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RESEARCH

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Biocontrol potential of some bacterial and fungal isolates against the terrestrial snail, *Monacha obstructa*, evaluating their laboratory and field efficiency

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Abstract

Background The land snail *Monacha obstructa*, commonly known as the glassy clover snail, poses a significant threat to crops, causing substantial damage in agriculture and horticulture. It particularly thrives in regions of Egypt, where it can reproduce rapidly, leading to substantial population increases and consequent damage. This study aimed to perform laboratory experiments and field trials to assess the effectiveness of bacterial (*Bacillus thuringiensis*) and fungal (*Metarhizium anisopliae* and *Trichoderma harzianum*) isolates, as well as methomyl, a carbamate compound approved by the Ministry of Agriculture in Egypt against the terrestrial snails. Various methods including contact and leaf dipping as well as baiting techniques were applied under both laboratory and field conditions, using spray and baiting techniques.

Results *Bacillus thuringiensis* (*Bt*) and *M. anisopliae* isolates showed the most effective results outcomes in comparison with methomyl against *M. obstructa*. Out of all the application approaches tested, the residual film (contact) technique of these microorganisms proved to be the most potent, with LC_{50} values of 6.49×10^{6} cells/ml *Bt* (after 14 days), 1.24×10^{8} and 1.49×10^{8} spores/ml (*M. anisopliae*, *T. harzianum,* respectively), after 21 days and 620 ppm, for methomyl after 7 days of treatments. Besides, the findings demonstrated that *Bt* exhibited the highest success rate in decreasing the number of snails in the field. It was also observed that pathological symptoms increased as the duration of exposure increased, and the snails ceased to feed and showed a noticeable decline in their activity. A slimy, almost tan-like substance coated the exterior of their shells for *Bt*, and part of the soft body came out of the shell for *M. anisopliae*. In the case of *T. harzianum*, fungus hypha growth took on a white color formed inside the shell, and in addition, a black substance formed on the mouth of the shell.

Conclusions The gathered data revealed that *Bt* and *M. anisopliae* effectively combatted the terrestrial snail, making them viable options in integrated pest control as substitutes for pesticides.

Keywords *Monacha obstructa*, Biological control, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Trichoderma harzianum*, Pathological symptoms

Background

Molluscs have been widely distributed worldwide. Terrestrial molluscs including snails and slugs that belong to class Gastropoda have a greatly economic importance as dangerous pests in many parts of the world. Recently, some land snails are regarded as pests attacking the most agricultural fields in Egypt, causing significant damage to

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greenhouses, nurseries, fruit crops, orchards, ornamental plants, vegetables, medicinal plants, navel oranges and apple trees (Ali and Robinson 2020). The damage is represented in nibbling the plant leaves on which they feed and bore into other parts such as the roots, tubers and fruits (Abdel-Rahman 2017). The terrestrial snail, *Monacha obstructa* (Family: Hygromiidae), is the most common snail species on cultivated crops, and it is recorded in high population density on Egyptian clover, cabbage, green beans, maize and cucumber (Shoieb 2008). Chemical molluscicides are the most widely used methods against land snails, but due to their toxicity to land and aquatic life, there is a growing interest in discovering acceptable biological, eco-friendly molluscicides as a natural and environmentally safe alternative to synthetic chemicals (Ahmed et al. 2023). Nowadays, the use of environmentally safe microbes in pest management has received more attention from many authors (Kumar et al. 2021). The pathogenicity of bacteria and fungi against some land snails in Egypt has been studied by several researchers (Soliman and Ghareeb 2021). *Bacillus thuringiensis* is a gram-positive, spore-forming bacterium that, during sporulation, produces protein crystals and chemical compounds toxic to pests. It is characterized as a widespread insect pathogen. *Metarhizium* species, an entomopathogenic fungus (EPF), have products being developed for the control of insect pests in agricultural systems. The genus *Trichoderma* is currently receiving a lot of interest since it is thought to be suitable for pest control strategies and aids in avoiding the health and environmental hazards of chemical pesticides (Kumar et al. 2019). *Trichoderma harzianum*, the most prevalent species in this genus, is extensively used as a biocontrol agent against several plant pathogens and insect pests (Napitupulu et al. 2019). The objective of this study was to evaluate molluscicidal activity of native isolates of EPFs, *M. anisopliae* and *T. harzianum*, and bacteria, *B. thuringiensis*, compared to methomyl against land snails, *M. obstructa*, under laboratory and field conditions.

Methods

Laboratory evaluation

Tested snails

Adults of the land snail, *Monacha obstructa* (12mm ± 1), were collected from different infested nurseries at Sohag Governorate, Egypt. The snails were transferred into plastic bags to the laboratory of the Agriculture Zoology and Nematology Department, Faculty of Agriculture, Al-Azhar University, Assiut Branch, Egypt. The plastic containers contained moist sterilized sandy loam soil 1:1 (v:v) and fed on fresh lettuce leaves (*Lactuca sativa* L.) for 14 days to be laboratory acclimatized.

Tested materials

See Table 1.

Inoculum preparation of bacteria and fungi isolates

Culture media

The media used during this study was potato dextrose agar (PDA), consisting of the extraction of 200 ml of potatoes, 20 g of dextrose and 20 g of agar. The media was sterilized in an autoclave at 121 °C for 20 min (Difco Manual 1984).

