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Full Length Article

Biocontrol Potential of *Trichoderma harzianum* against the Land Snail *Monacha cartusiana*: Lab and Field Trails

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Abstract

Monacha cartusiana, a land snail, is one of most damaging pests to horticultural and agricultural crops in farmed and recently reclaimed agroecosystems in Egypt. Chemical molluscicides are the first line of defence against land snails, but due to their toxicity to both land and aquatic life, interest in discovering acceptable biological ecofriendly molluscicides has increased. As a natural and environmentally safe alternative to synthetic chemicals, the molluscicidal activity of a fungal isolate (*Trichoderma harzianum*) against the land snail, *M. cartusiana*, was tested. Under laboratory and field conditions, *T. harzianum* exhibited molluscicidal activity 7 and 21 days after exposure, respectively. After being exposed to the LC_{50} dose of *T. harzianum*, histopathological changes were observed in the digestive system, ovotestis, foot, and kidney of *M. cartusiana*. Treated snails displayed higher lipid peroxidation levels, lower glutathione content, and a significant drop in total lipid, total protein, and alkaline phosphatase. Acetylcholinesterase was also reduced in snails treated with *T. harzianum*. In conclusion, the use of *T. harzianum* against *M. cartusiana* resulted in severe toxic effects associated with a variety of biochemical and histological alterations similar to those observed with methomyl. As a result, *T. harzianum* may be used as an eco-friendly bioagent molluscicide in land snail control program instead of harmful synthetic molluscicides. © 2023 Friends Science Publishers

Keywords: Mollusca; Bioagent; Biochemical; Histological; Acetylcholinesterase

Introduction

Mollusks, the second-largest invertebrate group in the animal kingdom after arthropods, contribute significantly to global biodiversity (Abbott 1989; South 1992; Rosenberg 2014). The gastropoda are the most successful terrestrial molluscan group (Smith and Kershaw 1979). Snails and slugs, for example, are serious agricultural pests that inflict major economic harm to field crops, vegetables, and horticultural plants (Godan 1983; Feldkamp 2002; Iglesias *et al*. 2003). Land snails spread bacteria, viruses, and fungi by scratching plant parts while feeding, which has an impact on plants both directly and indirectly (Raut and Barker 2002). Chemical molluscicides are the most popular method for controlling land snails (Geasa *et al*. 2013; Castle *et al*. 2017). Due to its effectiveness and simplicity of use, methomyl is the most popular molluscicide (Hendawy *et al*. 2015; Khalil 2016). However, this molluscicide is poisonous to beneficial invertebrates and dangerous to humans, other animals, and the environment as a whole (Gabr *et al*. 2006; Moustafa *et al*. 2016). Molluscicides of natural origin are thought to be the most effective alternative to chemical molluscicides since they are less expensive and pose fewer environmental risks (Norris *et al*. 2002; Schüder *et al*. 2003; *et al*. 2013). Natural enemies such as predators, illnesses, or parasites could also be an effective long-term method for snail biological control (Moazami 2008). A few years ago, biological management with microbial agents against various land snails and slugs attracted a lot of attention (Kramarz *et al*. 2007; Genena and Mostafa 2008; Shahawy 2018). *Trichoderma* is a genus of flamentous fungi that has been extensively studied in this context, and it has been concluded that many species of *Trichoderma* can exert biological control directly through parasitism and/or indirectly through the production of toxic metabolites, antifeedant compounds, and repellent metabolites (Tyśkiewicz *et al*. 2022). *Trichoderma harzianum* is a species of the genus *Trichoderma* that has a worldwide distribution and has been widely used as a biocontrol agent against a variety of crop plant pests as well as root, shoot, and postharvest diseases (Zin and Badaluddin 2020; Poveda 2021). *T. harzianum* has been investigated against a variety

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of insect pests of agricultural crops, including the elm bark beetle, *Scolytus* spp. (Jassim *et al*. 1990). Hazaa *et al*. (2019) found that using of *T. harzianum* at a concentration of 1×10^8 spore/mL caused 80% mortality in the larvae of the African cotton leaf worm *Spodoptera littoralis*. Also, at a concentration of 4×10^8 spore/mL, *Trichoderma* sp. displayed entomopathogenic activity against *Bemisia tabaci* nymph, with a 73% mortality rate (Anwar *et al*. 2016). The biochemical investigation of *T. hamatum* spore-treated adult cotton aphids revealed different quantitative modifications in total soluble protein, transaminase enzymes, and carbohydrates hydrolyzing enzymes as compared to untreated controls (Khaleel *et al*. 2016). Binod *et al*. (2007) investigated the bioefficacy of *T. harzianum* culture filtrate containing chitinase against the Cotton bollworm, *Helicoverpa armigera*, and discovered that *T. harzianum* inhibited the insect larvae's growth and metamorphosis. Ghosh and Pal (2016) discovered that spore suspension of *T. longibrachiatum* at a concentration of 1×10^8 spores/mL inhibited the brinjal fruit and shoot borer, *Leucinodes orbonalis*, to the greatest extent (80%). Continuing this endeavour, the current study investigated for the first time the potential actions of *T. harzianum* against *M. carutisiana*.

