are in agreement with those published by (Hend, 2013) indicating that the fungus, *V. albo-atrum* has significantly affected the juveniles (6 months) age of *E. vermiculata* snail and recorded the highest mortality 63.33% after one week of treatment. It was followed by *T. harzianum*, *T. album* and *M. anisopliae* which caused 56.66, 33.33 and 23.33% mortality after the same period, respectively. On the other hand, *P. lilacinus* achieved the lowest molluscicidal activity against juveniles with a record only 13.33% mortality. However, both *Paecilomyces variotii* and *Trichoderma viride* didn't cause any mortality in juveniles. The mortality of juveniles

increased gradually with increasing the experimental time. Similarly, *T. harzianum* attained 83% mortality of *Cochlicella acuta* juveniles at the age of 6 months. Moreover, it caused 71% mortality of *Theba pisana* juveniles at the same age. Whereas, the other fungi; *Aspergillus phoenicis* and *Aspergillus terreus* were recorded 50 & 37 and 71 & 40% mortality of *C. acuta* and *T. pisana* juveniles, respectively (Hend, 2007). In a related study, *Aspergillus flavus* showed 80% mortality of *Monacha cartusiana* juveniles after 21 days of treatment. It was followed by *A. terreus* and *Aspergillus kiliense* which caused 60 and 46.67% mortality of juveniles after the same period of treatment, respectively (Abd El-Magied, 2009). Another notable study reported by Roberts and Humber (1981) showed that *M. anisopliae* caused rapid death of juvenile snails by penetrating and multiplying inside it. It can invade the juvenile body, digest its tissues and turn them into diffusible nutrients and this is also accompanied by the release of toxins by the fungus. The other fungus, *P. lilacinus* caused a significant lethal effect against the juveniles of *Pomacea canaliculata* snail. Its molluscicidal potency is based on its secretion of hydrolysis enzymes that help fungal mycelium penetrate the cell walls of juveniles (Maketon *et al.*, 2009).

## 2. Molluscicidal Influence of Fungal Isolates Against Adult Snails Under Laboratory Conditions:

The susceptibility of adult snails to the tested fungal isolates was explored. As shown in Table 3. adults were significantly affected by the fungus *V. albo-atrum* which recorded the highest mortality 63.33% after 21 days of initial exposure at the highest concentration of  $6 \times 10^5$  spores/ml and this effect was still constant until the end of the experiment (one month). It was followed by *T. harzianum* and *M. anisopliae* which caused 60 and 43.33% mortality at the same concentration of  $6 \times 10^5$  spores/ml at the end of the experiment, respectively. Whereas, *P. lilacinus* was recorded the lowest impact against adult snails by achieving 36.66% mortality after 21 days and this percentage still constant until the end of the experiment. No mortality of snails was recorded in the control and high significant difference was recorded between the mortality percentages achieved by the tested fungi compared with the control.

