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Control of the Green Peach Aphid Insect, *Myzus persicae* and the Glassy Clover Snail, *Monacha cartusiana* (Müller) by using Fungus, *Trichoderma yunnanense* as a Safe Alternative to Pesticides and its Effect on Aminotransferase Enzymes Activity



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ABSTRACT

Studies disclosed that insecticide and molluscicidal activity of fungus, *Trichoderma yunnanense* against the green peach aphid insect, *Myzus persicae* and *Monacha cartusiana* snail (Müller), respectively using dipping and spraying techniques. Data showed that *T. yunnanense* metabolites was the most impact compound followed with *T. yunnanense* spore suspension against insect, *M. persicae* compared to Bioranza and Sumithion. In the contrary, results reported that *T. yunnanense* spore suspension was the most impact compound followed, with *T. yunnanense* metabolites versus juveniles and adults of *M. cartusiana* snail compared methomyl pesticide beneath laboratory conditions. Moreover, datum indicated that the residual impact on reduction percentages next 21 days were (19.15 & 33.08%) for *T. yunnanense* spore suspension at concentrations (10^6 & 10^8 spore/ml), respectively and (54.70 & 85.71%) for methomyl pesticide at concentrations (1 & 2%), respectively using spraying technique under field conditions. Furthermore, studies showed that fungal, *T. yunnanense* (10^8 spore/ml) was existence after 3 days and disappearance after 7 days using spray application in a field cultivated with lettuce compared control. Also, data reported that changes in activity of AST and ALT enzymes in adults of tested insect and snail treated with fungus, *T. yunnanense* spore suspension. At concentration 10^8 was very high decrease in AST and ALT enzyme compared to control recording (-76.57, -49.44, -38.06 %) and (-41.14, -21.75, -15.30 %) of AST enzyme (-26.35, -49.30, -70.42 %) and (-28.42, -55.88, -90.56%) of ALT enzyme of *M. persicae* and *M. cartusiana*, respectively. Finally, *T. yunnanense* was identified by using 18s rRNA and its accession OQ659412.

Keywords: *Myzus persicae* - *Monacha cartusiana* - Control - *Trichoderma yunnanense* - aminotransferase enzymes

INTRODUCTION

The aphid insect considered one of the most dangerous arthropod pests of greenhouse and field crops worldwide, which is one of the insect pests. As evidenced by data on decreased harvest yields, their incursions result in serious economic losses. Furthermore, aphids have been studied to be celebrated vectors of numerous viral diseases Soomro *et al.*, (1992) and Emden and Harrington, (2007). However, according to Nakhla *et al.* (1993) and Godan (1983), land snails cause expensive prejudice to field crops, fruit trees, vegetables and ornamental plants. While in several Egyptian districts, *Monacha cartusiana* snails have been observed attacking various plants (Eshra, 2013). Diverse tactics have been used to get rid of this pest. Because chemical pesticide application is quicker, farmers tend to favor it for pest management. However, due to reverse effects of pesticides on environment. So, one of the most committing bio-control approach that has extradited attention of numerous scientists is the development of toxins from *Bacillus thuringiensis* as insecticides (Belfiore *et al.*, 1994). *Trichoderma* species are well known for producing a wide variety of secondary metabolites, including polysaccharides, poisons and antibiotics (Gams and Bissett 1998). Additionally, this genus' strains from various species are frequently applied in the biocontrol of soil-borne plant

pathogenic fungus (Samuels 2006). *Helicella vestalis* land snail was insecure to chlorpyrifos and methiocarb, which caused changes in the biochemical parameters alanine amino transaminase (ALT), alkaline phosphatase (ALP), total protein (TP) and aspartate amino transaminase (AST), as well as several histopathological changes in a number of organs (Sharaf *et al.*, 2015).

Therefore, the present treatise was conducted in the laboratory and field to evaluate impact of fungus, *Trichoderma yunnanense* as a safe alternative to pesticides against the green peach aphid insect, *Myzus persicae* and the, *Monacha cartusiana* (Müller) snail.

MATERIALS AND METHODS

Tested insect: The *Myzus persicae* insect was gathered from several fields throughout Egypt's Sharkia Governorate, bagged in paper, and brought to the plant protection research institute's facility in Zagazig district. Individual insects were raised in a lab setting with environmental conditions of 25°C, 70% relative humidity, and a 12-hour photoperiod (Ahmed *et al.*, 1999).

Animal used in testing: Adult and juvenile *Monacha cartusiana* snails were collected from various lettuce-growing fields in the Banayous area of Egypt's Zagazig district and identified using the keys provided by Godan

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(1983). Snails were promptly transported to the plant protection research institute's lab in white fabric bags. Snails that were healthy and comparable in appearance were picking and preserved in a glass terrarium filled with humid mud soil that was set to 75% of the water field's capacity. Before starting treatment, tested snails were fed daily with lettuce leaves for 14 days for acclimatization.

Pesticides that were tested: Included molluscicidal methomyl 90% S.P. (Trade name), S-methyl-N-[(methyl carbamoyl) oxy] thioacetimidate (chemical name), lannate (common name) was created via Kafer El-Zayat firm for pesticides & chemicals, Egypt, bio-insecticide, Bioranza and chemical insecticide, Sumithion brought from plant protection research institute.

Fungus that was tested: *Trichoderma yunnanense* was isolated from the green peach aphid insect, *Myzus persicae*, at the Sharkia Governorate plant protection research institute using the homogenization technique, followed by dilution plating of the homogenate on modified Czapek-Dox's agar medium oxoid (Goettel and Inglis, 1997). Every 15 days, cultures were subcultured and safeguarded on PDA agar slants at 4 °C. Then, it was purified and morphologically recognized using a light microscope, accordance with Domsch *et al.* (1980), Bissett (1991), and Moubasher (1993). The animal health research institute, agricultural research center, Giza, Egypt performed the DNA sequence of a fungus isolate's 18s rRNA gene, following procedures as per the protocol developed by Tarini *et al.* (2010) for molecular characterisation.