Materials and Methods

Monacha cartusiana collection and their laboratory maintenance

Adult *M. cartusiana* snails were collected from Sids village, Beni-Suef governorate, Egypt. Snails were transported in muslin bags to the Harmful Animals Research Department at the Sids Research Station, Agriculture Research Centre (ARC), Beni-Suef, Egypt, where they were placed in plastic containers with moist sterile sandy loam soil 1:1 (v:v) and given fresh lettuce (*Lactuca sativa*) leaves for 14 days to acclimatize to the lab conditions.

Trichoderma harzianum isolation

Plant Pathology Institute (ARC, Egypt) graciously donated *T. harzianum* (Family: Hypocreaceae) cultivated on potato dextrose agar (PDA; Becton and Dickinson Co., MA, USA). Using the methods described by Barnett and Hunter (1972) and Ramirez (1982), the isolated fungus was identified at the Plant Pathology Department, National Research Centre, Egypt, and confirmed by the Fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. The culture was kept in an incubator at 25°C for mass spore production. After 15 days of inoculation, each conical flask received 100 mL of sterile water, and the solution was vigorously vortexed to dislodge the spores. Then, the spore suspensions were collected in 250 mL bottles. A hemocytometer was used to count the spores and select which ones would be used in the subsequent testing.

Gas chromatography-mass spectrometry (GC-MS) analysis

The volatile components of *T. harzianum* were examined using a Trace Ultra Gas Chromatographer paired with a DSQ II Mass Spectrometer (Thermo Scientific). Thermo Scientific's TR-5MS (30 m 0.25 mm 0.25 m) capillary column was used for the chromatographic separation of the components according to the method of Adams (1995). The compounds were identified from relevant data stored in databases from literature and equipment (Adams Book 07, Nist 98, Xcalibur). The Relative Retention Index was calculated using a range of n-alkanes (C8–C24). Relative % of the compounds have been obtained electronically from area percent data.

Toxicity test

Different spore concentrations (1×10^5, 10^6, 10^7, 10^8 conidia/mL) of the *T. harzianum* were prepared to evaluate their toxicity on the *M. cartusiana* adult snail using contact technique (thin layer film). Briefly, each concentration was distributed and gently spread in circles on the inside surface of a petri-dish (9 cm diameter). Then, petri-dishes were left for few minutes under the laboratory conditions allowing water to evaporate, leaving a thin-layer film of spores of each concentration and finally five individual snails were placed in each petri dish. Methomyl (20% SL), a carbamate compound approved for land snail management by Egypt's Ministry of Agriculture and Land Reclamation, was purchased from KZ Co. and used as a positive control treatment and to compare with *T. harzianum*. Each test was done in five replicates. After seven days of treatment, the mortality percentages were calculated and LC_{50} has been determined according to Finney (1971).

Experimental design

Snails were divided into three groups, each of five snails. The first group was treated with LC_{50} concentration of *T. harzianum* and served as *T. harzianum* treated group, the second groups was treated with the LC_{50} concentration of methomyl and served as methomyl treated group, while the third group was treated with sterilized water and served as control group. Snails were dissected after 7 days of treatment and the soft parts were isolated for the subsequent biochemical and histological studies as shown in Fig. 1.

For histological studies, samples of the digestive gland, ovotestis, kidney and foot of the surviving *M. cartusiana* snails were excised and fixed in Bouin's solution (Cas. No. 50-00-0; 64-19-7; 88-89-1). According to Mohamed and Saad (1990), these samples were first dehydrated in gradient ethanol, then embedded in paraffin wax, and finally sectioned and stained with hematoxylin and eosin. These organs' slides underwent microscopic examination for any histological changes.

For biochemical studies, 1 g of soft tissue from each

snail was homogenized in 10 mL of phosphate buffer saline. The homogenate was centrifuged for 15 min at 3000 rpm, and the supernatant was removed and stored at -30°C until the biochemical markers were detected. Alkaline phosphatase (ALP, Cat. No. AP 10 20) activity was evaluated according to Henry (1974) with commercial kit purchased from Biomed Diagnostics Company, Egypt. Total protein (Cat. No. TP 20 21) was assessed according to Kingsley (1939), meanwhile total lipids (Cat. No. TL 20 10) and cholesterol (Cat. No. CH 12 20) level were determined following the methods of Zöllner and Kirsch (1962) and Ellefson and Caraway (1976), respectively with aid of commercial kits purchased from Biomed Diagnostics Company, Egypt. Regarding the oxidative stress and antioxidant markers, glutathione (GSH, Cat. No GR 25 11) content and malondialdhyde (MDA, Cat. No MD 25 29) level were assessed colorimetrically according to the method of Beulter *et al.* (1963) and Ohkawa *et al.* (1979), respectively using commercial kits purchased from Biodiagnostic Company (Egypt).

Anti-acetylcholinesterase (AChE, Cat. No**.** E-BC-K174-M) activity was assessed according to Ellman *et al.* (1961) following the modification of Li *et al.* (2005). The inhibition percentage of AChE enzyme was determined as following: AChE inhibition (%) = 100 − ((As / Ac) × 100), where: As = AChE activity for either *T. harzianum* or methomyl treated snail, Ac = control snails.

