Egypt. J. Plant Prot. Res. Inst. (2023), 6 (2): 208 –220

Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Histological and biochemical studies of fungus, *Trichoderma yunnanense* on adults of the glassy clover snail *Monacha cartusiana* (Gastropoda: Hygromiidae) and the green peach aphid insect *Myzus persicae* (Hemiptera: Aphididae)

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ARTICLE INFO
Article History
Received: 26/4 /2023
Accepted: 28/6/2023

Keywords

Monacha cartusiana, Myzus persicae, Trichoderma yunnanense, Histological and biochemical studies.

Abstract

Fungus is thinking about promising biological pest control. These papers, focus on changes in the activity of amylase and invertase enzymes in adults of *Monacha cartusiana* Müller (Gastropoda: Hygromiidae) snail and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) aphid treated with fungus, *Trichoderma yunnanense* compared with control utilizing the dipping technique. The highest concentration 10^8 spore/ml showed a very high decrease in amylase and invertase enzymes which caused the highest reduction after one day, three days and seven days of treatment compared to the control recording (-50.12, - 40.30, -24.96 %) and (-48.01, -33.40, -12.73 %) of amylase enzyme (-29.73, - 39.94, -51.42 %) and (-25.48, -38.91, -54.16 %) of invertase enzyme of tested snail and aphid, respectively**.** Histological studies were conducted on digestive glands in adults of *M. cartusiana* snails to recognize the fine structure of digestive gland in the two healthy and infected snails treated to 10^8 spore/ml after five days of treatment with tested fungus. The digestive gland lost its normal appearance, became degenerated and more vacuolated. The lumina of the tubules appeared more branched shape than those of the normal. The digestive cells became swollen, and the outer tubular membranes of some tubules are damaged. Also, histological studies of treated aphids showed much disinformation in their tissues and showed fungal growth inside the aphid body compared with untreated aphids with natural tissues with perfect cuticles divided into epicuticle and endocuticle. Finally, *Trichoderma yunnanense* was identified by utilizing 18s rRNA and its accession OQ659412.

Introduction

The introduction of novel crops, the consolidation of agricultural production regulation, resulting expansion over human trade, and the trend of species adapting to these altered settings have all been connected to an increase in pest problems (Barker, 2002). Terrestrial snails are thought to be the

most harmful pest in Egypt's stylommatophora (Heiba et al., 2002 and Ali and Robinson, 2020). Also widely dispersed in the Egyptian Governorates were *Monacha cartusiana* (Müller, 1774), Mollusca, Gastropod Pulmonata, Stylomatophora, Helicoidae, and Monacheae. They are appeared in vegetable and fruit crops, orchards, decorative and medicinal plants (Shetaia *et al.*, 2009; Heikal, 2015; Kadry *et al.*, 2018; Shahawy, 2018 and Rady, 2019).

Likewise, one of the most dangerous insect species that causes financial losses in agricultural fields is aphids (Leclant and Deguine, 1994). Due to its vast host range and as a vector of numerous plant viruses, it is a serious pest. Therefore, synthetic chemical pesticides are generally utilized for pest control (Wang *et al.*, 2002). As one of the main causes of environmental pollution, chronic diseases in humans, and harm to most living things, attention is being devoted more and more to the use of synthetic chemical pesticides as a hazardous way of pest management. *Trichoderma* species are widely known for producing a wide variety of secondary metabolites, such as polysaccharides, toxins, and antibiotics (Gams and Bissett, 1998).

Additionally, strains from various species of this genus are frequently utilized in the bio control of fungi that cause plant diseases in soil (Samuels, 2006). Significant use in the sector is using entomopathogenic bacteria as biological control agents (Lacey and Shapiro-Ilan, 2008).

This study was striped to assay the effect of fungus, *Trichoderma yunnanense* on some biochemical activities and the histological structure of adults of the glassy clover snail *M. cartusiana* and the green peach aphid insect *M. persicae*.

Materials and methods

1. Tested animal:

Adults of the glassy clover snail, *M. cartusiana were* collected from fields cultivated with lettuce at Sheeba locality, Zagazig district, Sharkia Governorate, Egypt and identified according to the keys given via Godan (1983). The captured land snails were delivered right away to the Plant Protection Research Institute's lab in white linen bags. Healthy and similar snails were selected and kept in a glass terrarium filled with damp clay soil that was 75% of the water field's capacity. Snails were fed daily with lettuce leaves for two weeks before treatment for acclimatization.

2. Tested insect:

The green peach aphid insect *M. persicae,* was collected from several fields in the Sharkia governorate of Egypt, placed in paper bags, and taken to the lab at the plant protection research institute in the same governorate of Egypt. Individually, the studied insects were raised in a lab. at a temperature of 25 °C, relative humidity of 70 %, and a photoperiod of 12 hrs**.** (Ahmed *et al.*, 1999).