Media for culture: Media utilized for isolation, purification and identification of entomopathogenic fungi: First, Czapek-Dox's agar medium oxoid Goettel and Inglis, 1997 composed (g/L): 30 sucrose, 3.0 NaNO₃, 1.0 KH₂PO₄, 0.5 MgSO₄.7H₂O, 0.5 KCl, 0.01 FeSO₄.7H₂O, 0.5 yeast extract and 20 gm agar-agar and dissolved in distilled water pH 5.0. **Second, Potato-Dextrose** agar Bilgrami and Verma (1981); composed of (g/L); 250 peeled potato 20.0 dextrose and 20.0 agar-agar, dissolved in 1L. distilled water.

Preparation of inoculum: Spore suspension was made using *Trichoderma yunnanense* at various serial dilutions. The test fungus' seven-day-old plate culture was rubbed with disinfected distilled water that contained a drop of tween 80 to collect the spores (Krutmuang and Mekchay, 2005).

Metabolites: Erlenmeyer conical flasks (250 ml capacity), all containing 50 ml of fermented medium were utilized in the approaching study. The pH of the medium was adjusted to 5. Flasks were blocked with cotton plug and sterilized at 121°C for 20 min. Each flask, next being cooled was vaccinated with one disc of spore under septic conditions. The culture flasks were then incubated at 25°C in an electric incubator. Each treatment was carried out in triplicates and data reported through this treatise were the arithmetic average of at least two trials. Laboratory evaluation of *T. yunnanense* was cultured on Dox agar medium and take part in growth chamber at 25±1°C. Conidia imperturbable from 12 days old cultures diluted at (10², 10⁴, 10⁶ and 10⁸) conidia/ml and 50%, 25%, 12.5% and 6.250 ml of metabolites (filtrate) were taken. Mass rearing of insect *M. persicae* collected from cotton plants in a greenhouse and

laboratory culture established on broad bean. Colony of aphids was maintained at 25±1°C, 65±5% RH.

Identification of fungal, *T. yunnanense* spore suspension under field conditions: Tested fungal were examined after 3 and 7 days using spray application in a field cultivated with lettuce at Banayous locality, Sharkia Governorate, Egypt at 10⁸ colonial /ml for spore suspensions. Fungal were identified via observing and recording the cultural properties such as size of colony, color, mycelia growth and change in media color through growth. Isolates were also microscopically resolved for morphological characteristics (surface, shape, pigmentation and margin). Fungal isolates were specified using standard manuals. Thereafter it was identified according to Domsch *et al.* (1980); Bissett (1991) and Moubasher (1993).

Toxicity studies:

Insect rearing technique: The suitable conditions and feed for rearing laboratory culture of the green peach aphids, (*M. persicae*) was followed according to the method mentioned by Ramadan (1982). Aphid colonies were preserved according to Ramadan (1982) and El-Gendy (2009). Preparation of serial husbandry of cotton plants in plastic pots (30 cm diameter in 25 cm highest) at the laboratory conditions, specimens of infested plants by peach aphid were collected from field, introduced in paper bags, transformed to the laboratory. Peach aphids were transmitted from infested plants to non-infested one via utilizing fine brush. Aphid colonies were away from away infection via placing infested specimens in cages blocked with a muslin cloth, which were avert aphids from parasites and predators (El-Gendy, 2009).

Laboratory test of tested insect using dipping technique: To test to the effects of selected fungal spores and its metabolites as bio control agents on aphid insects mortality percentages, using leaf dipping technique, the best method used in studied as described by El-Gendy (2009). Thirty adult aphid were calculated and introduced in sterile petri dishes, four dishes for all treatment as replicates as well as control. The disc of cotton leaves (2 square inch) were prepared, dipped in tested concentrations for ten seconds, thereafter left to dry at room temperature and provided to aphid in petri dishes. The tested concentrations were destined as for tested fungus: four conc. 10⁸, 10⁶, 10⁴ and 10² colonial /ml for spore suspensions and 50, 25, 12.5 and 6.250% for metabolites of *T. yunnanense*, bio-insecticide, Bioranza and chemical insecticide, Sumithion per petri dishes. The died and a live number of aphids were counted after 1, 2, 3, 4 and 5 days of treatments sub laboratory conditions (25– 28°C and 70 – 80 pH) as described, Ghatwary (2000). The mortality percentage were calculated and courteous according to Abbott's formula (Abbott, 1925).

$$\text{Mortality (\%)} = [(X - Y)/X] 100$$

Whereas:

X = % survival in control, and Y = % survival in treated aphids.

Employing the dipping technique, a laboratory test on a snail: We used the dipping method to compare the molluscicidal abilities of the fungus *T. yunnanense* to those of the insecticide methomyl. Four concentrations of 2, 1, 0.50 and 0.25 % were prepared by adding 2 g of each material + 98 ml sterile water (2%) then diluted to reach 1, 0.5 and 0.25 % for methomyl pesticide, four conc. 10⁸, 10⁶,