Field study

T. harzianum at 1×10^8 was tested as a spray against *M. cartusiana* in comparison to methomyl (20 mL/L) using a knapsack sprayer, CP3 (Coopper Pegler Co. Ltd., Northumberland, England) on lettuce plants cultivated in lines with width of 50 cm in Sids village, Beni-Suef Governorate, Egypt. Throughout the experiment, spore suspension of T. harzianum was sprayed twice. Twelve plots were selected for each test (each of 30 m^2; 3/1000 ha) and each test were done in four replicates. A distance of two meters was maintained between each plot and the next. Live snails were counted on each plot's plants before treatment, as well as on days 1, 3, 7, 15 and 21 after treatment. The reduction in snail population was calculated at day 21 post-treatment using Henderson and Tilton's (1952) formula:

$$\text{Population Reduction \%} = 1 - \frac{C1 \times T2}{C2 \times T1} \times 100$$

Where, C1=number of snails in control before application, C2=number of snails in control after application, T1=number of snails in treatment before application and T2=number of snails in treatment after application.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) in SPSS (version 20) (SPSS Inc., Chicago, IL, USA). Duncan's multiple range and two tailed paired t-test were used to analyse variations between the means of different groups. Probit analysis was used to calculate the lethal concentration values and respective 95% confidence limit (CL) of LC_{50} (Finney 1971).

Results

Volatile constituents of *T. harzianum*

GC/MS analysis revealed 14 components in *T. harzianum*, with levomenthol (28.51%) being the major constituent (Table 1).

Toxicity test

The results showed that *T. harzianum*'s molluscicidal effect against the *M. cartusiana* snail was concentration dependent. It was discovered that as *T. harzianum* concentration increased, the survival rate of *M. cartusiana* snails significantly decreased (Fig. 2). The highest mortality (87.5%) was attained at the dose of 1×10^8 and the LC_{50} was reached at concentration of 1.4×10^7 (Table 2).

Acetylcholinesterase inhibition and the oxidative stress biomarkers

The GSH content of *M. cartusiana* significantly decreased ($P \leq 0.05$) following the exposure to the LC_{50} dose of *T. harzianum* while the decrease in the methomyl treated snails was non-significant. Meanwhile, *T. harzianum* and methomyl caused significant elevation in MDA level ($P \leq 0.001$). In addition, methomyl significantly inhibited the AChE; however, *the* inhibition with *T. harzianum* was not significant (Fig. 3). *T. harzianum* and methomyl both caused significant decline ($P \leq 0.05$, $P \leq 0.001$, respectively) in total protein, and alkaline phosphatase. Meanwhile, both of them don't show any effect on the cholesterol level (Fig. 4).

Histological studies

Digestive gland: The gland in control snails is composed of several digestive tubules that are lined with excretory cells, calcium cells, and digestive cells, with the latter being the most prevalent type. The different cell types are grouped in the tubules surrounding a narrow lumen (Fig. 5a). Tubular disruption and degeneration were seen in *T. harzianum*-treated snails as a result of abnormal lining cell arrangement. Some cells' apical portions detached to produce blebs, indicating cell death (Fig. 5b). As a sign of an inflammatory response, hemocytes infiltration with many vacuolated epithelial cells was observed. In the methomyl group, similar results were seen, as well as an increase in the number of secretory cells (Fig. 5c).

Table 1: The phytochemical composition of *T. harzianum* by GC-MS

Peak	R.t*	Name	Area %	Molecular Weight	Molecular formula	MF**
1	4.91	2,2'-BIPYRIDINE, 6,6'-BIS[1-(METHOXYMETHYL)ET HENYL]-	1.22	296	C18H20N2O2	772
2	5.98	1,4-Bis(trimethylsilyl)benzene	0.96	222	C12H22Si2	677
3	7.19	Benzenepropanoic acid, à-(hydroxyimino)-	1.37	179	C9H9NO3	851
4	7.51	Benzene, 1-isocyano-2-methyl-	21.96	117	C8H7N	919
5	8.12	Levomenthol	28.51	156	C10H20O	945
6	8.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	3.03	144	C6H8O4	718
7	9.75	5-Hydroxymethylfurfural	25.54	126	C6H6O3	912
8	11.76	Benzene, (isothiocyanatomethyl)-	4.45	149	C8H7NS	834
9	15.60	PHENOL, (1,1-DIMETHYLETHYL)-4-METH OXY-	3.79	180	C11H16O2	762
10	21.28	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	0.83	374	C25H42O2	776
11	27.99	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIMETHOXYPHENYL)-3,5-DIHYDROXY-7-METHOXY-	0.50	344	C18H16O7	706
12	29.05	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	0.94	324	C23H32O	778
13	31.19	Ethyl iso-allocholate	6.06	436	C26H44O5	764
14	32.24	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	0.83	440	C30H48O2	744