Bacterial inoculum preparation

The isolate was cultured in Petri dishes on potato dextrose agar (PDA) medium for two days. An agar plug (5 mm in diameter) of the isolate was then taken from the margin of the bacterial colonies and transferred to an autoclaved flask (500 ml) filled with 300 ml of potato dextrose broth (PDB). The inoculated flasks were incubated at 25 °C for 24 h (Genena and Mostafa 2008). Developmental stages of bacterial isolates are illustrated in Fig. 1.

Fungi inoculum preparation

The isolates were cultured in Petri dishes on potato dextrose agar (PDA) medium for 7 days. An agar plug (5 mm diameter) of each isolate was then taken from the margin of the fungus and transferred to an autoclaved flask (500 ml) filled with 300 ml of potato dextrose

Table 1 Bacterial and fungal strains, and origin of materials used

Groups	Type	Origin
Microorganisms	Bacteria	
	Bacillus thuringiensis	Assiut university, Moubasher Mycological Center (AUMMC B-122) Assiut—Egypt
	Fungi	
	Metarhizium anisopliae	Assiut University, Moubasher Mycological Center (AUMMC5130) Assiut—Egypt
	Trichoderma harzianum	Al-Azhar University (Assiut branch) Faculty of Agriculture, Department of Agricultural Botany (Plant Pathology) Assiut – Egypt
Synthetic chemicals	Methomyl 90 SP	Origin: China and the importing company: Al-Huda Pesticides and Chemicals Factory. Address: Beheira—Egypt

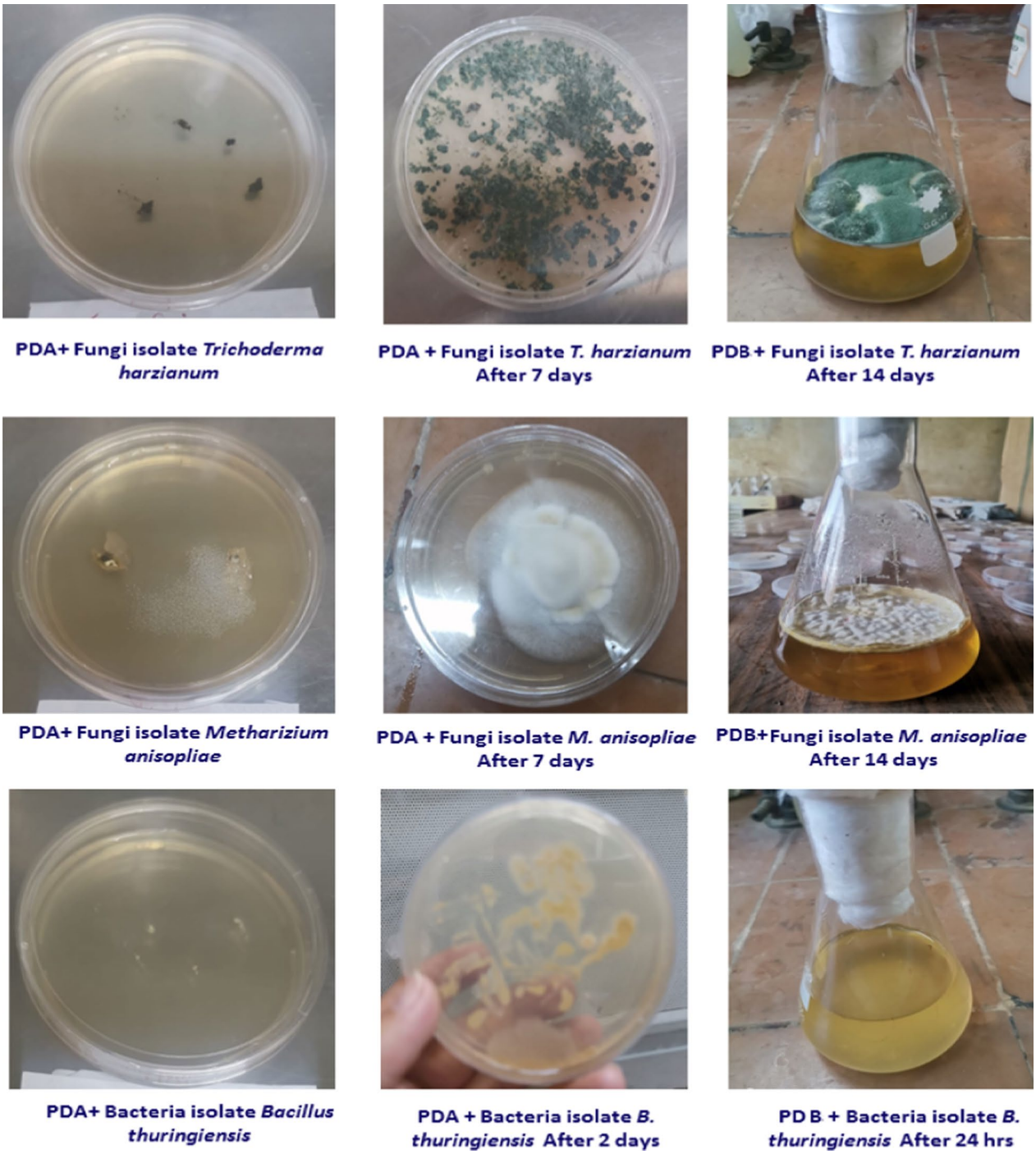

Fig. 1 Stages of development of fungal and bacterial isolates used. (PDB: Potato dextrose broth; PDA: Potato dextrose agar)

broth. The inoculums were obtained from two-week old cultures grown in the inoculated flasks, which were incubated at 25 °C (Moussa et al. 2014). The developmental stages of fungal isolates are illustrated in Fig. 1.

