https://dx.doi.org/10.4314/ejppri.v7i3.8

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (3):352-361

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Fungal and biochemical impacts of the best fungal isolate against the adult cabbage aphid insect *Brevicoryne brassicae* (Hemiptera:Aphididae) and the white garden snail *Theba pisana* (Gastropoda: Helicidae)

Noha, Lokma; Moshiera, A. S. Ahmed; Asmaa, M. A. El-Sayd and Farag, M. F. N. G.

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received:17 /7/2024

Accepted:19 /9/2024

Keywords

Fungal species, *Brevicoryne brassicae*, *Theba pisana*, amylase enzyme and biocontrol.

Abstract

Several fungal species were isolated from various samples of soil and cabbage aphids in this investigation using Czapek-dox agar conditions, tested its toxicity, and compared *Trichoderma asperellum* with accession number (OQ616502) against cabbage aphids and white garden snail, which gave the high biocontrol effect against cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), while giving the lowly effect against the white garden snail, *Theba pisana* (Müller) (Gastropoda : Helicidae) at 10^8 spore suspension after 5 days as 89.42% and 28 days as 26.67%, respectively. On the other hand, for an enzyme (three and seven days of treatment), biochemical studies revealed a very high decrease in the concentrations of amylase and invertase enzymes at the highest concentration of 10^8 spore/ml, which caused the highest reduction compared to the control recording (-39.94, - 40.32, -41.16%) and (-26.48, - 27.34, -28.32%) of the amylase enzyme (-16.53, -17.37, -34.19%) and (-24.42, -29.31, -45.76%) of the invertase enzyme of *T. pisana* and *B. brassicae*, respectively.

Introduction

Insects are the main pests in agricultural systems, causing significant losses in crop productivity and storage. Aphids are major insect pests of crops around the world. Aphids cause serious damage to a variety of crops throughout the world, both directly through feeding and indirectly by transmitting several viruses (Rauquet, 2004). Cabbage aphids are small, 2.0 to 2.5 mm, and covered with a waxy covering. These aphids suck plant juices, causing the infected leaves to become withered or dead patches. Furthermore, honey secreted by the aphid may accumulate in the plant, facilitating Mold growth and giving the leaves a purplish-black appearance (Frank *et al*., 2018). Also, terrestrial gastropods pose one of the biggest risks to sustainable agriculture (Barker, 2002). Land snails such as *Theba pisana* (Müller) (Gastropoda: Helicidae) snails eat a broad range of plants. They also kill seedlings, slow growth, and lower harvests. In addition to causing direct harm to the plants, they also let plant pathogenic fungi infect plants through the wounds they

leave behind. The mucous trails left by the snails can contaminate grains, vegetables, fruits, and plants. They can also act as vectors for a variety of plant diseases. Their bodies and shells, when present in significant quantities, can contaminate crops that are harvested mechanically (Godan, 1983; Garthwaite and Thomas, 1996; and Barker, 2002). Biological control refers to the employment of particular microorganisms as an alternative to pesticides in protecting plants against pests and plant diseases. Other pest management methods were discovered as a result of the pest population's development of insecticide resistance and the growing negative consequences of pesticide use on human health (Foster and Devonshire, 1996). *Trichoderma* is a genus of filamentous fungi that have been extensively studied and utilized as a biocontrol agent in agriculture. It has the ability to produce insecticide secondary metabolites and parasitize insects to directly control pest insects (Jorge, 2021). *Trichoderma yunnanense* is a safe alternative to pesticides for the control of *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae) snail and *Myzus persicae* Sulzer (Hemiptera: Aphididae) insect (El-Sayd *et al.*, 2023). Lokma *et al.* (2023) showed that studies on the histology and biochemistry of *T. yunnanense* have been carried out on *M. persicae* and *M. cartusiana*.

Therefore, the present paper was conducted in the laboratory to evaluate the impact of some fungal isolates as a safe alternative to pesticides against adult cabbage aphid insects, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), and the white garden snail *T. pisana*.