10⁴ and 10² colonial /ml for spore suspensions and 50, 25, 12.5 and 6.250 % for metabolites of *T. yunnanense*. Comparable pieces of fresh lettuce leaves were downward for 10 seconds in tested fungus and pesticide. Then left to harsh prior being offered to tested snails. Also 15 juveniles, 15 adult individuals of *M. cartusiana* snail were dipped in each tested solution of the tested compounds for 10 seconds in each concentration of tested fungus and pesticide. Five juveniles and adults were introduced into plastic boxes (3/4 kg capacity) and then keep the snail from escaping, wrapped it with muslin cloth and fastened it with a rubber band. Each concentration was replicated three times. After the next two days of exposition period, the treated leaves were supplement daily with untreated leaves for 21 sequential days. For the control test the lettuce fresh leaves were sloping in water suspension free from any compounds. Mortality percentages were counted after 1, 3, 7, 14, 21 and 28 days and corrected via Abbott's formula (1925). Field application of tested snail: The study was carried out on a lettuce-growing field at Banayous locality, Sharkia Governorate, Egypt. The field area was discordant four plots, each including control, each plot was splitted into three replicates for all treatments. Fungus, *Trichoderma yunnanense* solution concentrations (10⁶ and 10⁸) colonial /ml for spore suspensions were prepared by incorporating the calculated weight with water (V/V). 1 and 2 % were prepared by adding 2 g of each material + 98 ml sterile water (2%) then diluted to reach 1 % for methomyl pesticide. Each plot was 3 × 3.5m = 10.5 m² Abd El-Rahman (2020). All spray applications were made once on April, 2022 using knapsack sprayer. Alive land snails inside each plot were counted prior just treatment and after 1, 3, 7, 14 and 21 days of spray enforcement. Reduction percentages were deliberate according to the formula of Henderson and Tilton (1955) as follows:

$$\% \text{ Reduction} = [1 - (t_2 \times r_1) / (t_1 \times r_2)] \times 100$$

Whereas:

r₁ = Number of alive land snails before treatment in untreated plots.

r₂ = Number of alive land snails after treatment in untreated plots.

t₁ = Number of alive land snails before treatment in treated plots.

t₂ = Number of alive land snails after treatment in treated plots.

Biochemical studies:

Preparation of samples for the biochemical assay:

Samples weighing 1gm were collected from the treated (10⁶ and 10⁸ spore/ml) and untreated (control) groups of snails

and aphids at 1, 3, and 7 days after treatment. Adult shells of *Monacha cartusiana* snails were extracted. In little bottles, snails and aphids were kept in the freezer until analysis. The frozen samples of the snails and aphids under study were mixed with 5ml distilled water per sample in a Teflon homogenizer. In distilled water, the soft tissues of snails and aphids were weighed, collected, and homogenised at a ratio of 1:10 (w/v). The homogenates were centrifuged at 5000 r.p.m. for 20 minutes at 5°C, according to Abd El-Haleim *et al.* (2006). The supernatants of tested aphids and snails were used as enzyme source for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Enzymes activities were measured according to the method described by Reitman and Frankel (1957).

Statistical analysis: The statistical analysis was decided by using one way test, (ANOVA), COHORT SOFTWARE (2005).

RESULTS AND DISCUSSION

Molecular identification of the tested fungus: Utilizing the primers ITS (1) and (4), the PCR result of the showed *Trichoderma yunnanense* fungus was sequenced. The resulting DNA sequences from the PCR were compared to the published sequences using the Basic Local Alignment Search Tool (BLAST) programme, Altschul *et al.* (1990), and Altschul *et al.* (1997) to check if any homologs to the Gen Bank data existed. The PCR results from the examined fungus had sequences that were 99.63% identical to those of *T. yunnanense* (Figure 1). *T. yunnanense* was identified in the same position on the phylogenetic tree of the tested isolate and was arranged in accordance with an evolutionary distance matrix based on imperfect 18S rRNA gene sequences (Figure 2). According to (Figure 3), the sequence of the PCR products from the selected fungus was 99% identical to that of *T. yunnanense*.

Trichoderma yunnanense CBS 121219 ITS region; from TYPE material Sequence ID: NR_134419.1 Length: 599 Number of Matches: 1. See 1 more title(s) See all Identical Proteins (IPG) Range 1: 11 to 567 GenBank Graphics Next Match Previous Match Alignment statistics for match #1 Score Expect Identities Gaps Strand 971 bits (1076) 0.0 552/559 (99%) 3/559 (0%) Plus/Plus Query 2.

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input type="checkbox"/>	Trichoderma sp. XD-2018b isolate YMF1.04629 small subunit ribosomal RNA gene partial sequence internal transcribed spacer 1	Trichoderma pseu...	992	992	96%	0.0	99.63%	604	MH383059.1
<input type="checkbox"/>	Trichoderma yunnanense CBS 121219 ITS region from TYPE material	Trichoderma yunn...	992	992	96%	0.0	99.63%	599	NR_134419.1
<input type="checkbox"/>	Trichoderma yunnanense strain YMF1.01694 internal transcribed spacer 1 partial sequence 5.8S ribosomal RNA gene	Trichoderma yunn...	992	992	96%	0.0	99.63%	585	AY941823.1
<input type="checkbox"/>	Trichoderma lieckfeldiae CBS 123049 ITS region from TYPE material	Trichoderma lieck...	970	970	95%	0.0	99.08%	577	NR_138438.1
<input type="checkbox"/>	Trichoderma pubescens strain DAOM 166162 from USA 18S ribosomal RNA gene partial sequence internal transcribed spacer 1	Trichoderma pube...	963	963	96%	0.0	98.71%	572	DQ083016.1
<input type="checkbox"/>	Trichoderma poronideum BPI GJS 01-203 ITS region from TYPE material	Trichoderma poro...	959	959	96%	0.0	98.53%	578	NR_134446.1
<input type="checkbox"/>	Trichoderma pubescens DAOM 166162 ITS region from TYPE material	Trichoderma pube...	959	959	96%	0.0	98.53%	627	NR_077179.1
<input type="checkbox"/>	Trichoderma anisohamatum small subunit ribosomal RNA gene partial sequence internal transcribed spacer 1 5.8S ribosomal RNA gene	Trichoderma ania...	957	957	96%	0.0	98.53%	599	MH113926.1
<input type="checkbox"/>	Trichoderma insignis small subunit ribosomal RNA gene partial sequence internal transcribed spacer 1 5.8S ribosomal RNA gene	Trichoderma insignis	957	957	96%	0.0	98.53%	577	MH113925.1
<input type="checkbox"/>	Trichoderma cerebiforme BPI GJS85-245 ITS region from reference material	Trichoderma cere...	955	955	96%	0.0	98.35%	594	NR_134447.1
<input type="checkbox"/>	Trichoderma hamatum DAOM 167057 ITS region from TYPE material	Trichoderma ham...	952	952	96%	0.0	98.35%	625	NR_134371.1
<input type="checkbox"/>	Trichoderma paucisporum BPI GJS 01-13 ITS region from TYPE material	Trichoderma pauc...	952	952	94%	0.0	98.88%	535	NR_134360.1
<input type="checkbox"/>	T. hamatum rRNA genes and ITS1 and ITS2 DNA	Trichoderma ham...	952	952	96%	0.0	98.35%	614	Z48816.1
<input type="checkbox"/>	Trichoderma theobromicola CBS 119120 ITS region from TYPE material	Trichoderma theo...	950	950	93%	0.0	99.06%	533	NR_134359.1
<input type="checkbox"/>	Trichoderma theobromicola culture CBS 119120 strain CBS 119120 internal transcribed spacer 1 partial sequence	Trichoderma theo...	942	942	93%	0.0	98.87%	530	MH863052.1
<input type="checkbox"/>	Trichoderma strigosum strain DAOM 166121 from USA 18S ribosomal RNA gene partial sequence internal transcribed spacer 1	Trichoderma strig...	941	941	95%	0.0	97.81%	577	DQ083027.1