Application methods

Residual film technique

The residual film (contact) technique, as a method of application, was utilized in this investigation as described by Ascher and Mirian (1981). Ten adult snails with

approximately similar sizes were introduced in each Petri dish for the control group using free water suspension. The tested concentrations were 0.25×10^8, 0.5×10^8, 1×10^8 and 2×10^8 spores/ml for (*M. anisopliae* and *T. harzianum*), and 1.75×10^6, 3.5×10^6, 7×10^6 and 14×10^6 cells/ml for (*Bt*), while methomyl (90% SP) was used at concentrations of 500, 1000, 2000 and 2500 ppm. Two ml of each concentration were gently spread on the inner surface of a Petri dish. The water evaporated under room conditions within a few min, leaving a thin layer film at different concentrations for each material.

Leaf dipping technique

Three plastic boxes were used for each concentration, with each box containing 1/2 kg of sterilized clay soil. The tested concentrations for microorganisms were 0.25×10^8, 0.5×10^8, 1×10^8 and 2×10^8 spores/ml for (*M. anisopliae* and *T. harzianum*), and 1.75×10^6, 3.5×10^6, 7×10^6 and 14×10^6 cells/ml for (*Bt*), while methomyl (90% SP) was used at concentrations of 500, 1000, 2000 and 2500 ppm. For microorganisms, the soil was incorporated with 20 ml at different concentrations for each isolate and then sprayed on the soil of each treated plastic box (Aboul-nasr 2014). The soil moisture content was 75% of the water field capacity. Ten adult snails with approximately (similar sizes) were introduced into the box. Similar pieces of green lettuce leaves were dipped in glass jars containing 100ml of the tested material for 5 s at different concentrations for each material and air-dried before being introduced to land snails. For the control test, the lettuce fresh leaves were dipped in a water suspension free from any material. Each box was then covered with muslin cloth held by rubber bands.

Poisonous baiting technique

For the poisonous baits experiment, four concentrations of microorganisms (0.25×10^8, 0.5×10^8, 1×10^8 and 2×10^8 spores/g) were used for fungi (*M. anisopliae* and *T. harzianum*), and 1.75×10^6, 3.5×10^6, 7×10^6 and 14×10^6 cells/g for bacteria (*Bt*). Moreover, four concentrations of methomyl 90% SP (5000, 10,000, 20,000 and 30,000 ppm) were examined. The poisonous baits were prepared by mixing a concentration of each pathogen with 5 parts of black sugarcane syrup; then, the mixture was incorporating with wheat bran to be finally 100 parts (Said et al. 2023). The bait was moistened with the appropriate amount of water to form a crumbly mash mixture. Three plastic boxes were used for each concentration. Each box contained 1/2 kg of sterilized clay soil. For microorganisms, the soil was incorporated with 20 ml at different concentrations for each isolate and sprayed on the soil of each treated plastic box (Aboul-nasr 2014). The soil moisture content was 75% of the water field capacity. About 20 g of bait was weighed and spread into the box. The control treatment was prepared using bran bait free from any material. Ten adult snail individuals with approximately similar sizes were introduced into the box. Each box was then covered with muslin cloth held by rubber bands.

Field evaluation

Bacillus thuringiensis and *M. anisopliae* strains which exhibited the highest toxicity in the laboratory were chosen to be compared to the methomyl compound for evaluation against *M. obstructa* under field conditions using spray and poisonous baiting techniques.

Spray technique

The trial was performed in the Dar El-Salam district, Sohag Governorate, Egypt, during April 2023. The spraying method was used in a heavily infested area (two feddans cultivated with orange and mandarin trees). *Bt* and *M. anisopliae* were applied at the concentrations of 14×10^6 cells/ml and 2×10^8 spores/ml respectively, while methomyl was applied at the concentration of 2500 ppm. The orchard was irrigated four days before treatment. Five trees were selected for each treatment. Four treatments were carried out, including *Bt*, *M. anisopliae* and methomyl, in addition to the control (Mostafa 2020).

Poisonous baiting technique

A concentration of 14×10^6 cells/g for *Bt* and 2×10^8 spores/g for *M. anisopliae*, and a concentration of 30,000 ppm for methomyl were used as baits in an Egyptian clover field heavily infested with *M. obstructa* snails at Dar El-Salam district, Sohag Governorate, Egypt. An area of two feddans (four plots), including a control, and an area were left as a buffer between each plot and the other (Mortada et al. 2013). Poison baits were prepared as follows: the amount of the tested material + an appropriate weight of bran + 5% sugar cane syrup to give 100 parts of bait, about 200 g of the bait was placed on plastic sheets (50 × 50 cm) and distributed in the experimental plots at known distances (El-Sayed 2010). The number of live snails per 0.25 m^2 was recorded before application and after 7, 14 and 21 days post-treatment.

Data analysis

Mortality percentages were determined and corrected employing Abbott's formula (1925). Toxicity lines and LC_{50} values were statistically analyzed after 21 days for fungi and 14 days for bacteria, while methomyl compound was analyzed after 7 days post-treatment according to Finney (1971) using "LdP Line"® software. Furthermore, we used Costat Statically computer program to determine LSD test at a significance level of

0.05. Regarding the field evaluation, population reduction percentages were calculated according to the formula given by Henderson & Tillton (1955).