Materials and methods

1. Tested insect:

Samples of infested plants with cabbage aphid *Brevicoryne brassicae* were collected from field cabbage plants, put in paper bags, and transported to the plant protection research institute's lab. Individually, the studied insects were raised in a lab setting and reared for three generations at a temperature of 25±2˚C and 70±5% RH (Ahmed *et al.*, 1999). Aphids were collected from the laboratory culture using a fine hairbrush and placed on host leaf discs.

2. Tested snail:

Adult land snail, white garden snail *T. pisana*, was collected from an orchard in the Menia El-Kamh area, Sharkia Governorate, Egypt, that was planted with navel oranges, *Citrus sinensis* L. The gathered snails were sent straight to the laboratory in white cotton bags and recognized using Godan's (1983) given keys. Selected and comparable healthy snails were kept in glass terrariums with damp clay soil that had been calibrated to occupy 75% of the water field capacity. Before treatment, cabbage leaves were given to snails every day for two weeks to help with acclimation.

3. Microbiological analysis:

3.1. Fungi isolation technique:

Various fungal species were separated from 3.1.1. Aphids: By using the homogenization method and dilution plating of the homogenate. It is described to surface disinfest cadavers to remove potential contaminates on their integument. This takes place using ethanol (70%) for 10 seconds, then passed through 5 separate washings with saline solution. Sterilized aphids were left to dry, then transferred aseptically. The surface disinfested cadavers can be homogenized by using mechanical grinders. The suspension is diluted as required (Usually in a 3 to 4

fold dilution serious) and plated on Czapek- Dox agar medium for growth incubating at 28±2˚C for 7 days according to Goettel and Inglis (1997). Every day, the growth of the colonies on the incubated plates was observed. The colonies were subsequently purified, kept on slants of the appropriate artificial media at 4º C, and subcultured every 15 days at 4 °C., until they were suitable for use in these investigations. 3.1.2. Soil samples: By the diluting methods described by Johnson *et al.* (1959), in plant protection research institute in Sharkia governorate. Cultures were maintained on PDA agar slants at 4ºC and subcultured every 15 days. Finally, the colonies of fungal isolates appeared and were identified manually according to Bissett (1991), who described and differentiated them on the basis of conidiophore and conidium morphology.

3.2. The basal culture medium used:

3.2.1. Czapek- Dox's medium (Oxoid, 1982) is composed of (g/L) 20 sucrose, 2.0 $NaNO_3$, 0.5 $MgSO_4.7H_2O$, 1.0 KH_2PO_4, 0.5 KCl, and 0.001 $FeSO_4.7H_2O$.

3.2.2. Potato-Dextrose (Bilgrami and Verma, 1981): Composed of (g/L) 250 pealed potatoes, 20.0 dextrose.

3.3. Inoculum preparation and culture conditions:

1 ml of 10^8 conidia of different isolates was used to inoculate 50 ml of both basal liquid mediums separately in a 250 ml Erlenmeyer flask and 20 g/agar dox media in a petri dish at 28±2ºC.

3.4. Laboratory test on a snail:

Three concentrations of 50 % were then diluted to reach 25 and 12.50 % for metabolites and three conc. 10^8, 10^4 and 10^2 colonial/ml for spore suspensions of *T. asperellum***.** Comparable pieces of fresh cabbage leaves were downward for 10 seconds in the tested fungus. Then left to harsh prior to being offered to tested snails. Also, 15 adult individuals of *T. pisana* snail were dipped in each tested solution for 10 seconds in each concentration. Five adults were introduced into plastic boxes (3/4 kg capacity) and then kept the snail from escaping, wrapped it with muslin cloth, and fastened it with a rubber band. Each concentration was replicated three times. Over the next two days of exposition period, the treated leaves were supplemented daily with untreated leaves for 28 sequential days. For the control test, the cabbage 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)**.

3.5. Laboratory test on an aphid:

The pathogenicity of different fungal isolates was evaluated on the adult cabbage aphids by dipping leaves as follows: Ghatwary (2000) and Krutmuang and Mekchay (2005). Thirty aphid mothers were tallied and placed in sterile Petri dishes, three for each treatment serving as duplicates and controls. The discs of host leaves (2 square inches) were prepared, dipped in the tested treatments (50% concentrations of liquid dox and potato media separately) for 10 seconds, then left to dry at room temperature and provided to the aphid in Petri dishes. Prepare 50% of the metabolites isolated fungi cultivated on both liquid potato and dox medium separately. Controls were prepared in a similar manner using sterile distilled water. The concentration mortality regression analysis was computed for the tested fungus according to Finney (1971).