**Table 3.** Molluscicidal activity of fungal isolates against adult snails under laboratory conditions:

Fungal isolates	Conc. (spores/ml)	Mortality % after periods in days				
		3	7	14	21	30
<i>M. anisopliae</i>	$2 \times 10^5$	16.66 <sup>cde</sup>	16.66 <sup>ef</sup>	23.33 <sup>ef</sup>	26.66 <sup>gh</sup>	30.00 <sup>fg</sup>
	$4 \times 10^5$	23.33 <sup>bcd</sup>	26.66 <sup>cdef</sup>	30.00 <sup>def</sup>	36.66 <sup>efg</sup>	36.66 <sup>ef</sup>
	$6 \times 10^5$	30.00 <sup>abc</sup>	33.33 <sup>cd</sup>	40.00 <sup>bcd</sup>	40.00 <sup>def</sup>	43.33 <sup>cde</sup>
<i>P. lilacinus</i>	$2 \times 10^5$	10.00 <sup>de</sup>	13.33 <sup>fg</sup>	20.00 <sup>f</sup>	20.00 <sup>h</sup>	23.33 <sup>g</sup>
	$4 \times 10^5$	16.66 <sup>cde</sup>	20.00 <sup>def</sup>	26.66 <sup>ef</sup>	30.00 <sup>fgh</sup>	30.00 <sup>fg</sup>
	$6 \times 10^5$	23.33 <sup>bcd</sup>	30.00 <sup>cde</sup>	33.33 <sup>cde</sup>	36.66 <sup>efg</sup>	36.66 <sup>ef</sup>
<i>T. harzianum</i>	$2 \times 10^5$	20.00 <sup>cd</sup>	26.66 <sup>cdef</sup>	33.33 <sup>cde</sup>	40.00 <sup>def</sup>	40.00 <sup>def</sup>
	$4 \times 10^5$	26.66 <sup>bcd</sup>	36.66 <sup>bc</sup>	50.00 <sup>ab</sup>	53.33 <sup>abc</sup>	53.33 <sup>abc</sup>
	$6 \times 10^5$	40.00 <sup>ab</sup>	50.00 <sup>ab</sup>	56.66 <sup>a</sup>	56.66 <sup>ab</sup>	60.00 <sup>ab</sup>
<i>V. albo-atrum</i>	$2 \times 10^5$	26.66 <sup>bcd</sup>	33.33 <sup>cd</sup>	43.33 <sup>bc</sup>	43.33 <sup>cde</sup>	46.66 <sup>cde</sup>
	$4 \times 10^5$	40.00 <sup>ab</sup>	40.00 <sup>abc</sup>	43.33 <sup>bc</sup>	50.00 <sup>bcd</sup>	50.00 <sup>bcd</sup>
	$6 \times 10^5$	46.66 <sup>a</sup>	53.33 <sup>a</sup>	60.00 <sup>a</sup>	63.33 <sup>a</sup>	63.33 <sup>a</sup>
<b>Control</b>		0.00 <sup>e</sup>	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 <sup>i</sup>	0.00 <sup>h</sup>
<b>P</b>		.0000 <sup>**</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>
<b>LSD<sub>0.05</sub></b>		1.82	1.44	1.26	1.17	1.07

These results were supported by Almeida (2000) who stated that *V. albo-atrum* was the most potent fungus against the adults of *Helix aspersa* and *Helix pomatia* snails. The other fungus, *M. anisopliae* considered also a successful biocontrol agent against snails. At the same trend, Abd El-Aal (2001) demonstrated that the adult snails of *M. cartusiana* were more susceptible to *Beauveria bassiana* and *T. harzianum* fungi. They caused 29.06 and 22.09% mortality of both snail species after 7 days of the infection, respectively. Moreover, the adults of *C. acuta* and *T. pisana* were more affected also by *T. harzianum*. It was recorded 100 and 40% mortality at the concentration  $3 \times 10^5$  spores/ml for the two snails consecutively. The pathogenic ability of this fungus against pests is due to its production of hydrolytic enzymes, toxins and antibiotics which play a major role in the exterminatory action against pests (Hend, 2007). On the other hand, *Fusarium oxysporum*, *Aspergillus versicolor* and *Mucor hiemalis* fungi recorded a high potential against the adult individuals of *H. aspersa* snail (Sanchez *et al.*, 2012). Similarly, *M. anisopliae* recorded 56.66 and 71.66% mortality of *Helicella vestalis* adults after four weeks of the infection with concentrations of  $2.5 \times 10^{10}$  and  $5 \times 10^{10}$  conidia/ml, respectively (Ismail, 2011). This fungus has been observed ability to kill land snails due to its production of volatile organic compounds such as 1-octene, 3-octanone and 1-octen-3-ol that showed significant molluscicidal potency against the adults of *Cornu aspersum* snail (Salim *et al.*, 2019). Interestingly, the lethal effect of each fungus differs from the other and this depends on the quantity and speed of secretion of hydrolytic enzymes which play a main role in the pathogenic activity of fungus against pests (Bekheit and Abo El-Abbas, 2001).