Results

Efficiency of some microorganisms on *Monacha obstructa* snails using residual film technique under laboratory conditions

As shown in Table 2, the results showed that *Bt*, *M. anisopliae* and *T. harzianum* exhibit molluscicidal effects against the *M. obstructa* snail, and they were concentration-dependent. The survival rate of *M. obstructa* snails significantly decreased with increasing concentrations. The highest mortalities were recorded at the highest concentrations (14×10^6 cells/ml for *Bt* and 2×10^8 spores/ml for the two fungus) with values 60.00, 53.33 and 36.67% for *Bt*, *M. anisopliae* and *T. harzianum*, after 14 days post-treatment, respectively, while there were 63.33 and 60.00% mortality rates for *M. anisopliae* and *T. harzianum*, respectively, after 21 days. The tested concentrations of *Bt*, *M. anisopliae* and *T. harzianum* in this study did not cause any mortalities after one day of treatment. The lowest concentrations (1.75×10^6 cells/ml for *Bt* and 0.25×10^8 spores/ml for 2 fungi) of *Bt*, *M. anisopliae* and *T. harzianum* resulted in mortality rates of 16.67, 10.00 and 0.0%, respectively, after 14 days. After 21 days, *M. anisopliae* and *T. harzianum* recorded 16.67 and 6.67% mortalities, respectively.

Microorganisms efficiency against *Monacha obstructa* snails using leaf dipping technique under laboratory conditions

The results showed that the mortality rates increased with increasing concentrations of microorganisms and exposure time (Table 3). After 14 days post-treatment, the mortality percentages of *M. obstructa* were recorded with *Bt*, *M. anisopliae* and *T. harzianum* with values of 46.67, 46.67 and 40.00% at the highest concentration, respectively, while the mortality rates after 21 days were 53.33, 46.67 for *M. anisopliae* and *T. harzianum*, respectively.

Microorganisms efficiency against *Monacha obstructa* snails using poisonous baiting technique under laboratory conditions

Data in Table 4 revealed that at day 7 post-treatment, *Bt*, *M. anisopliae* and *T. harzianum* exhibited molluscicidal efficacy against *M. obstructa*. The percentages of mortality were 33.33, 30.00 and 30.00 % at the highest concentrations ($14x10^6$ cells/ml for *Bt* and $2x10^8$ spores/ml for the 2 fungi), respectively, after 14 days of treatments. The results showed that mortalities increased with increasing microorganism concentrations and exposure time. *Bt* was found to be more efficient against *M. obstructa*, than *M. anisopliae* and *T. harzianum* 14 days post-treatment. Moreover, after 21 days, the mortality rates for *M. anisopliae* and *T. harzianum* were 50.00 and 46.67%, respectively.

Table 2 Impact of some microorganisms against *Monacha obstructa* using residual film technique under laboratory conditions after 1, 7, 14 days for bacteria and 21 days for fungi

Isolates	Concentrations cells/ml	% Mortality (/Days)			
Bacteria		1day	7days	14days	21days
Bacillus thuringiensis	1.75×10^6	0.00	13.33	16.67	–
	3.5×10^6	0.00	40.00	50.00	–
	7×10^6	0.00	43.33	53.33	–
	14×10^6	0.00	46.67	60.00	–
Fungi	Spores/ml	1day	7days	14days	21days
Metarhizium anisopliae	0.25×10^8	0.00	6.67	10.00	16.67
	0.5×10^8	0.00	13.33	13.33	26.67
	1×10^8	0.00	20.00	23.33	43.33
	2×10^8	0.00	36.67	53.33	63.33
Trichoderma harzianum	0.25×10^8	0.00	0.00	0.00	6.67
	0.5×10^8	0.00	6.67	6.67	16.67
	1×10^8	0.00	13.33	23.33	36.67
	2×10^8	0.00	30.00	36.67	60.00

Table 3 Impact of some microorganisms against *Monacha obstructa* using leaf dipping technique under laboratory conditions after 1, 7, 14 days for bacteria and 21 days for fungi

Isolates	Concentrations cells/ml	% Mortality			
Bacteria		1day	7days	14days	21days
Bacillus thuringiensis	1.75×10^6	0.00	10.00	13.33	–
	3.5×10^6	0.00	13.33	16.67	–
	7×10^6	0.00	26.67	30.00	–
	14×10^6	0.00	43.33	46.67	–
Fungi	Spores/ml	1day	7days	14days	21days
Metarhizium anisopliae	0.25×10^8	0.00	13.33	23.33	23.33
	0.5×10^8	0.00	16.67	20.00	33.33
	1×10^8	0.00	20.00	36.67	43.33
	2×10^8	0.00	30.00	46.67	53.33
Trichoderma harzianum	0.25×10^8	0.00	6.67	10.00	16.67
	0.5×10^8	0.00	10.00	13.33	26.67
	1×10^8	0.00	20.00	23.33	30.00
	2×10^8	0.00	30.00	40.00	46.67

Table 4 Impact of some microorganisms against *Monacha obstructa* using bait technique under laboratory conditions after 1, 7, 14 days for bacteria and 21 days for fungi