Also, the mortality ratios of 50, 25, and 12.5% concentrations of both metabolites of tested fungi cultivated on dox and potato liquid media separately, and 10^8,10^4 and 10^2 conidia of different isolates cultivated on agar dox media, were studied, such as tested snail.

4. Biochemical studies:

Preparation of snails and aphids for biochemical assay:

After their mollusca shells were removed, adult *T. pisana* snails and *Brevicoryne brassicae* insects were weighed, pooled, and homogenized as 1:10 (w/v) in distilled water. The homogenates were centrifuged for 20 minutes at 5 °C at 5000 r. p. m. (Abd El-Haleim *et al*., 2006). The supernatants were subjected to an instantaneous analysis using Ishaaya and Swiriski's technique (1976) to ascertain the activity of amylase and invertase enzymes.

5. Statistical analysis:

ANOVA, a one-way test, was used in statistical analysis (**Cohort Software, 2005).**

Results and discussion

In this study, we tested seven fungal isolates, which were collected from different sources in Table (1). We extracted four fungal isolates from soil samples and two from cabbage aphids. As shown in Table (1) recovered in this study, fungi belong to three classes of ascomycetes, zygomycetes, and deuteromycetes, represented as seven isolates. Ascomycetes were represented by two genera; *Aspergilus* was represented by three isolates, and *Penicillium* was represented by one isolate. Deutromycetes were represented by two genera; *Trichoderma*, which is represented by one isolate, and *Humicola*, which is represented by one isolate. Four isolates were isolated from soil samples, namely *Aspergillus flavus*, *A. fumigatus*, *Penicillium chrysogenum*, and *Rhizopus oryzae*, while *Humicola sp*. and *A. niger* isolated from cabbage aphids, *Brevicoryne brassicae*. Our results about isolation from soil and insects are in agreement with Garrett, 1951, and El-Sayd *et al.*, 2023.

Table (1): List of different isolated, tested fungal species and their sources.

Class	Genus	Serial	Fungal species	Isolation source
Ascomycetes	*Aspergillus*	1	*A. flavus*	Soil
		2	*A. niger*	Cabbage aphid
		3	*A. fumigates*	Soil
	Penicillium	4	*P. chrysogenum*	Soil
Deuteromycetes	*Trichoderma*	5	*T.asperellum*	Plant protection research Institute
	Humicola	6	*Humicola* sp.	Cabbage aphid
Zygomycetes	*Rhizopus*	7	*Rhizopus oryzae*	Soil

1. Toxicological study for aphids:

Toxicity data of 7 selected related fungi against cabbage aphid at 50% conc. Of both metabolites of tested fungal cultivated on liquid DOX, PDA, and 10^8 spores/ml conidial suspensions. Thirty aphid mothers were counted and put into Petri dishes, four dishes of each treatment as well as control, the discs of radish leaves (2 square inch) were prepared and dipped in 50% conc. of both liquid DOX and PDA separately, and 10^8 spores/ml conidial suspension of each fungal species were left to dry and provided to the aphids. The dead and alive numbers of aphids were calculated 120 hrs after treatment, and the mortality percentages were

calculated. Data are presented in Table (2) show that the most tested fungal species revealed an obvious aphicidal effect expressed as mortality percentages at experimental concentrations in comparison with control. Maximum inhibition of aphid growth was observed with 50% conc. of *T. asperellum* metabolites cultivated on liquid Dox media recorded a mortality percentage (69.64%), followed by that of *Penicillium chrysogenum* recording a mortality percentage (60.33%) while *A. flavus* recording the minimum inhibition recording 3.61%, while the fungi cultivated on PDA media had the maximum inhibition aphid growth recorded by *T. asperellum* at 65.18%, while the minimum inhibition was recorded by *A. flavus*, when the 10^8 spores/ml conidial suspension the maximum inhibition was recorded by *T. asperellum* (61.94%) followed by *Penicillium chrysogenum* (59.41%) while the minimum inhibition recorded by *A. flavus* as (3.94%). Data mentioned that the best fungal isolate was *T. asperellum*. Also, results found that its metabolites on 50% Dox medium gave the highest inhibition compared to its metabolites on 50% PDA, followed by 10^8 spore/ml spore suspension. Our findings are consistent with those of other studies, which demonstrate that entomopathogenic fungi infect insects directly through the cuticle, a process that requires adhesions and lytic enzymes (Lipases, proteases, and chytinases). The fungus subdues the insect's defenses and infiltrates its body, generating and dispersing fresh conidia from its decomposing host. Entomopathogenic fungi must generate a diverse range of insecticidal secondary metabolites to finish their life cycle (Qu and Wang, 2018 and Litwin *et al*., 2020).