R.T, Retention time (min), MF** Match factor

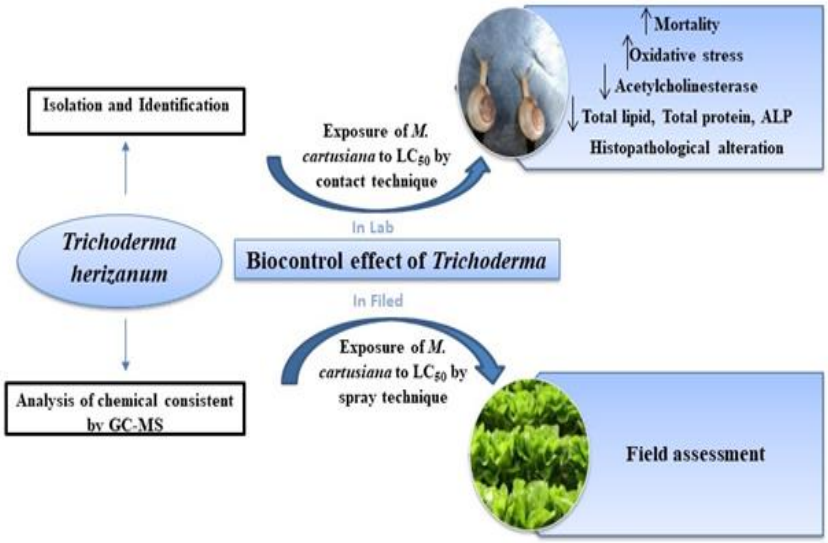

Fig. 1: Schematic diagram of the experimental setup for bio-controlling of *M. cartusiana* using *T. harzianum*

Ovotestis

The ovotestis of the snails in the untreated group was histologically normal (Fig. 5d). The ovotestis is made up of numerous finger-shaped follicles lined with follicular epithelium and separated by a thin basement membrane. The follicular germinal epithelium, which produces both gametes, lines the follicles. The follicles contain a large number of mature spermatozoa as well as spermatozoa at various stages of development. Oocytes, on the other hand, have numerous nucleoli and a large amount of yolk in their granular cytoplasm. Sertoli cells are present and line the follicles' boundary. The ovotestis of *T. harzianum* and methomyl treated snails had many malformed oocytes, degenerative abnormalities in the sperms, a decrease in sperm density in the acini, and necrosis in the sertoli cells (Figs. 5e–f).

Kidney

The kidney is situated dorsoposterior on the visceral mass's surface. The renal wall is made up of numerous and large folds that are vessels (Fig. 6b–c). Nephrocytic dilatation, signs supported by connective axes with hemolymphatic lacunae. The nephrocytes line these folds (Fig. 6a). Following *T. harzianum* and methomyl exposure, the kidney revealed cellular and conjunctival hyperplasia with significantly dilated lymphatic of cellular distress, local dilatation, and the onset of degeneration.

Foot

In the control group, the foot displayed normal histology with an external cuticular layer acting as a barrier (Fig. 7a). A layer of pseudostratifed columnar epithelium with

Table 2: Molluscicidal activity and lethal concentrations of *T. harzianum* against *M. cartusiana* snails. Data represented as means ± SEM, n = 5. Means within the same column followed by different superscripts are significantly different (Duncan's multiple range test: P: $P \leq 0.05$). (f) frequency. (df) degree of freedom

Treatment	Conc. conidia/ml.	Mortality % Mean ± SE	LC_{50} (95% CL)	LC_{90} (95% CL)	F (df = 4)	*P*
T. harzianum	1×10^5	37.5 ± 3.23^c	1.4×10^7 (- 2.9 x 10^8- 1.9 x 10^8)	1.1×10^9 (7.5 x 10^8 - 2.1 x 109)	38.31	*0.001*
	1×10^6	$43.75 \pm 3.75^{b,c}$				
	1×10^7	50.00 ± 4.56^b				
	1×10^8	87.5 ± 4.79^a				
Methomyl mg/mL	0.08	30.00 ± 12.24^b	0.18 (0.113- 0.245)	0.383(0.299-0.629)	10.92	*0.001*
	0.16	40.00 ± 10.00^b				
	0.31	80.00 ± 12.24^a				
	0.63	100.00 ± 0.00^a				
De. H_2o		00.00 ± 00.00				

Data represented as means ± SEM, n = 5. Means within the same column followed by different superscripts are significantly different (Duncan's multiple range test: P: $P \leq 0.05$). (f) frequency. (df) degree of freedom

Table 3: Field performance of *T. harzianum* against land snails, *M. cartusiana*, comparing with control after 21 days of treatment. Data represented as means ± SEM, n=5 (paired t-test P: $P \leq 0.05$)

Treatment	No. pretreatment	No. post treatment	t	P	Percent of change
Trichoderma harzianum	95.75 ± 4.89	44.75 ± 2.87	6.69	0.01	52.7%
Methomyl	90.50 ± 6.89	22.50 ± 2.10	13.56	0.001	74.00%
control	91.00 ±9.03	90.00 ± 7.55	0.28	0.79	

Data represented as means ± SEM, n = 5 (paired t-test P: $P \leq 0.05$)