Isolates	Concentration cells/g	% Mortality			
Bacteria		1day	7days	14days	21days
Bacillus thuringiensis	1.75×10^6	0.00	0.00	6.67	–
	3.5×10^6	0.00	3.33	13.33	–
	7×10^6	0.00	10.00	16.67	–
	14×10^6	0.00	16.67	33.33	–
Fungi	Spores/g	1day	7days	14days	21days
Metarhizium anisopliae	0.25×10^8	0.00	0.00	0.00	6.67
	0.5×10^8	0.00	0.00	10.00	13.33
	1×10^8	0.00	10.00	16.67	20.00
	2×10^8	0.00	26.67	30.00	50.00
Trichoderma harzianum	0.25×10^8	0.00	0.00	0.00	13.33
	0.5×10^8	0.00	0.00	0.00	16.67
	1×10^8	0.00	3.33	6.67	23.33
	2×10^8	0.00	20.00	30.00	46.67

Comparative efficacy of the tested fungal and bacterial isolates and methomyl against *Monacha obstructa*

Data in Table 5 demonstrate the pathogenicity of certain microorganisms as compared to methomyl using three different methods. The effect of *Bt*, *M. anisopliae* and *T. harzianum* and the methomyl compound on the land snails *M. obstructa* was determined under laboratory conditions after 14 days for *Bt*, 21 days for *M. anisopliae* and *T. harzianum* and after 7 days for methomyl. In the case of residual film method, the highest LC_{50} values were observed by methomyl (620 ppm), followed by *Bt* (6.49×10^6 cells/ml), *M. anisopliae* (1.24×10^8 spores/ml) and *T. harzianum* (1.49×10^8 spores/ml). Furthermore, when applied using the leaf dipping technique under laboratory conditions, the LC_{50} values were 17.97×10^6 cells/ml, 1.57×10^8 spores/ml, 2.80×10^8 spores/ml and 750 ppm for *Bt*, *M. anisopliae*, *T. harzianum* and methomyl, respectively. On the other hand, in the poisonous baiting technique, methomyl exhibited the highest toxic effect with an LC_{50} value of 2900 ppm. *Bt* and *M. anisopliae* had a moderate effect with an LC_{50} value of 37.50×10^6 cells/g, 2.34×10^8 spores/g, respectively, while *T. harzianum* had a slight effect, with LC_{50} value of 2.98×10^8 spores/g. In the present results, microorganisms exhibited the highest pathogenicity against the tested snail using the residual film method than poison baits and leaf dipping methods. Methomyl was the most toxic in all techniques.

Pathological symptoms of *Monacha obstructa* snails treated with tested microorganisms

After exposure to the bacteria *Bt*, it was noticed that they ceased feeding, and their activity decreased significantly with increasing exposure time. A light brown mucous secretion and a light brown unknown substance were present on the outer surface of the shell (Fig. 2B). Regarding *M. anisopliae*, it was observed that a part of the soft body came out of the shell. As the exposure period increased, fungus hypha growth took on a white color formed inside the shell, which could reach inside the soft part through the natural openings, consuming its internal tissues, leading to its death in the end (Fig. 2A). After exposure to *T. harzianum*, it was observed that a part of the soft body emerged from the shell, as increase in the exposure period, a black substance formed around the mouth of the shell (Fig. 2C).

Table 5 Effect of the microorganisms and methomyl against *Monacha obstructa* using three techniques under laboratory conditions after 21 days for fungi, 14 days for bacteria and 7 days for methomyl post-treatment

Technique	Microorganism/materials	LC_{50}	Slope ± SE	X^2 value
Residual film	*Bacillus thuringiensis*	6.49×10^6	0.92 ± 0.19	1.99
	Metarhizium anisopliae	1.24×10^8	1.46 ± 0.20	0.37
	Trichoderma harzianum	1.49×10^8	1.96 ± 0.23	0.06
	Methomyl	620	2.37 ± 0.27	15.57
Leaf dipping	*B. thuringiensis*	17.97×10^6	0.93 ± 0.19	1.87
	M. anisopliae	1.57×10^8	0.89 ± 0.19	0.03
	T. harzianum	2.80×10^8	0.91 ± 0.20	1.13
	Methomyl	750	2.55 ± 0.26	21.99
Poisonous baiting	*B. thuringiensis*	37.50×10^6	1.53 ± 0.21	2.37
	M. anisopliae	2.34×10^8	1.67 ± 0.24	3.66
	T. harzianum	2.98×10^8	1.15 ± 0.21	3.50
	Methomyl	2900	3.31 ± 0.71	0.23