Figure 1. 18S ribosomal RNA gene of *T. yunnanense*.

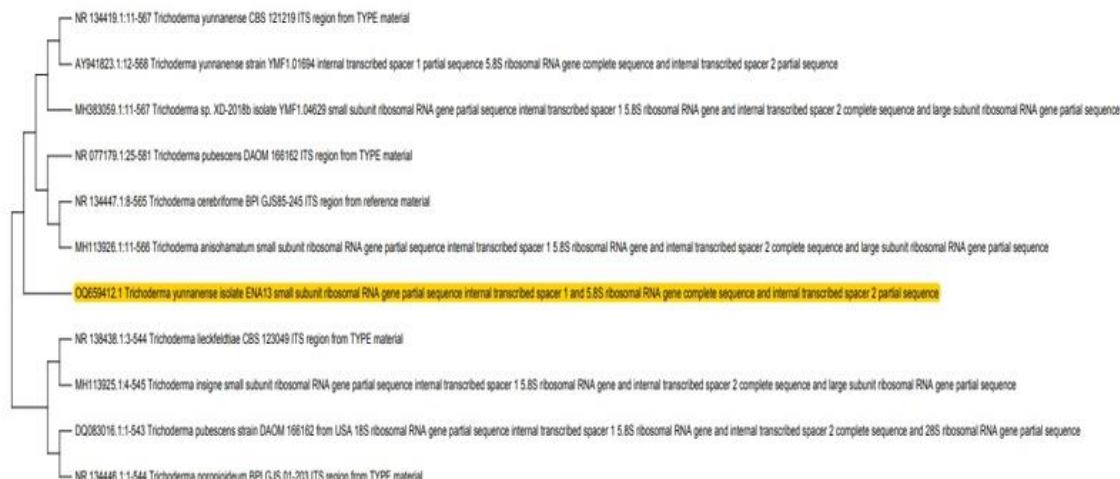


Figure 2. Phylogenetic dendrogram of several fungal isolates accessions detected via average correlation cluster analysis based on 18S rRNA partial sequence.



Figure 3. 18S ribosomal RNA gene, partial sequence; internal transcribed spacer1 and 1.5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

Large subunit partial sequence of 18S rRNA gene of DNA

TGGGGAATTCTACCTGATCCGAGGTCAACATTTAGAAAGTTGGGTGTTTACGGACGTGGACGCGCCGC
GCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCCGGG
GCCGGCACCCGTGTGAGGGGTCCCGATCCCCAAGCCGATCCCCCGAGGGGTTTCGAGGGTTGAAATGA
CGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTTCGATGATTCACTGA
ATTC

Trichoderma yunnanense ENA13 Accession no. OQ65941

Effect of fungus, *Trichoderma yunnanense* spore suspension and metabolites, bio-insecticide, Bioranza and chemical insecticide, Sumithion Metabolites on *Myzus persicae* insect using dipping technique under laboratory conditions: Using dipping technique, data presented in Table (1) revealed where mortality percentages were 31.13, 42.76, 55.84 and 57.36% at concentrations (10^2 , 10^4 , 10^6 & 10^8 spore/ml) of *T. yunnanense* spore Suspension, respectively after five days of treatment compared control. While, mortality percentages of *T.*

yunnanense metabolites, metabolites of bio-insecticide, Bioranza (2g/L) and metabolites of chemical insecticide, Sumithion (3.73cm/100L) were (30.60, 44.55, 59.26 & 64.71 %), (55.96, 58.26, 68.73 & 87.36 %) and (67.63, 68.81, 77.94 and 97.87 %) at the four concentrations 6.250, 12.5, 25 & 50%, respectively compared control. The data demonstrated a strong correlation between the concentrations in the examined insect and the passage of time. Results are in agreement with the finding of Verma *et al.* (2007) reported that *Trichoderma* species have been

widely used as antagonistic fungal agents against various pests. According to Sahebani and Hadavi (2008), different *T. harzianum* concentrations (10^2 – 10^8 spore/ml) considerably reduced nematode infection when compared to control. Additionally, *T. album* predicted 100% mortality

within five days of inoculation against poultry red mites Kaoud (2010). According to Khaleil *et al.* (2016), the *T. hamatum* caused a high level of mortality in cotton aphids

Table 1. Effect of fungus, *Trichoderma yunnanense* spore suspension and metabolites, bio-insecticide, Bioranza and chemical insecticide, Sumithion on *Myzus persicae* insect using dipping technique under laboratory conditions.