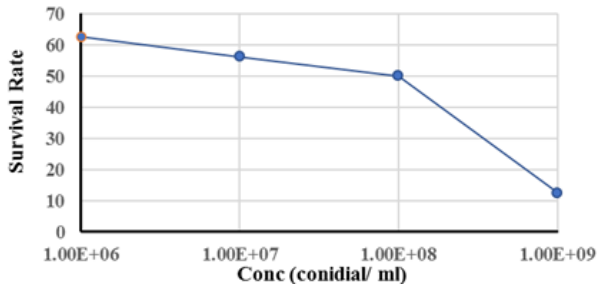

Fig. 2: Survival rate of *M. cartusiana* after exposure to different doses of *T. herizanum* conidial

unicellular glands (mucous gland) that contain basophilic secretory materials and pigment glands that contain acidophilic secretory materials is located inside of this lining. Finally, muscular fibres make up the innermost layer (Fig. 7b). *Trichoderma* or methomyl treatment resulted in the destruction of the epithelial covering migration, cellular hyperplasia, and accumulation of the pigment cells in the treated snails. Additionally, the gland and the muscle below the epithelium both showed localized necrosis (Fig. 7c).

Field studies

The field performance of *T. harzianum* against the *M. cartusiana* population is shown in Table 3. *T. harzianum* and methomyl dramatically decreased the population of *M. cartusiana* snails by 52.7 and 74%, respectively as compared to the untreated control group.

Discussion

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). *T. 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). Its potential for use was correlated with the formation of several volatile, non-volatile, antifeedant, and repellent secondary metabolites, including pyrones, sesquiterpenes, and peptaibols (Reino *et al.* 2008). As a result, the goal of this study was to identify the chemical components of *T. harzianum* and to evaluate its molluscicidal effectiveness against *M. cartusiana* in the laboratory and in the field.

The volatile compounds in *T. harzianum* have been identified in the current work using GC/MS, according to Hynes *et al*. (2007). The identified compounds belonged to the classes of monoterpenes, furanes, isocyano metabolites, alkanes, and alcohols, similar to those reported by Siddiquee *et al*. (2012). Due to the existence of several bioactive components, it is not possible to assign the biocontrol actions of *Trichoderma* spp. to a single bioactive constituent, but rather to the synergism among these various bioactive constituents (Yassin 2022).

At day 7 post treatment, *T. harzianum* exhibited molluscicidal efficacy against *M. cartusiana*. Statistically, the percentage of mortality rose as *T. harzianum* concentration increased. *T. harzianum* caused 87.5% mortality at a concentration of 1×10^8, with an LC_{50} attained

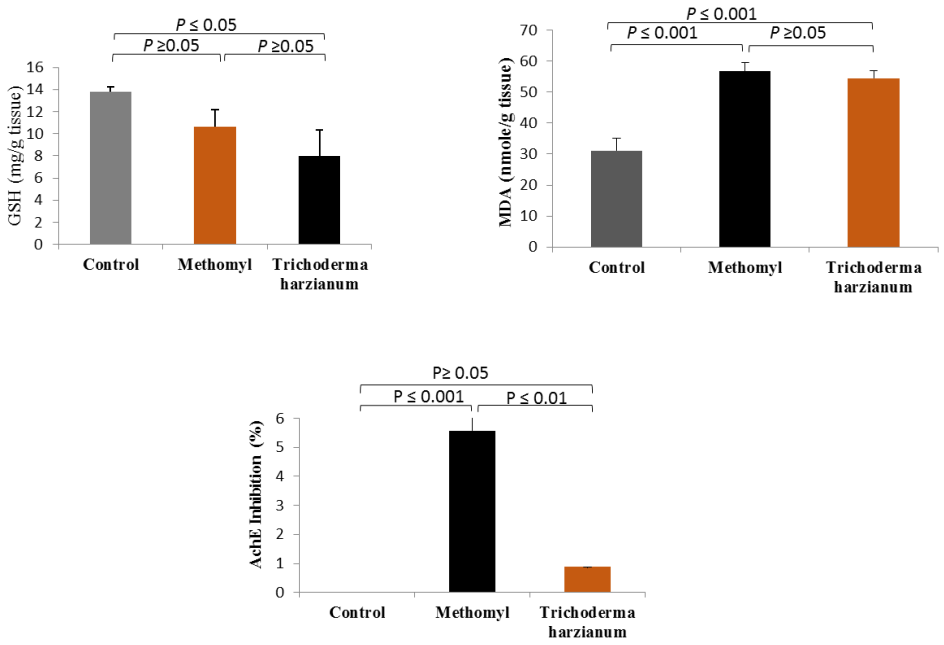

Fig. 3: Oxidative enzymes and acetylcholinesterase inhibition of treated *M. cartusiana* snails by *T. harzianum*. The column represented mean of three replicates ± SE. statistical significance at $P \leq 0.05$ using Duncan's multiple range test