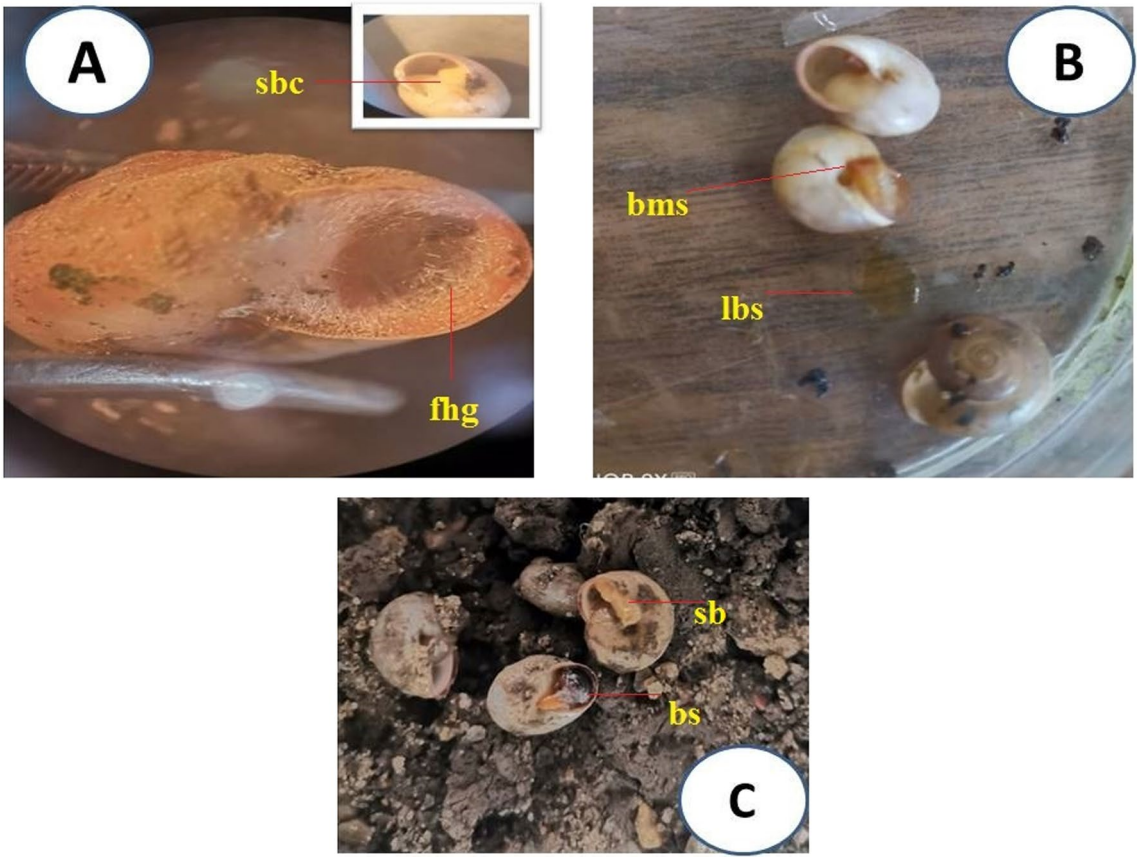

Fig. 2 Pathological symptoms of *Monacha obstructa* snails treated with tested microorganisms **A** Effect of *Metarhizium anisopliae* (sbc: soft body came out; fhg: fungus hypha growth); **B** effect of *Bacillus thuringiensis* (bms: brown mucous secretion; lbs: light brown substance); **C** effect of *Trichoderma harzianum* (bs: black substance; sb: soft body)

Field evaluation

Laboratory experiments indicated that *Bt and M. anisopliae* were the most effective microorganisms against adults of *M. obstructa*. Therefore, the two microorganisms, compared to methomyl, were applied in Egyptian clover fields using the poisonous bait technique and in orange and mandarin orchards using the spray technique for three weeks in the Dar El-Salam district, Sohag Governorate, Egypt. The reduction percentages of *M. obstructa* infesting orange and mandarin trees and Egyptian clover plants, treated with microorganisms (*Bt* and *M. anisopliae*) and methomyl after 21 days of exposure as compared to control, were 62.29, 58.67 and 73.17%, respectively, for the spray technique, while they were 43.83, 42.93 and 83.55% for the bait technique (Table 6).

Discussion

Land snails have become increasingly significant as crop pests in agriculture. Land snails that cause damage to plants have received the attention of many studies to find appropriate control measures. The use of microbial control presents a potentially valuable alternative to the high cost, potential pest resurgence, development of resistance and environmental contamination associated with chemical compounds. Microorganisms represent a crucial component of biological control techniques (Moussa et al. 2014). They have low pathogenicity to the environment and ecosystem, low probability of target pests building up resistance and low cost (Rodrigues et al. 2016). Our findings are consistent with Genena and Mostafa (2008) who investigated the molluscicidal activity of the bacterial strain *B. thuringiensis*, which was the most toxic and killed 53% of the land snail *M. cantiana* after two weeks from exposure at a concentration of 1×10^8 cfu/ml. Similarly, Mortada et al. (2012) demonstrated that the biocides Biogard (*Bacillus thuringiensis*) reduced the population density of *Monacha sp.* on pea plantations to reach 23.25% after 14 days. Furthermore, El-Sabbagh et al. (2013) found that under laboratory conditions, *B. thuringiensis* caused 60% mortality against *M. cartusiana* after 14 days at a concentration of 2×10^5 cfu/

Table 6 Molluscicidal activity of certain microorganisms and methomyl against *Monacha obstructa* snails under filed conditions