Insecticides	Conc.	One day	Two days	Three days	Four days	Five days
<i>T. yunnanense</i> spore suspension	10^2	1.74 ^k	4.91 ^m	11.51 ^l	19.38 ⁱ	31.13 ^g
	10^4	6.25 ^j	11.69 ^l	17.03 ^h	27.45 ^h	42.76 ^f
	10^6	17.51 ⁱ	21.51 ^j	26.77 ^g	50.45 ^g	55.84 ^e
	10^8	20.51 ⁱ	27.55 ⁱ	27.99 ^g	52.75 ^{fg}	57.36 ^e
<i>T. yunnanense</i> metabolites	6.250%	1.12 ^k	6.19 ^m	17.04 ^j	21.35 ⁱ	30.60 ^g
	12.5%	6.57 ^j	16.41 ^k	26.21 ^g	29.39 ^h	44.55 ^f
	25%	24.49 ^h	31.23 ^h	39.93 ^f	52.67 ^{fg}	59.26 ^e
	50%	29.36 ^f	40.26 ^f	47.94 ^e	55.00 ^{ef}	64.71 ^d
Bio-insecticide, Bioranza (2g/L)	6.250%	25.98 ^{gh}	34.37 ^g	38.04 ^f	52.26 ^{fg}	55.96 ^e
	12.5%	28.22 ^{fg}	36.68 ^g	41.27 ^f	54.56 ^{ef}	58.26 ^e
	25%	34.94 ^{de}	48.17 ^e	50.81 ^{de}	65.03 ^d	68.73 ^d
	50%	56.26 ^b	66.28 ^b	79.82 ^b	84.80 ^b	87.36 ^b
Chemical insecticide, Sumithion (3.73cm/100L)	6.250%	36.09 ^d	51.91 ^d	53.27 ^d	57.16 ^c	67.63 ^d
	12.5%	32.72 ^e	53.04 ^d	54.19 ^d	63.00 ^d	68.81 ^d
	25%	43.94 ^c	61.07 ^c	66.84 ^c	71.81 ^c	77.94 ^c
	50%	73.96 ^a	85.22 ^a	88.66 ^a	95.21 ^a	97.87 ^a
Control	—	0.00 ^k	0.00 ^k	0.00 ^j	0.00 ⁱ	0.00 ^h
P		0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
L.S.D. _{0.05}		3.20	3.04	3.56	3.22	4.80

Effect of fungus, *Trichoderma yunnanense* spore suspension and methomyl pesticide on *Monacha cartusiana* snail

Effect of fungus, *Trichoderma yunnanense* spore suspension and metabolites and methomyl pesticide on juveniles and adults of *Monacha cartusiana* snail using dipping technique under laboratory conditions: Data in Table (2) reported that, mortality percentages of *M. cartusiana* snail (juveniles and adults) were (6.67&0.00%), (13.33& 0.00%), (20.00& 6.67%) and (26.67& 13.33%) at concentrations 6.250, 12.5, 25 & 50%, respectively after 28 days of treatment using *T. yunnanense* metabolites. In the case of *T. yunnanense* spore suspension, the mortality was (13.33& 0.00%), (20.00& 6.67%), (26.67& 13.33%) and (46.67& 33.33%) at 10^2 , 10^4 , 10^6 & 10^8 spore/ml, respectively after 28 days of treatment. On the other hand, mortality percentages of methomyl pesticide were (66.67& 60.00%), (100& 93.33%), (100& 100%) and (100& 100%)

at concentrations 0.25, 0.50, 1 & 2%, respectively after 28 days of treatment. Also, these data reported that juveniles were more sensitive than adults. Moreover, data showed that a highly significance between the four concentrations in tested snail by the passage of time. These data are in harmony with the results obtained by several authors, Ghamry (1997) studied two varieties of *B. thuringiensis* [Kurstaki (B.T.K.) and Israelensis (B.T.I.)] under laboratory conditions for biological control of the three land snails, *Helicella vestalis*, *Monacha cartusiana* and *Eobania vermiculata*. Results found that *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* to killing *Pomaceacanaliculata* snails Wimol and Amaret (2003). Ismail and Hegab (2006) demonstrated

Table 2. Effect of fungus, *Trichoderma yunnanense* spore suspension and metabolites and methomyl pesticide on juveniles and adults of *Monacha cartusiana* snail using dipping technique under laboratory conditions.

Tested Fungus	Conc. %	Mortality percentages											
		1 day		3 days		7 days		14days		21days		28 days	
		J.	A.	J.	A.	J.	A.	J.	A.	J.	A.	J.	A.
<i>T.yunnanense</i> metabolites	6.250	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	0.00 ^e	0.00 ^f	6.67 ^{fg}	0.00 ^e
	12.50	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	0.00 ^e	0.00 ^f	13.33 ^{ef}	0.00 ^e
	25	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	6.67 ^{de}	0.00 ^f	20.00 ^{de}	6.67 ^{de}
	50	0.00 ^e	0.00 ^e	6.67 ^{de}	0.00 ^e	6.67 ^{fg}	0.00 ^f	6.67 ^{ef}	0.00 ^e	13.33 ^d	6.67 ^{ef}	26.67 ^d	13.33 ^d
<i>T.yunnanense</i> spore suspension	10^2	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	6.67 ^{de}	0.00 ^f	13.33 ^{ef}	0.00 ^e
	10^4	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	13.33 ^e	0.00 ^e	13.33 ^d	6.67 ^{ef}	20.00 ^{de}	6.67 ^{de}
	10^6	6.67 ^{de}	0.00 ^e	6.67 ^{de}	0.00 ^e	13.33 ^{ef}	6.67 ^{ef}	26.67 ^d	6.67 ^e	26.67 ^c	13.33 ^e	26.67 ^d	13.33 ^d
	10^8	13.33 ^d	0.00 ^e	13.33 ^d	6.67 ^{de}	20.00 ^e	13.33 ^e	40.00 ^c	20.00 ^d	46.67 ^b	26.67 ^d	46.67 ^c	33.33 ^c
Methomyl pesticide	0.25	13.33 ^d	6.67 ^d	26.67 ^c	13.33 ^d	33.33 ^d	26.67 ^d	46.67 ^c	40.00 ^c	53.33 ^b	46.67 ^c	66.67 ^b	60.00 ^b
	0.50	26.67 ^c	20.00 ^c	33.33 ^c	26.67 ^c	46.67 ^c	40.00 ^c	73.33 ^b	66.67 ^b	93.33 ^a	80.00 ^b	100 ^a	93.33 ^a
	1	46.67 ^b	33.33 ^b	53.33 ^b	40.00 ^b	80.00 ^b	73.33 ^b	100 ^a	93.33 ^a	100 ^a	100 ^a	100 ^a	100 ^a
	2	86.67 ^a	73.33 ^a	93.33 ^a	80.00 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
Control	—	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	0.00 ^e	0.00 ^f	0.00 ^g	0.00 ^e
P		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
		***	***	***	***	***	***	***	***	***	***	***	***
L.S.D. _{0.05}		7.61	6.21	8.21	6.94	7.60	6.93	7.59	6.93	8.78	7.60	9.31	8.20