Table (2): Toxicity data of some tested fungi against Aphids under laboratory conditions after 120 hrs.

Fungus name	Mortality%		
	50% conc. liquid Dox media	50% conc. liquid PDA media	10^8/ spore/ml conidial suspension
Rhizopus oryzae	4.31±0.25	5.37±0.15	4.08±0.33
A. fumigatus	9.00±0.22	8.89±0.04	6.01±0.42
Penicillium chrysogenum	60.33±2.91	56.55±2.05	59.41±3.27
Trichoderma asperellum	69.64±1.68	65.18±4.5	61.94±1.83
***Humicola* sp.**	29.08±0.03	25.41±1.05	23.17±0.98
A. niger	49.41±2.29	58.99±3.65	47.12±1.16
A. flavus	3.61±0.56	3.58±0.32	3.94±0.15
Control	5.26±0.15	5.26±1.80	5.26±0.48

2. Effect of fungus, *Trichoderma asperellum* metabolites and spore suspension on adults of *Theba pisana* snail and *Brevicoryne brassicae* insect under laboratory conditions:

Results in Table (3) showed that mortality percentages of *T. pisana* snail and *B. brassicae* insect were (0.00 and 44.67%), (6.67 and 61.60%) and (20.00 and 99.13%) at concentrations 12.5, 25 and 50% respectively, after 28 and 5 days of treatment respectively, using *T. asperellum* metabolites. In the case of *T. asperellum* spore suspension, the mortality was (6.67 and 29.53%), (13.33 and 59.61%) and (26.67 and 89.42%) at 10^2, 10^4 and 10^8 spore/ml, respectively, after 28 and 5 days of treatment, respectively. Also, these data show that insects were more sensitive

than snails. Moreover, data showed that there was a highly significant difference over time between the three concentrations in the tested snail and aphid. 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 (*Locard) (Gastropoda : Geomitridae) , *M. cartusiana*, and *Eobania vermiculata (*Gastropoda :Helicidae). Also, results found that *Pseudomonas aeruginosa* and *P. fluorescens* kill *Pomacea canaliculata* snails (Wimol and Amaret, 2003). Aina *et al.* (2012) showed the snail-killing effects of *Streptomyces* 218 powder against *Oncomelania hupensis* snail. Furthermore, *Candidatus paenibacillus glabratella* kills 90% of the *Biomphalaria glabrata* snail, the snail intermediate host of *Schistosomiasis mansoni* (Duval *et al*., 2015). Finally, El-Sayd *et al.* (2023) showed mortality percentages of *M. cartusiana* snail and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) aphid using *T. yunnanense* spore suspension and metabolites.

Table (3): Impact of fungus *Trichoderma asperellum* metabolites, and spore suspension on adults of *Theba pisana* snail and *Brevicoryne brassicae* under laboratory conditions.