Microorganism/materials	Conc	Population reduction percentage			Mean
		7day	14days	21days	
Spray technique					
Bacillus thuringiensis	14×10^6 cells/ml	36.03	57.70	62.29	52.00
Metarhizium anisopliae	2×10^8 spores/ml	28.26	41.40	58.67	42.78
Methomyl 90% SP	2500 ppm	59.74	70.37	73.17	67.76
Baits technique					
B. thuringiensis	14×10^6 cells/g	22.31	54.69	43.83	40.27
M. anisopliae	2×10^8 spores/g	33.78	34.96	42.93	37.22
Methomyl 90% SP	30,000 ppm	91.53	93.83	83.55	89.63

ml. Ghareeb and Lokma (2017) reported that methomyl gave a 56.60% reduction of *M. cartusiana* snails seven days after application. In the present study, it is obvious that there are variations in pathogenicity according to the type of pathogen and method of application. These differences in pathogenicity levels may be attributed to various classes of microorganisms responsible for molluscicidal activities. Several authors have previously investigated the impact of microorganisms on terrestrial gastropods. For instance, Hendawy et al. (2015) assessed the molluscicidal activity of native isolates of EPFs, specifically comparing *M. anisopliae* to methomyl against land snails *Monacha* spp. under laboratory and field conditions. The laboratory results indicated that *M. anisopliae* had a moderate effect compared to the methomyl. Moreover, under field conditions, methomyl emerged as the most potent compound for reducing the population of *Monacha cantiana*.

Ahmed et al. (2023) tested the fungal isolate *T. harzianum* against the land snail *M. cartusiana* under laboratory and field conditions. *T. harzianum* exhibited molluscicidal activity 7 and 21 days after exposure, respectively. It was also found that as the concentration of *T. harzianum* increased, the survival rate of *M. cartusiana* snails significantly decreased. Ali et al. (2017) reported mortality rates of 56.67 and 39.98%, respectively, against the land snails *Succinea putris* and *Eobania vermiculata*, four weeks post-treatment with *Trichoderma album.* Abd El-Atti et al. (2020) found that the fungus biozed has molluscicidal action against *M. cartusiana*, with a 66.6% mortality rate at high concentrations. In a recent study by Gaber et al. (2022), it was found that *Bt* demonstrated potent pathogenicity against the land snail *M. cartusiana*; interestingly, the mortality rate was found to significantly increase with both the concentration of bacteria and the duration of exposure. Ghareeb (2023) investigated the molluscicidal activity of *Bacillus subtilis* and *T. album* against *M. cartusiana* snails. No mortalities of snails were recorded by each bio-agent until the third day of the experiment. *B. subtilis*, after the seventh day, caused 40% mortality of snails, and the mortality increased up to 63.33% on day 21 of the treatment. On the other hand, *T. album* showed only 16.66% mortality at the lowest concentration after the seventh day of the experiment. Few studies have generally examined the pathogenic symptoms of snails treated with microorganisms, particularly those involving the microorganisms under investigation. Duval et al. (2015) found white nodules in various regions (the ovotestis, hepatopancreas and mantle) of *Biomphalaria glabrata* snails with bacterial infections, leading to high mortality in breeding tanks.

Conclusions

Microorganisms, such as fungi and bacteria, have been shown a significant impact against clover snails *Monacha obstructa.* Utilizing these microorganisms as a means of controlling land snails presents an effective solution without resorting to the harmful use of chemical molluscicides. The use of *M. anisopliae* and *Bt* has proven effective in integrated pest control programs, reducing the reliance on chemical pesticides that pose risks to both humans and the environment. More studies are needed to detect the histological, biochemical and even ultrastructural changes.

Abbreviations

°C	Degree Celsius
Bt	*Bacillus thuringiensis*
EPF	Entomopathogenic fungus
Hrs	Hour
LC_{50}	Lethal concentrations, which cause 50% mortality in the population
L.S.D	Least significant different
PDB	Potato dextrose broth
PDA	Potato dextrose agar
SP	Soluble powder