J. = Juveniles A. = Adults

***Trichoderma yunnanense* ENA13 Accession no. OQ65941**

how juvenile and adult *E. vermiculata* snails react to various chemicals under laboratory and field conditions.

They reported that juveniles were more sensitive than adults for abamectine and methomyl pesticides. Aina *et al.*, (2012) showed that the snail-killing effects of *Streptomyces* 218

powder against *Oncomelania hupensis* snails. *Candidatus Paenibacillus glabrata* killing 90% of *Biomphalaria glabrata* snails, the snail intermediate host of *Schistosomiasis mansoni* Duval et al., (2015). The molluscicidal activity of four chemical pesticides, namely methomyl, avaut, pestban, and herbazed against *Monacha cartusiana* snail adults utilizing baits technique under laboratory conditions, in terms of comparative toxicity, methomyl pesticide came up on top, followed by avaut, pestban, and herbazed pesticides Abd-El-Haleem et al. (2022).

using spraying technique under field conditions:

Data in Table (3) reported that the initial effect after one day were (1.34 & 2.79 %) for *T. yunnanense* spore suspension at concentrations (10^6 & 10^8 spore/ml), respectively. Likewise, methomyl pesticide was (5.22 & 11.82 %) at concentrations (1&2 %), respectively. The residual effect on reduction percentages after 21 days was (19.15& 33.08%) for *T. yunnanense* spore suspension at the same concentrations, respectively and (54.70& 85.71 %) for methomyl pesticide at the same concentrations,

respectively. Furthermore, results reported significance between the two concentrations *T. yunnanense* spore suspension and methomyl pesticide

by time elapsing. Similar observations were mentioned by Ismail et al. (2005) reported that methomyl pesticide gave the highest reduction percentages for *M. cartusiana* snail under field conditions. Abd El- Aal (2007) studied the molluscicidal activity of methomyl and other pesticides including protecto under field conditions. He claimed that during the 15 days following treatment, methomyl had the most effect on *M. cartusiana* and *Eobania vermiculata* snails, whereas protecto had the least impact. Hendawy et al. (2015) reported that methomyl has the highest influence against *M. cantiana* and *M. cartusiana* under field conditions. El-Sayed (2017) showed that reduction percentages of certain compounds used under field conditions can be arranged descending according to its efficacy as follows: Newmeal (64.7)> Gastrotax (46.95)> *Streptomyces heliomycini* (26.05). Finally, Abd-El-Haleem (2021)

Table 3. Effect of fungus, *Trichoderma yunnanense* spore suspension and methomyl pesticide on *Monacha cartusiana* snail utilizing spraying technique under field conditions.

Tested Fungus	Conc. (%)	Number of snails before treatment	Initial effect		Residual effect								Mean for red.
			1 day		3 days		7 days		14 days		21 days		
			No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	
<i>Trichoderma</i>	10 ⁶	36.04	35.96	1.34 ^d	35.93	5.97 ^c	35.67	7.96 ^d	35.54	14.40 ^d	35.32	19.15 ^d	9.76 ^d
<i>yunnanense</i>	10 ⁸	48.24	47.43	2.79 ^c	46.78	8.54 ^c	43.13	16.85 ^c	41.56	25.22 ^c	39.13	33.08 ^c	17.30 ^c
Methomyl	1	65.43	62.72	5.22 ^b	56.98	17.87 ^b	47.52	32.46 ^b	42.86	43.14 ^b	35.93	54.70 ^b	30.68 ^b
pesticide	2	63.98	57.06	11.82 ^a	50.12	26.12 ^a	26.13	62.02 ^a	19.72	73.25 ^a	11.08	85.71 ^a	51.78 ^a
Control	—	52.74	53.34		55.92		56.71		60.76		63.93		
P				0.0001 ***		0.0001 ***		0.0001 ***		0.0001 ***		0.0001 ***	0.0001 ***
L.S.D _{0.05}				0.98		3.65		3.64		5.81		4.32	5.16

mentioned that chemical control of some chemical components, i.e. methomyl, avaut, pestban, and herbazed pesticides were used under field conditions using the poisonous baits technique. Toxicity studies showed that methomyl was the most impact compound.