### 3. Molluscicidal Efficacy of Fungal Isolates Against Adult Snails Under Field Conditions:

The results in Table 4. indicated that by increasing the concentration of the two fungi and the exposure period, the reduction of snails increased. Both *V. albo-atrum* and *T. harzianum* achieved their highest potency at the highest concentration of  $6 \times 10^5$  spores/ml by giving 58.06 and 55.10% reduction after one month of the experiment, sequentially. On the other hand, both fungal species recorded their lowest molluscicidal activity at the lowest concentration  $2 \times 10^5$  spores/ml by achieving 41.66 and 38.02% reduction after the same period of the trial, respectively. There is a highly significant difference between the reduction caused by both fungi through the experimental period. These findings are in accordance with Hend (2007) who indicated that *T. harzianum* reduced 66.8, 69.5 and 73.2% of *C. acuta* snails at  $0.5 \times 10^5$ ,  $1.5 \times 10^5$ ,  $3 \times 10^5$  spores/ml concentrations of the fungus after 21 days of spraying in the field, respectively. On the contrary, Abd El-Aal (2001) confirmed *B. bassiana* and *T. harzianum* reduced only 20 and 11% of *M. cartusiana* snails after 15 days of the field trial, respectively. The same fungal species caused a 20.01 and 11.09% reduction of *M. cartusiana* snails in the field, respectively.

**Table 4.** Molluscicidal activity of fungal isolates against adult snails under field conditions:

Fungal isolates	Conc. (spores/ml)	Reduction % after periods in days				
		3	7	14	21	30
<i>T. harzianum</i>	$2 \times 10^5$	0.00 <sup>e</sup>	16.50 <sup>e</sup>	29.16 <sup>e</sup>	33.59 <sup>e</sup>	38.02 <sup>e</sup>
	$4 \times 10^5$	13.20 <sup>d</sup>	26.13 <sup>c</sup>	33.79 <sup>d</sup>	40.23 <sup>c</sup>	46.87 <sup>c</sup>
	$6 \times 10^5$	25.70 <sup>b</sup>	32.56 <sup>b</sup>	45.40 <sup>b</sup>	48.70 <sup>b</sup>	55.10 <sup>b</sup>
<i>V. albo-atrum</i>	$2 \times 10^5$	13.88 <sup>d</sup>	22.22 <sup>d</sup>	30.55 <sup>e</sup>	36.11 <sup>d</sup>	41.66 <sup>d</sup>
	$4 \times 10^5$	21.42 <sup>c</sup>	30.95 <sup>b</sup>	35.71 <sup>c</sup>	40.47 <sup>c</sup>	45.23 <sup>c</sup>
	$6 \times 10^5$	38.70 <sup>a</sup>	45.16 <sup>a</sup>	48.38 <sup>a</sup>	54.83 <sup>a</sup>	58.06 <sup>a</sup>
<b>P</b>		.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>	.0000 <sup>***</sup>
<b>LSD<sub>0.05</sub></b>		1.75	2.05	1.68	1.54	2.04



Moreover, the biocide abamectin achieved slight molluscicidal activity against *E. vermiculata* and *M. obstructa* snails under field conditions (Mahrous *et al.*, 2002). This may be due to the effect of weather factors on the compound decomposition, which reduces its lethal effect against snails (Ahmed, 2008). In contrast, Gabr *et al.* (2006) revealed that *E. vermiculata* snails were more susceptible to spinosad when it was applied by spraying in the field than the other snail species, *T. pisana* and *M. obstructa*. Another related study reported that *B. bassiana* and *A. flavus* caused a 45.19 and 41.68% reduction of *M. cartusiana* snails after 28 days of the field application, respectively (Abd El-Magied). On the other hand, *M. anisopliae* killed 59.99 and 69.92% of *H. vestalis* snails at  $2.5 \times 10^{10}$  and  $5 \times 10^{10}$  conidia/ml after 4 weeks of the field experiment, respectively (Ismail, 2011). In the same trend, Babou and Jayakumar (2009) stated that *Trichoderma viride* has a significant molluscicidal impact against land snails in the field. The other fungal species; *P. lilacinus* and *Trichoderma album* at  $4 \times 10^3$  spores/ml caused 21.66 & 53.33% and 22.21 & 24.55% reduction of *E. vermiculata* and *M. cartusiana* snails after four weeks of the field trial, respectively. The lowest concentrations of  $2 \times 10^3$  and  $1 \times 10^3$  spores/ml of both fungi achieved 20 & 13.33% and 38.33 & 20% reduction of *E. vermiculata* snails after the same period of application, respectively. While, the same two concentrations recorded 16.66 & 9.71% and 14.03 & 12.27% reduction of *M. cartusiana* snails after the same period of the field experiment, respectively (Hend, 2013).