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Author contributions
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References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18(2):265–267
Abd El-Atti MS, Khalil AM, Elsheakh AM, Elgohary WS (2020) Biological control of *Monacha cartusiana* "glassy clover land snails" by microbial biopesticides Biozed and Biogard, using bait technique. Biocata Agric Biotechnol 25:101572. https://doi.org/10.1016/j.bcab.2020.101572
Abdel-Rahman AHE (2017) Efficiency of some natural plant extracts and ferrous sulphate in controlling the land snail (*Monacha cartusiana*) under laboratory and field conditions at Sharkia Governorate, A. R. Egypt. J Plant Prot Path 8(12):647–650
Ahmed HY, Al-Quraishy S, Hassan AO, Abdel-Baki AS, Abdel-Tawab H (2023) Biocontrol potential of *Trichoderma harzianum* against the land snail *Monacha cartusiana*: lab and field trails. Intl J Agric Biol 30:79–89
Ali RF, Robinson DG (2020) Four records of new to Egypt gastropod species including the first reported tropical leather leaf slug *Laevicaulis alte* (Dade Ferussac, 1822) (*pulmonata*: *Veronicellidae*). Zool Ecol 30(2):138–156. https://doi.org/10.35513/21658005.2020.2.8
Ali MA, Al-Ghnam HA, Abou El Atta D (2017) Efficiency of certain bio-agents as biological control against two land snail species, *Eobania vermiculata* (Müller) and *Succinea putris* (Linnaeus) under laboratory conditions. J Plant Prot Path 8:265–269
Ascher RS, Mirian F (1981) The residual contact toxicity of Bay Sir 8514to *Spodoptera littoralis* larva. Phytoparasitica 9(2):133–137
Duval D, Galinier R, Mouahid G, Toulza E, Allienne JF, Portela J, Calvayrac C, Rognon A, Arancibia N, Mitta G, Théron A, Gourbal B (2015) A novel bacterial pathogen of *Biomphalaria glabrata*: a potential weapon for schistosomiasis control? PLoS Negl Trop Dis 9(2):e0003489. https://doi.org/10.1371/journal.pntd.0003489
El-Sabbagh SM, Adayel SA, El-Masry SAA, Alazazy HM (2013) Biological control of some species of land snails infesting citrus trees. New York Sci J 6(7):5–12
El-Sayed AH (2010) Molluscicidal effects of some chemical compounds against *Monacha cartusiana* (Muller) and *Eobania vermiculata* (Muller) land snails under laboratory and field conditions. Egypt J Agric Res 88(4):1197–1207
Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, London, p 318
Gaber OA, Asran AA, Khider FK, El-Shahawy G, Abdel-Tawab H, Elfayoumi HMK (2022) Efficacy of biopesticide Protecto (*Bacillus thuringiensis*) (*BT*) on certain biochemical activities and histological structures of land snail *Monacha cartusiana* (Muller, 1774). Egypt J Biol Pest Control 32:36. https://doi.org/10.1186/s41938-022-00534-6
Genena MAM, Mostafa FAM (2008) Impact of eight bacterial isolates of *Bacillus thuringiensis* against the two land snails *Monacha cantiana* and *Eobania vermiculata* (Gastropoda: Helicidae). J Plant Prot Path 33:2853–2861
Ghareeb HSh (2023) Effects of some microbial agents and their mixtures against the terrestrial snails *Eobania vermiculata* and *Monacha cartusiana*. Adv Crop Sci Tech 11:623
Handerson GF, Tillton EW (1955) Test with acarides against the brown wheat mite. J Econ Entomol 48:157–161
Hendawy AS, El-Fakharany SK, Samy MA (2015) Laboratory and field evaluation of molluscicidal activity of native biological isolates compared to an insecticide against the land snails, *Monacha* spp. on lettuce and cabbage plantations. Egypt J Biol Pest Control 25:675–678
Kumar R, Kumari K, Hembram KC, Kandha L, Bindhani BK (2019) Expression of an endo α-1, 3-Glucanase gene from *Trichoderma harzianum* in rice induces resistance against sheath blight. J Plant Biochem Biotechnol 28:84–90. https://doi.org/10.1007/s13562-018-0465-7
Kumar P, Kamle M, Borah R, Mahato DK, Sharma B (2021) *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable Agriculture. Egypt J Biol Pest Control 31(95):2–7. https://doi.org/10.1186/s41938-021-00440-3
Mortada MM, Mourad AAM, Abo-Hashem AM, Keshta TMS (2012) Efficiency of certain biocides and molluscicides against *Monacha* Sp. land snails at Dakahlia Governorate. J Plant Prot and Path 3(7):717–723
Mortada MM, Daoud MI, Ali MMA, Sahawy WWI (2013) Molluscicidal activity of certain pesticides against *Monacha obstructa* Montago (Fam: Helicidae) land snails under laboratory and field conditions. J Plant Prot Path 4(12):1115–1121
Mostafa NM (2020) Evaluation of some environmentally safe methods for controlling common land snail species at Kafr El-Sheikh Governorate. J Plant Prot Res Inst 3(2):648–653
Moussa S, Abouelmaaty HG, Hamada HA, Hemieda EA (2014) Evaluation of *Bacillus thuringiensis* Cry1Ca strain and *Metarhizium anisopliae* fungus against potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera : Gelechiidae). Egypt J Biol Pest Control 24(2):515–521
Napitupulu TP, Ilyas M, Kanti A, Sudiana IM (2019) In vitro evaluation of *Trichoderma harzianum* strains for the control of *Fusarium oxysporum* f.sp. *cubense*. Plant Pathol Quar 9:152–159. https://doi.org/10.5943/ppq/9/1/13
Rodrigues IM, Forim MR, Silva MF, Fernandes JB, Filho AB (2016) Effect of ultraviolet radiation on fungi *Beauveria bassiana* and *Metarhizium anisopliae*, pure and encapsulated and bio-insecticide action on *Diatraea saccharalis*. Adv Entomol 4:151–162. https://doi.org/10.4236/ae.2016.43016
Said RM, Al-Badwy AH, Mohamed AA (2023) Oxidative and histological effects of herbicide glufosinate ammonium and cyanobacteria extracted anatoxin-a on land snails *Monacha cartusiana*. Jordan J Biol Sci 16(1):13–20. https://doi.org/10.54319/jjbs/160103
Shoieb MA (2008) Occurrence distribution of terrestrial molluscs in Suez Canal Governorate and North of Sinai. Egypt J Agric Res 86(3):989–994
Soliman MHA, Ghareeb HSh (2021) Molluscicidal effect of ammonium nitrate and some biopesticides on the brown garden snail *Eobania vermiculata* (Gastropoda: Helicidae). Egypt J Plant Prot Res Inst 4(2):322–340
Aboul-nasr GMN (2014) A study on the effect of some bacterial isolates on the land snail *Monacha obstructa*, (Mollusca, Gastropoda) as a biological control. Ph.D. Thesis, Faculty of Science, Assiut University, Egypt, p 152
Difco Manual (1984) Dehydrated culture media and reagents for microbiology, 10th Edition, Difco Laboratories, Detroit, Michigan, USA, pp 689–691
Ghareeb HSh, and Lokma MHE (2017) Antagonistic effect of some antifungal substances against fungi causing rots of the glassy clover snail baits. Nat Sci 15(7)

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