Tested fungus	Conc. %	Mortality percentages									
		Theba pisana snail						*Brevicoryne brassicae*			
		1 day	3 days	7 days	14 days	21 days	28 days	1 day	3 days	4 days	5 days
Trichoderma Asperellum metabolites	12.50	0.00	0.00	0.00	0.00^{b}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	22.55^{d}	44.67^{d}
	25	0.00	0.00	0.00	0.00^{b}	0.00^{b}	6.67^{cd}	5.17^{b}	17.87^{b}	30.42^{c}	61.60^{c}
	50	0.00	0.00	0.00	0.00^{b}	13.33^{a}	20.00^{ab}	13.26^{a}	26.80^{a}	79.35^{a}	99.13^{a}
Trichoderma Asperellum spore suspension	10^{2}	0.00	0.00	0.00	0.00^{b}	0.00^{b}	6.67^{cd}	0.00^{c}	0.00^{c}	18.67^{d}	29.53^{e}
	10^{4}	0.00	0.00	0.00	0.00^{b}	6.67^{ab}	13.33^{bc}	2.61^{bc}	14.54^{b}	29.04^{c}	59.61^{c}
	10^{8}	0.00	0.00	0.00	6.67^{a}	13.33^{a}	26.67^{a}	11.78^{a}	25.80^{a}	62.82^{b}	89.42^{b}
Control	___	0.00	0.00	0.00	0.00^{b}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{e}	0.00^{f}
P		___	___	___	0.0423*	0.0022**	0.0002***	0.0001***	0.0001***	0.0001***	0.0001***
$L.S.D._{0.05}$		___	___	___	4.41	7.64	9.42	3.03	3.50	5.50	4.68

3. Biochemical studies:

Carbohydrates are essential to the structure and function of pest tissues. Wyatt (1967) states that the enzymes amylase, trehalase, and invertase play a part in the digestion and utilization of carbohydrates by pests, essentially regulating their metabolism. For the breakdown and utilization of carbohydrates as energy, enzymes such as amylase and invertase are essential (Naveed *et al*., 2009). The data in Tables (4 and 5) show that, in comparison to the control using the dipping approach, the fungus *T. asperellum* altered the activity of the enzyme's amylase and invertase in the adults of the *T. pisana* snail and *Brevicoryne brassicae* insect. All treatments resulted in a decrease in the activity of amylase and invertase when compared to the control. At concentration 10^{8}, exhibited a very high decrease in amylase enzyme, which

caused the highest reduction at different time intervals compared to control recording (-39.94, -40.32, -41.16%) and (-26.48, -27.34, -28.32%) of amylase enzyme (-16.53, - 17.37, -34.19%) and (-24.42, -29.31, -45.76%) of invertase enzyme of tested snail and aphid, respectively. While concentration 10^6 gave (-1.38, - 1.47, -4.77%) and (-4.32, -6.27, -11.05%) amylase enzyme (-2.75, - 9.28, -25.05%) and (-1.22, -6.04, -32.16%) of invertase enzyme of tested snail and aphid, respectively. Results demonstrated a highly significant difference over time between the two concentrations of snails and aphids, except for seven days for the insect's amylase enzyme. Previous research supported the findings of Khaleil *et al*. (2016), who mentioned various quantitative changes in the relative activity of the enzyme's amylase and invertase in adult cotton aphids treated with *Trichoderma hamatum* fungus. Also, Lokma *et al.* (2023) showed the effect of the fungus *T. yunnanense* on the activity of the enzymes amylase and invertase in *M. cartusiana* snail and *M. persicae* aphid.

Table (4): Activities of the enzymes (Amylase and invertase) change in adults of *Theba pisana* snail after treatment with the fungus, *Trichoderma asperellum*.

Tested fungus	Conc. (Spore/ml)		Amylase			Invertase		
			1 day	3 days	7 days	1 day	3 days	7 days
Trichoderma asperellum	10^4	SA	34.96[a]	35.56[a]	35.72[a]	42.47[a]	38.12[b]	32.64[b]
		RA%	(-1.38)	(-1.47)	(-4.77)	(-2.75)	(-9.28)	(-25.05)
	10^8	SA	21.29[b]	21.54[b]	22.07[b]	36.45[b]	34.72 [c]	28.66[c]
		RA%	(-39.94)	(-40.32)	(-41.16)	(-16.53)	(-17.37)	(-34.19)
Control		SA	35.45[a]	36.09[a]	37.51[a]	43.67[a]	42.02[a]	43.55[a]
P			0.0001 ***	0.0002 ***	0.0001 ***	0.0010 **	0.0001 ***	0.0001 ***
L.S.D.$_{0.05}$			1.63	4.16	3.26	2.58	1.62	1.61

SA = Specific activity as (ml glucose /ml)
RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100.

Table (5): Activities of the enzymes (Amylase and invertase) change in adults of *Brevicoryne brassicae* insect treated with fungus, *Trichoderma asperellum*.