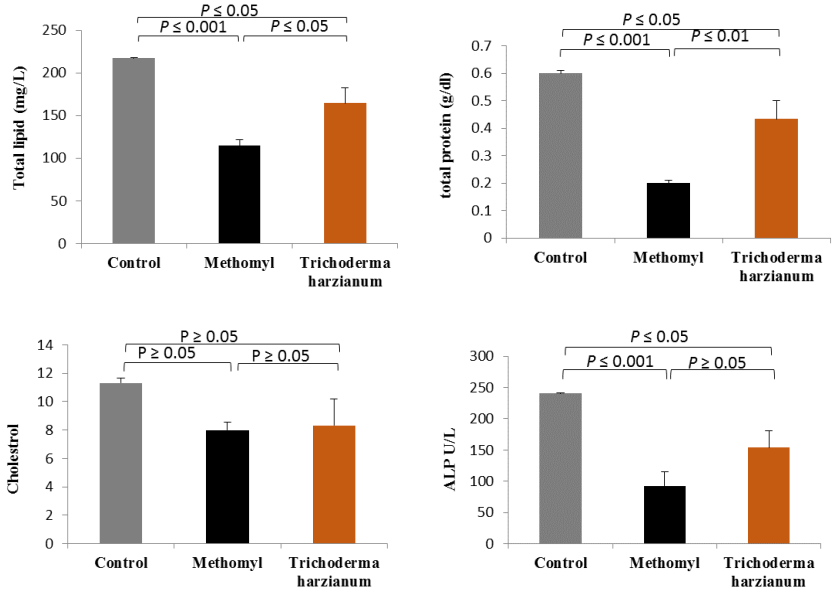

Fig. 4: Total protein, total lipid, cholesterol and ALP of treated *M. cartusiana* snails by *T. harzianum* after exposure to LC_{50}. The column represented mean of three replicates ± SE. statistical significance at $P \leq 0.05$ using Duncan's multiple range test

at concentration of 1.4×10^7. This is consistent with the findings of Ali *et al.* (2017b), who found mortality rates of 56.66 and 39.98%, respectively, in the land snails *Succinea putris* and *Eobania vermiculata* 4 weeks post treatment with *T. album*. Similarly, El-Atti *et al*. (2020) discovered that the fungus biozed has molluscicidal action against *M. cartusiana*, with 66.6% mortality at the high concentrations. According to Ali *et al*. (2017a), the toxic effects of *Trichoderma* spp. may be connected to their menthol metabolites, since menthol caused 80% mortality in *M. obstructa* in the laboratory.

The AChE activity significantly decreased in the *T. harzianum* and methomyl treated groups in the current investigation. These findings are consistent with the findings

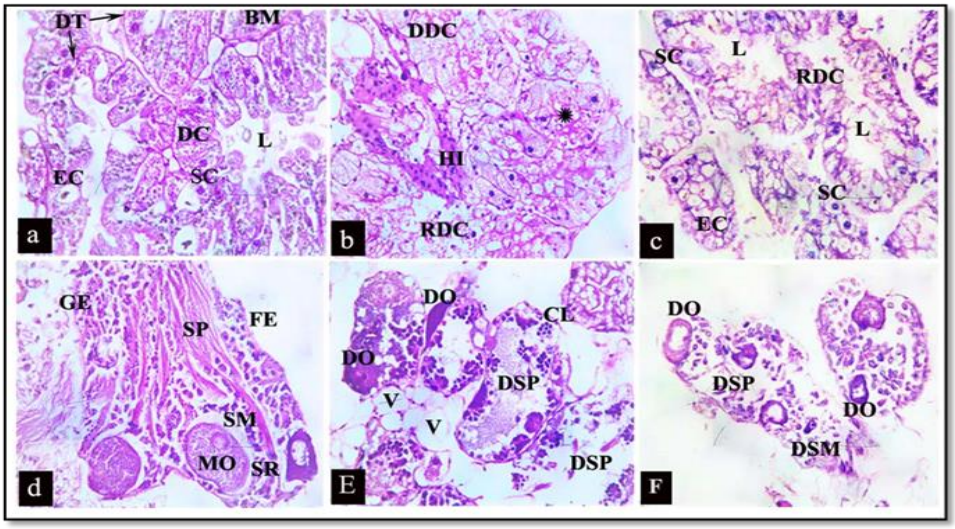

Fig. 5: Light micrographs showing effect of *T. harzianum* at LC_{50} on the digestive gland and ovotests of *M. cartusiana* snails (**a**) control, digestive tubule (DT), digestive cell (DC), secretory cell (SC), excretory cell (EC), basement membrane (BM), lumen (L). (**b**) TRH treated snails showing presence of cellular blebs, hemocytic infiltration (HI), rupture (RDC) and degeneration (DDC) digestive cell. (**c**) methomyl treated snails (**d**) control ovotestis, follicular epithelium (FE), germinal epithelium (GE), sperms (SP), spermatocyte (SM), sertoli cell (SR) and mature ova ((H&E; × 40). (**e**) Treated snails showing de formed sperms (DSP), deformed ova (DO), and vacuoles (V). (**f**) methomyl treated snails