Identification of fungal, *T. yunnanense* spore suspension under field conditions: Figure (4) showed that fungal, *T. yunnanense* (10^8 spore/ml) was existence after 3 days using spray application in a field cultivated with lettuce compared control. Otherwise, Figure (5) demonstrated that tested fungal was disappearance after 7 days at the same concentration using the same application compared control where due to time elapsing under field conditions. Similar observations were mentioned by Ossowski and Duchmann (1997) reported that *Trichophyton rubrum* was eliminated through a washing temperature at 30°C. Hijnen et al. (2006) studied a wavelength of 200 to 300 nm that corresponds to peak absorption of DNA is effective and the absorption of UV light via the DNA molecule reasons the death of microorganisms. Kim et al. (2013) showed that impacts of light on secondary metabolism and fungal development of *Fusarium graminearum*. Amichai et al. (2014) reported that sun exposure decreased fungal contamination. Also, authors mentioned that it was established in the early 1890s that UV radiation particularly UVC through a wavelength range of 250–280 nm is highly germicidal.

Biochemical studies: The biochemical responses of adults *M. persicae* and *M. cartusiana* (Müller) expressed as transaminase enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using fungus, *Trichoderma yunnanense* spore suspension via dipping technique under laboratory conditions. According to the findings in Tables (4&5), *T. yunnanense* spore suspension at concentrations of 10^6 and 10^8

(spore/ml) caused variations in the activity of the AST and ALT enzymes in adults of the examined insect and snail compared to the control. All treatments reduced the activity of AST and ALT, comparison to control. At concentration 10^8 exhibited very high decrease in AST and ALT enzyme which caused the highest reduction at different time intervals compared to control recording (-76.57, - 49.44, -38.06 %) and (-41.14, -21.75, - 15.30 %) of AST enzyme (-26.35, - 49.30, -70.42 %) and (-28.42, -55.88, -90.56%) of ALT enzyme of *M. persicae* and *M. cartusiana*, respectively. While concentration 10^6 gave (-64.93, - 42.47, -10.80 %) and (-31.10, -7.37, -1.42%) of AST enzyme (-2.04, - 21.63, -36.95 %) and (-8.53, -16.31, - 27.04%) of ALT enzyme of tested aphid and snail, respectively compared to the same control. Data showed that a highly significance between the two concentrations via the passage of time of *Myzus persicae* insect. In contrast, data reported no significance between the two concentrations by time elapsing of *M. cartusiana* snail except one week of ALT enzyme. Previous data are in agreement with those showed via Lebsack et al. (1980) mentioned that tissue injury may be the likely mechanism underlying the rise of AST and ALT levels. According to Tilkian et al. (1983), the pathophysiology influences which response enzymes are activated and that the amount of AST is inversely correlated with the number of injured cells. Amer et al. (1994) investigated the rise of AST and ALT enzymes activities was caused via the diffusion of these

enzymes from its intracellular sites due to damage caused via the insecticide on the subcellular level. Generally, alteration in the activity of AST and ALT enzymes are known to be helpful in the diagnosis of hepatic infarcts or

damage. Khaleil *et al.* (2016) reported that the biochemical responses of the cotton aphid, *Aphis gossypii* expressed as AST and ALT enzymes using *Trichoderma hamatum* spore suspension (10^8 spores/ml).

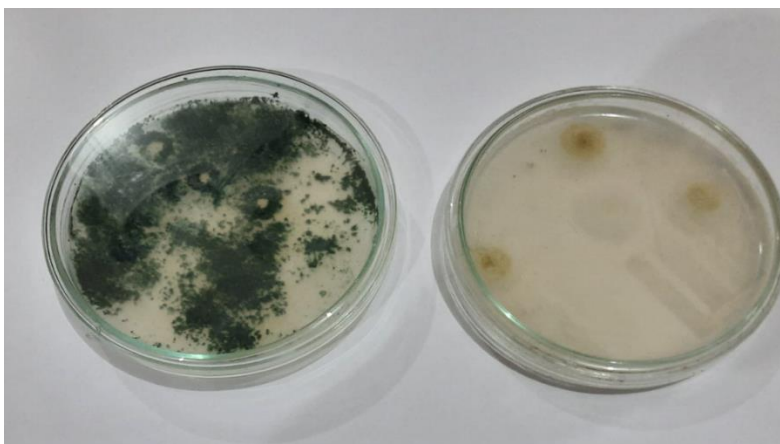


Figure 4. View of appearance of *Trichoderma yunnanense* spore suspensions (A) at 10^8 colonial /ml after 3 days of treatment using spray application compared control (B) under field conditions.

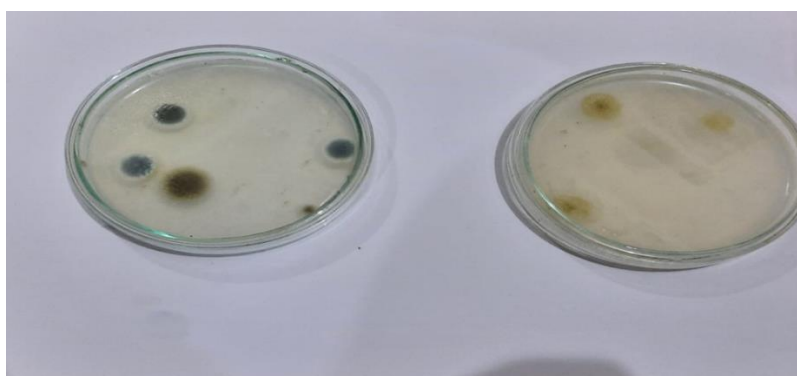


Figure 5. View of disappearance of *Trichoderma yunnanense* spore suspensions (A) at 10^8 colonial /ml after 7 days of treatment using spray application compared control (B) under field conditions.

Table 4. Changes in (AST and ALT) enzymes activities in adults of *Myzus persicae* insect treated with fungus, *Trichoderma yunnanense* spore suspension compared with control using dipping technique.