Tested fungus	Conc. (Spore/ml)		Amylase			Invertase		
			1 day	3 days	7 days	1 day	3 days	7 days
Trichoderma asperellum	10^4	SA	23.49[a]	23.76[a]	24.88	25.16[a]	24.75[a]	17.61[b]
		RA%	(-4.32)	(-6.27)	(-11.05)	(-1.22)	(-6.04)	(-32.16)
	10^8	SA	18.05[b]	18.42[b]	20.05	19.25[b]	18.62[b]	14.08[c]
		RA%	(-26.48)	(-27.34)	(-28.32)	(-24.42)	(-29.31)	(-45.76)
Control		SA	24.55[a]	25.35[a]	27.97	25.47[a]	26.34[a]	25.96[a]
P			0.0029 **	0.0014 **	0.0538[ns]	0.0435 *	0.0008 ***	0.0001 ***
L.S.D.$_{0.05}$			2.58	2.58	6.21	5.16	2.57	2.58

SA = Specific activity as (ml glucose /ml)
RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100.

Studies looked at the possibility of employing the fungus *T. asperellum* as a cheap and safe substitute for pesticides to manage the adult population of *T. pisana* snail and *B. brassicae* insects. According to data, *T. asperellum* had a low biocontrol effect on *T. pisana* and a strong biocontrol effect on *B. brassicae*.

References

Abbott, W. S. (1925): Methods for computing the effectiveness of an insecticide. J. Econ. Entomol., 18 (2): 256 – 267.

Abd El-Haleim, K. Y; Abou El-Khear, R. K. and Hussein, A. A. (2006): Molluscicidal efficacy and toxicity of some pesticides under laboratory and field conditions. Arab Univ. J. Agric. Sci., 14 (2): 861-870. DOI:10.21608/ajs.2006.15530

Ahmed, M.; Taylor, P.; Maingon, R. and Hurd, H. (1999): The effect of *Plasmodium yoelii* nigeriensis on the reproductive fitness of *Anopheles gambiae*. Inverteb. Reprod. Develop., 36: 217 – 222.

Aina,V. O.; Adewumi, A. A. J.; Yao, C. O.; Shi, M. Z.; Hu, D. Y. and Chai, W. H. (2012): Snail-killing effects of *Streptomyces* 218 powder. British Journal of Pharmacology and Toxicology, 3 (6): 263 – 266.

Barker, G. M. (2002): Molluscs as crop pests. CAB, International, Walling Forti Oxon 10 DE.UK, pp. 468.

Bilgrami, K. S. and Verma, R. N. (1981): Physiology of fungi 2nd eds., Vilkas Publishing. PVT., Ltd. Indian, p. 23 – 27.

Bissett, J. (1991): A revision of the genus *Trichoderma* 111.Section pachybasium. Can. J. Bot., 69 (1): 2373 – 2417.

Cohort Software (2005): Costat program v. 6. 311 (780 lighthouse, Ave. PMB 320, Montery, CA, USA.

Duval, D.; Galinier, R.; Mouahid, G.; Toulza, E.; Allienne, J.; Portela, J.; Calvayrac, C.; Rognon, A.; Arancibia, N.; Mitta, G.; Theron, A. and Gourbal, B. (2015): A novel bacterial pathogen of *Biomphalaria glabrata*: A potential weapon for Schistosomiasis control. Plos Neglected Tropical Diseases, 9 (2): 1-16. doi: 10.1371/journal.pntd.0003489.

El-Sayd, A. M. A; Farag, M. F. N. G. and Lokma, N. (2023): 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. J. of Plant Protection and Pathology, Mansoura Univ., 14 (8): 213-221. DOI: 10.21608/jppp.2023.216886.1157.

Finney, D.J. (1971): Probit analysis (Cambridge Univ. Press, London), p. 333.

Foster, S. P. and Devonshire A. L. (1996): Comparative survival of insecticide susceptible and resistant peach potato aphids, *Myzus persicae* (sulzer) (Homiptera: *Aphididae*) in low temperature field trials. Bulletin of Entomological Research, 86: 17–27.

Frank, B. P.; Gary, L. H. and Michael, J. B. (2018): Corn leaf aphids. Entomology, 20: pp.19.