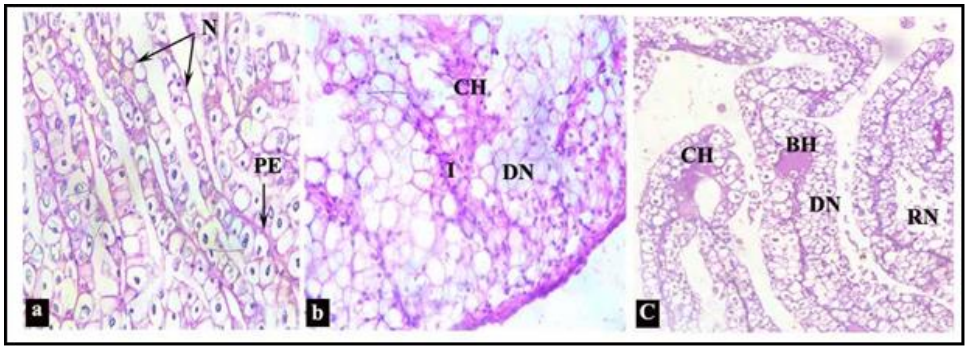

Fig. 6: Light micrographs showing effect of *T. harzianum* at LC_{50} on kidney of *M. cartusiana* snails. (**a**) Untreated snails, Plasma Epithelium (PE) and nephrocyte (N). (**b**) *T. harzianum* treated snails showing cellular hyperplasia (CH), degeneration nephrocyte (DN), and hymecyte infiltration (I) (**c**) methomyl treated snails (H&E; ×40)

of Eshra *et al*. (2014), who discovered that methomyl administration significantly decreased AChE activity in *Eobania vermiculata* and *Theba pisana* snails. AChE is known to be involved in the mechanisms of nerve impulse transmission throughout the body (Cassaneli *et al*. 2006). Inhibiting this enzyme with various neurotoxic chemicals causes a buildup of the chemical messenger acetylcholine in the synaptic region, resulting in continuous nerve impulse transmission and, eventually, death (Alout *et al*. 2007).

Overproduction of ROS has been shown to have a deleterious influence on a variety of biomolecules, including lipids, proteins, and nucleic acids, leading to an increase in oxidative stress (Phaniendra *et al*. 2015; Sharifi-Rad *et al*. 2020). MDA is an oxidative stress biomarker produced by lipid peroxidation of polyunsaturated fatty acids. MDA levels in tissues can be used to assess the degree of lipid peroxidation (Davey *et al*. 2005). GSH is essential for many cellular processes associated with changes in the maintenance and control of the thiol-redox state since it can exist in a variety of redox species (Forman *et al*. 2009). In the present investigation, *M. cartusiana* snails treated with *T. harzianum* displayed a highly significant drop in the level of MDA and a significant depletion in the content of GSH. Additionally, the administration of methomyl resulted in a discernible change in the oxidant-antioxidant state of *M. cartusiana* via an increase in MDA level and a decrease in GSH content. These results are consistent with those from Sharaf *et al*. (2015). Increased MDA levels and decreased GSH levels in snails

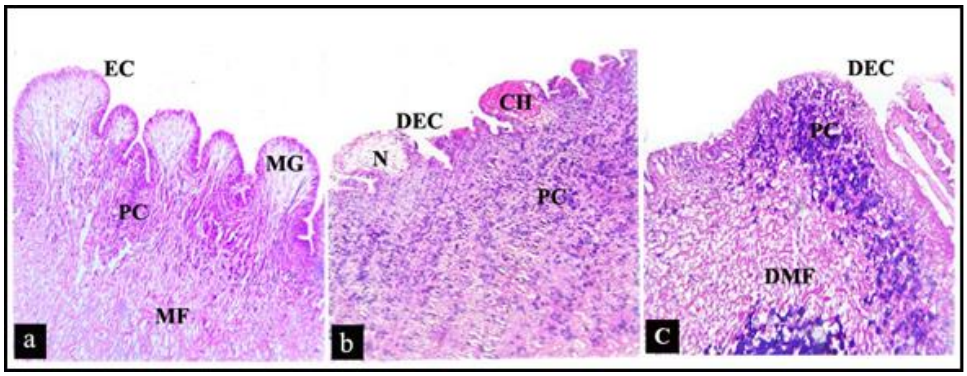

Fig. 7: Light micrographs showing effect of *T. harzianum* at LC_{50} on foot of *M. cartusiana* snails. (**a**) Normal foot, EC, epithelium cell, MG: moucus gland, MF: Mussel fiber, pigment cell (PC). (**b**) *T. harzianum* treated snails showing destruction epithelium cell (DEC), necrosis (N), cellular hyperplasia (CH). (**c**) methomyl treated snails (H&E; ×10)

exposed to the LC_{50} dose of *T. harzianum* in this study show that the oxidative cell damage caused by free radicals may play a role in mediating the toxicity of *T. harzianum* as suggested by (Khalil *et al.* (2017).