Tested Fungus	Conc. (spore/ml)		AST			ALT		
			One day	Three days	One week	One day	Three days	One week
<i>Trichoderma yunnanense</i>	10^6	SA	6.93 ^b	10.73 ^b	16.85 ^a	12.49 ^a	10.65 ^b	7.61 ^b
		RA%	(-64.93)	(-42.47)	(-10.80)	(-2.04)	(-21.63)	(-36.95)
	10^8	SA	4.63 ^c	9.43 ^b	11.7 ^b	9.39 ^b	6.89 ^c	3.57 ^c
		RA%	(-76.57)	(-49.44)	(-38.06)	(-26.35)	(-49.30)	(-70.42)
Control	—	SA	19.76 ^a	18.65 ^a	18.89 ^a	12.75 ^a	13.59 ^a	12.07 ^a
P			0.0001***	0.0001***	0.0013**	0.0216*	0.0002***	0.0001***
L.S.D _{0.05}			1.63	2.32	2.58	2.31	1.67	1.65

SA= Specific activity. AST expressed as μg oxaloacetate/g. and ALT as μg pyruvate/g. RA% = (Relative activity %) = [(Treatment – Control) / Control] \times 100.

Table 5. Changes in (AST and ALT) enzymes activity in adults of *Monacha cartusiana* snail treated with fungus, *Trichoderma yunnanense* spore suspension compared with control using dipping technique.

Tested Fungus	Conc. (spore/ml)		AST			ALT		
			One day	Three days	One week	One day	Three days	One week
<i>Trichoderma yunnanense</i>	10^6	SA	2.06	2.64	2.77	3.54	3.13	2.86 ^a
		RA%	(-31.10)	(-7.37)	(-1.42)	(-8.53)	(-16.31)	(-27.04)
	10^8	SA	1.76	2.23	2.38	2.77	1.65	0.37 ^b
		RA%	(-41.14)	(-21.75)	(-15.30)	(-28.42)	(-55.88)	(-90.56)
Control	—	SA	2.99	2.85	2.81	3.87	3.74	3.92 ^a
P			0.5184 ^{ns}	0.4868 ^{ns}	0.9392 ^{ns}	0.3157 ^{ns}	0.1608 ^{ns}	0.0049**
L.S.D _{0.05}			2.59	1.21	3.26	1.65	2.34	1.64

SA= Specific activity. AST expressed as μg oxaloacetate/g. and ALT as μg pyruvate/g. RA% = (Relative activity %) = [(Treatment – Control) / Control] \times 100.

CONCLUSION

Papers examined likelihood of using fungus, *Trichoderma yunnanense* as a safe alternative to pesticides as a safe and inexpensive manner to control the green peach aphid insect,

Myzus persicae and the glassy clover snail, *Monacha cartusiana* (Müller). Data reported that *T. yunnanense* metabolites and spore suspension are the most toxic on *Myzus persicae* insect followed with *Monacha cartusiana* snail. Finally, the relationship between the fungus *T.*

yumanense and the aspartate aminotransferase and alanine aminotransferase enzyme activities of the tested insect and snail were also examined.

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مكافحة حشرة من الخوخ الأخضر *Myzus persicae* ووقوع البرسيم الزجاجة *Monacha cartusiana* (Müller) باستخدام فطر *Trichoderma yunnanense* كبديل آمن للمبيدات وتأثيره على نشاط الإنزيمات الناقلّة للأمين

اسماء محمد عبدالمجيد السيد ، محمد فرج نور الدين غازي فرج ونهي حسن عصام

معهد بحوث وقاية النباتات مركز البحوث الزراعية الدقى – جيزه - مصر

الملخص

أظهرت الدراسات تأثير فطر *Trichoderma yunnanense* كمبيد حشري ورخوى ضد حشرة *Myzus persicae* ووقوع البرسيم الزجاجة *Monacha cartusiana* على الترتيب وذلك باستخدام تقنية الغمر والرش. وكذلك أظهرت النتائج ان نواتج التمثيل للفطر أكثر تأثيراً من جراثيمه وذلك ضد حشرة من الخوخ الأخضر مقارنة بالمبيدات Bioranza و Sumithion وعلى العكس من ذلك توصلت الدراسات الى ان جراثيم الفطر المختبر كانت أكثر تأثيراً من نواتج التمثيل لفطر ذلك ضد *Monacha cartusiana* مقارنة بال Methomyl وذلك تحت الظروف المعملية. وسجلت النتائج أيضاً انخفاض تعداد القواقع تحت الظروف الحقلية عند الرش باستخدام *T. yunnanense* حيث كانت (١٩,١٥ و ٣٣,٠٨٪) عند التركيزين ١٠ و ١٠٠ جرثوم/مل على الترتيب و (٥٤,٧٠ و ٨٥,٧١٪) لمبيد الميثوميل عند التركيزين (١ و ٢٪)، علاوة على ماسبق أشارت النتائج لوجود جراثيم الفطر محل الدراسة عند ثالث يوم من رش الخس تحت الظروف الحقلية ثم اختفاء الجراثيم بعد سابع يوم من الرش بالجراثيم عند تركيز ١٠ جرثوم/مل مقارنة بالكنترول، وأيضاً توصلت البيانات لحدوث انخفاض في نشاط انزيمات AST و ALT لطور البالغ للحشرة من الخوخ ووقوع البرسيم الزجاجة نتيجة لمعاملة بجراثيم الفطر محل الدراسة بتركيز ١٠ جرثوم/مل وذلك مقارنة بالكنترول حيث كانت النتائج (٧٦,٥٧ - ٩٤,٤٤ و ٣٨,٠٦ و ٣٨,٠٦٪) و (٤١,١٤ - ٢١,٧٥ و ١٥,٣٠٪) لانزيم AST (٢٦,٣٥ - ٩,٣٠ و ٧٠,٤٢ و ٢٨,٤٢ - ٥٥,٨٨ و ٩٠,٥٦٪) لانزيم ALT لحشرة من الخوخ ووقوع البرسيم الزجاجة على الترتيب وأخيراً تم التعرف على الفطر باستخدام تحليل حمضه النووي حيث تم تسجيله في بنك الجينات برقم OQ659412.