## CONCLUSION

The fungal isolates in the current study especially *Verticillium albo-atrum* and *Trichoderma harzianum* caused significantly molluscicidal potency against the different ages of *Eobania vermiculata* snail. The safety and efficacy of these isolates, make them excellent candidates for use as environmentally friendly molluscicidal agents for control land snails.

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## ARABIC SUMMARY

## الفطريات كعوامل مكافحه بيولوجيه ضد القوقع الأرضي إيوبانيا فيرميكبولاتا

هند شكري غريب

معهد بحوث وقايه النباتات - مركز البحوث الزراعيه - الدقي - جيزه - مصر

تم إجراء هذه الدراسه بهدف تقييم فعاليه بعض العزلات الفطريه الموصى بها في مجال مكافحه الأفات وهي ميتاريزيم أنيسوبلي - باسيلومييس ليلاسينس - تريكوديرما هارزيانم و فيرتيسلم ألبواترم ضد الأفراد الصغيره لقوقع إيوبانيا فيرميكبولاتا عند عمر شهرين و أربعة شهور و أيضا ضد الأفراد البالغه لنفس نوع القوقع. تم اختبار التأثير الإبادي لهذه العزلات الفطريه ضد المراحل العمرية المختلفه للقوقع و باستخدام ثلاثه تركيزات  $510 \times 2$  و  $510 \times 4$  و  $510 \times 6$  جرثومه/ملل لمدته شهر تحت الظروف المعملية و تم إختيار أكثر العزلات تأثيرا في المعمل لإختبار تأثيرها القاتل على الأفراد البالغه لنفس نوع القوقع تحت الظروف الحقلية. أظهرت النتائج أن بزيادة تركيزات جميع الفطريات المختبره و زياده فتره التعرض إزدادت نسبه موت القواقع عند جميع الأعمار و قد سبب فطر فيرتيسلم ألبواترم أعلى نسبه موت لجميع أعمار القواقع حيث سجل 90 و 86,66% موت للأفراد الصغيره عند عمر شهرين و أربعة شهور عند أعلى تركيز  $510 \times 6$  جرثومه/ملل بعد 21 يوم من المعامله بالتتابع. حقق نفس الفطر عند نفس التركيز المختبر و نفس فتره التجربه أعلى نسبه موت أيضا للأفراد البالغه بتسجيل 63,33% موت للقواقع يليه فطر تريكوديرما هارزيانم الذي حقق 83,33 و 76,66 و 60% موت للأفراد الصغيره عمر شهرين و أربعة شهور و الأفراد البالغه أيضا عند أعلى تركيز  $510 \times 6$  جرثومه/ملل بعد شهر من المعامله. في الإتجاه الأخر حقق فطر باسيلومييس ليلاسينس أقل تأثير ضد جميع أعمار القواقع حيث سجل 70 و 66,66 و 36,66% موت للأفراد الصغيره عمر شهرين و أربعة شهور و الأفراد البالغه لنفس القواقع عند أعلى تركيز مختبر  $510 \times 6$  جرثومه/ملل بعد شهر من التجربه. أوضحت النتائج أيضا أن تحت الظروف الحقلية كان التأثير الإبادي لفطر فيرتيسلم ألبواترم على الأفراد البالغه للقوقع يفوق تأثير فطر تريكوديرما هارزيانم حيث سجل 58,06% موت للقواقع عند أعلى تركيز مختبر  $510 \times 6$  جرثومه/ملل بعد شهر من التجربه الحقلية مقارنة بفطر تريكوديرما هارزيانم الذي حقق 55,10% موت للقواقع عند نفس التركيز المختبر و نفس فتره التجربه.