The treatment of *M. cartusiana* with *T. harzianum* resulted in a significant decrease in ALP levels in the current investigation. Furthermore, methomyl treatment resulted in ALP depletion comparable to that reported by Gabriel *et al.* (2011) and Kandil *et al.* (2014). The decrease in ALP levels may be attributed to a reduction in glycogen production (Shaffi 1979). ALP is also required for the conversion of the energy molecules NADP to NAD (Morton 1995). As a result, a decrease in ALP levels may cause a biosynthetic shift and an alteration in the exposed organism's energy metabolic pathway (Ovuru and Mgbere 2000). The current study also revealed that *T. harzianum* treatment of *M. cartusiana* resulted in significant reductions in total lipids and total protein levels. This drop could be attributed to a decrease in lipid synthesising ability or hydrolysis of hepatic fats in response to stress (Gabr *et al*. 2007; Sayed and El-Sayed 2020). In a comparable manner, Shahawy *et al*. (2018) found a significant drop in the levels of total lipids and total protein in the snails *Helicella vestalis* and *Theba pisana* following treatment with *Metarhizium anisopliae* fungus spores. Additionally, El-Atti *et al.* (2020) showed a significant reduction in the total lipid of *M. cartusiana* following treatment with *T. album* spores. MethomLy treatment in the current study also markedly decreased levels of total lipids and total protein. These findings correspond with those of Kandil *et al.* (2014) and Gaber *et al.* (2022), who discovered that the use of methomyl significantly reduced the levels of total lipids and total protein in *Eobania vermiculata* and *M. obstructa*. The decrease in protein levels in snails was hypothesized to be caused by the breakdown of carbohydrates, which pointed to changes in protein production and metabolism (Khalil 2016). Additionally, Gaber *et al*. (2007) claimed that the drop in total lipids was caused by a loss in the ability to synthesise lipids and/or by an increase in the hydrolysis of hepatic lipid in response to the stress circumstances.

The findings of the current investigation revealed that the digestive and hermaphrodite glands of *M. cartusiana* snails treated with LC_{50} doses of *T. harzianum* showed considerable histological abnormalities, including tubular disruptions, DC degeneration, and inflammation. Additionally, the tubular glands lost their regular shape. Sperms with abnormal shapes and vacuolated oocytes were seen in the ovotestis. El-Atti *et al*. (2020) discovered similar results in in the digestive and ovotestis glands of *M. cartusiana* following treatment with *T. album*. The testis and ovaries of the crayfish *Procambarus clarkii* were also found to have some structural abnormalities four weeks after exposure to *T. harzianum* (Sheir *et al.* 2015). Similarly, the treatment with methomyl caused disruptions and degeneration in DC, increasing the number of SC, fusion of tubular glands, deformed sperms, spermatocytes, and oocytes (Gaber *et al*. 2022).

The nephrocytes in the kidney of snails treated with *T. harzianum* and with methomyl both exhibited dilatation and degradation. These findings concur with those of Zaidi *et al*. (2021), who report that exposure to cyanobacteria causes necrosis and a loss of cell structure in the kidney of the land snail Helix aspersa. Additionally, Lance *et al*. (2010) noted several histological alterations in a freshwater snail, *Lymnaea stagnalis*, following exposure to *Planktothrix agardhii* extracts.

Destruction of the foot tissues leads to disturbance and great loss in the total body water content and consequently the death of snails (Abdl-Kader 2001). In the present study, the foot of snails treated with either *T. harzianum* or methomyl showed destruction of the outer layer, desquamation of the epithelium, necrosis of mucus and connective tissue and also, deformity of the muscle fibers. Same results have been recorded by Gaber *et al.* (2022), after exposer of the *M. cartusiana* to different doses of methomyl.

Field application of *T. harzianum* caused 52.7% reduction in *M. cartusiana* infesting lettuce plants after 21 days. This reduction may be due to the volatile organic compounds of *T. harzianum* that were known to have repellant effects (Xiong *et al*. 2018). Menthol which is the main component of *T. harzianum* was found to have repellent activity against the cowpea seed beetle *Callosobruchus maculatus* (Saeidi and Mirfakhraie 2017). Similarly, the application of *Trichoderma* spores reduced the beetle *A. obtectus* to 10% in the stored bean seeds due to its repellent action (Rodríguez-Gonz´alez *et al*. 2018, 2019, 2020). On the other hand, methomyl field application caused reduction of 74% in *M. cartusiana*. This reduction was similar to those reported by Hendawy *et al*. (2015) as they reported that methomyl is the most efficient compound for reducing the population of *M. cantiana* and *M. cartusiana* in lettuce and cabbage plantations, followed by *Metarhizium anisopliae*, while *Beauveria bassiana* was the least effective agent.

Conclusion

T. harzianum is a very promising candidate that could be used to control land snails, or exploited as a starting point to develop new, effective, and environmentally friendly molluscicides. *T. harzianum* showed molluscicidal activity almost comparable to that of methomyl, the recommended chemical molluscicides, and in contrast, *T. harzianum* is less harmful to environment and health. Land snails are affected through various biochemical, histological and oxidant/antioxidant disturbances; therefore, the exact mode of action should be investigated.

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Author Contributions

Heba Younes Ahmed: Experimental design, Methodology, Investigation, Data Analysis; Heba Abdel-Tawab: Methodology, Investigation, Data Analysis; Abdel-Azeem S. Abdel-Baki: Supervision, Methodology, Writing/ review; Ahmed O. Hassan: Supervision, Methodology, editing; Saleh Al-Quraishyd: Project administration, Funding acquisition.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Not applicable

Ethics Approval

Not applicable

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