Garrett, S. D. (1951): Ecological group of soil fungi: a survey of substrate relationships. New Phytologist, 50: 149 –166.

Garthwaite, D. G. and Thomas, M. R. (1996): The use of molluscicides in agriculture and horticulture in Great Britain over the last 30 years. In: Henderson, British Crop Protection Council, Farnham UK, 39 – 46.

Ghamry, E. M. (1997): Bioassay for two strains of bacteria *Bacillus thuringiensis* against certain land snails under laboratory conditions. Zag. J. Agric. Res., 24 (5): 815-821.

Ghatwary, W. G. T. (2000): Integrated management of certain piercing sucking insects infesting some vegetables crops. Ph.D. Thesis Fac. Agric. Zagazig University.

Godan, D. (1983): Pest Slugs and Snails, Biology and Control. Springer Verlag: Berlin, 191 – 192.

Goettel, M. S. and Inglis, G. D. (1997): Hyphomycetes, In: Manual of techniques in insect pathology (Lacey, L.A. eds.), Academic Press. USA, pp. 225–229.

Ishaaya, I. and Swiriski. E. (1976): Trehalase, invertase and amylase activities in the black scle, *Saissetia oleae* and their relation to host adablebility. J. Ins. Physiol., 16: 1025-1029**.**

Johnson, L. E.; Curl, E. A.; Bond, J. H. and Fribourg, H. A. (1959): Methods for studying soil microflora-plant diseases relationship. Burgess Publ. Co. Minn. USA, pp.178.

Jorge, P. (2021): *Trichoderma* as biocontrol agent against pests: New uses biocontrol against pests: New uses for amycoparasite. Biological control. 159, August 2021, 104634. https://doi.org/10.1016/j.biocontrol.2021.104634

Khaleil, M.; El-Mougith, A.; Hashem, H. and Lokma, N. (2016): Biocontrol potential of entomopathogenic fungus, *Trichoderma hamatum* against the cotton aphid, *Aphis gossypii*. Journal of Environmental Science, Toxicology and Food Technology, 10 (5): 11-20. DOI: 10.9790/2402-105021120

Krutmuang, P. and Mekchay, S. (2005): Pathogenicity of Entomopathogenic Fungi *Metarhizium anisopliae* Against Termites Conference on International Agricultural Research for Development Stuttgart-Hohenheim, 11-13 October 2005.

Litwin, A.; Nowak, M. and Rozalska, S. (2020): Entemopathogenic fungi unconventional applications. Rev. Environ. Sci. Biotechnol., 19: 23-42. DOI:10.1007/s11157-020-09525-1

Lokma, N; Farag, M. F. N. G. and El-Sayd, A. M. A. (2023): Histological and biochemical studies of fugus, *Trichoderma yunnanense* on adults of the glassy clover snail *Monacha cartusiana* (Gastropoda: Hygromiidae) and the green peach aphid insect *Myzus persicae* (Hemiptera: Aphididae). Egypt J. Plant Prot. Res. Inst., 6 (2): 208-220.

Naveed, A.; Dayananda, G. Y. and Hosetti, B. B. (2009): Effect of some selected insecticides on the activity of invertase at different stages of pentatomid bug *Cyclopelta siccifolia*. Our Nature J., 7: 222-225.

Oxoid, L. (1982): The Oxoid manual of culture media, ingredients and other laboratory services Turnergraphic Ltd., England, 5th eds.

Qu, S. and Wang (2018): Interaction of entimopathogenic fungi with the host immune system. Dev. Comp. Immunol., 83: 96 – 103.

https://doi.org/10.1016/j.dci.2018.01.010

Rauquet, V. (2004): Phylogénie morphologique des Myodocarpaceae: Famille sub-endémique de Nouvelle-Calédonie. Thesis, DEA de Systématique Animale et Végétale, Muséum National d'Histoire Naturelle Paris.

Wimol, C. and Amaret, B. (2003): Isolation and characterization of pathogens attacking *Pomacea canaliculata*. World Journal of Microbiology and Biotechnology, 19 (9): 903-906.

Wyatt, G. R. (1967): The biochemistry of sugars and polysaccharides in insects. Adv. Insect Physiol., 4: 287-360**.**