3. Tested fungus:

In the plant protection research institute in Sharkia Governorate, *Trichoderma yunnanense* was isolated from the green peach aphid insect, *M. persicae*, using the homogenization technique, followed by dilution plating of the homogenate on modified Czapek-Dox's agar medium oxoid (Goettel and Inglis, 1997). Cultures were protected on PDA agar slants at 4°C and subcultured every 15 days. Then it was purified and identified morphologically using a light microscope according to Domsch and Gams (1980), Bissett (1991) and Moubasher (1993). A Sequence of 18s rRNA gene of DNA of fungal isolate was done at animal health research institute, Agricultural Research Center, Giza, Egypt. Molecular characterization included the following steps as per the Tarini *et al*. (2010) protocol.

4. Preparation of inoculum:

T. yunnanense was used to prepare spore suspension at various serial dilutions. The spores were collected by rubbing the surface of seven days old plate culture of the tested fungus utilizing sterile distilled water containing a drop of tween 80 (Krutmuang and Mekchay , 2005). The suspensions using a haemocytometer10^6 and 10^8 spore/ml.

5. Biochemical studies:

5.1. Preparation of samples for the biochemical assay:

After 1, 3, and 7 days post-treatment, samples of aphids and snails weighing 1gm were taken from the treated (10^6 and 10^8 spore/ml) and untreated (control) groups of aphids and snails. *M. cartusiana* snails had their adult mollusca shells removed. In tiny vials, samples are stored in the freezer until analysis. Using a Teflon homogenizer, the frozen samples of the studied insects and snails were diluted in distilled water to a volume of 5 ml for each sample. The soft tissues of snails and insects were measured, collected, and homogenized in distilled water at a ratio of 1:10 (w/v). According to Abd El-Haleim *et al.* (2006), the homogenates were centrifuged at 5000 r.p.m. for 20 minutes at 5 °C. The enzyme sources for the amylase and invertase enzymes were the supernatants of the studied snails and aphids. The method outlined by Ishaaya and Swiriski (1976) was used to measure the activities of the enzymes under test.

6. Histological studies:

6.1. Preparation of tested snail:

Two groups of adult *M. cartusiana* snails 30 each were used. Land snails in a group (1) served as control while those in group (2) were dipped in 10^8 spores/ml of fungus, *T. yunnanense* and treated lettuce leaves with tested fungus for 30 seconds. The digestive gland (Hepatopancreas) of treated tested alive snails as well as control after five days of application was dissected. The shell was broken gently utilizing a small hammer, and then the soft specimen was quickly dissected in a saline solution, Hedon Fleig's saline solution (Lee, 1965).

6.2. Preparation of tested insect:

According to Khaleil *et al.* (2016), two groups of peach aphids (*M. persicae*) were infected with spore suspension (10^8 spores/ml) of *T. yunnanense*, while the other groups received water as a control. After being cultured for five days at 25°C and 70% R.H., the groups were then processed for light microscopy.

6.3. Light microscopy:

The digestive gland of tested snail and insect organs were quickly isolated and transferred directly into 10 % formalin, to overcome the problem of the immediate autolysis. The isolated tissues of snail and insect were fixed for about 24 hrs. in 10 % formalin. The fixed tissues were dehydrated and cleared by utilizing alcohol as a dehydrator and xylene as a clearing agent. Embedding in paraffin wax with a melting point of 55 – 56ºC was followed. Sections were cut at 2 µm. The prepared sections were stained by using eosin-haematoxylene (Drury and Wallington, 1980). Stained sections were critically examined and some of the examined parts were photographed at different magnifications. Scoping slides were achieved using a light microscope of a Plant Protection Research Institute in Sharkia Governorate, Egypt.

Results and discussion

1. Molecular identification of the tested fungus:

Using the primers ITS1 (1) and (4), the PCR product of the studied fungus (*Trichoderma yunnanense*) was sequenced. Utilizing the Basic Local Alignment Search Tool (BLAST) program, Altschul *et al.* (1990) and Altschul *et al.* (1997), the resultant DNA sequences from the PCR were compared to the published sequences to see if any homologs to the Gen Bank data existed. The sequences of the PCR products from the

tested fungus were 99.63 % identical to the sequence of *T. yunnanense* Figure (1). The tested isolate's phylogenetic tree revealed that *T. yunnanense* was in the same position and was organized according to an evolutional distance matrix based on incomplete 18S rRNA gene sequences (Figure 2). Figure (3) showed that the sequence of the PCR products from the chosen fungus was highly homologous to that of *T. yunnanense* (99 %).

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

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

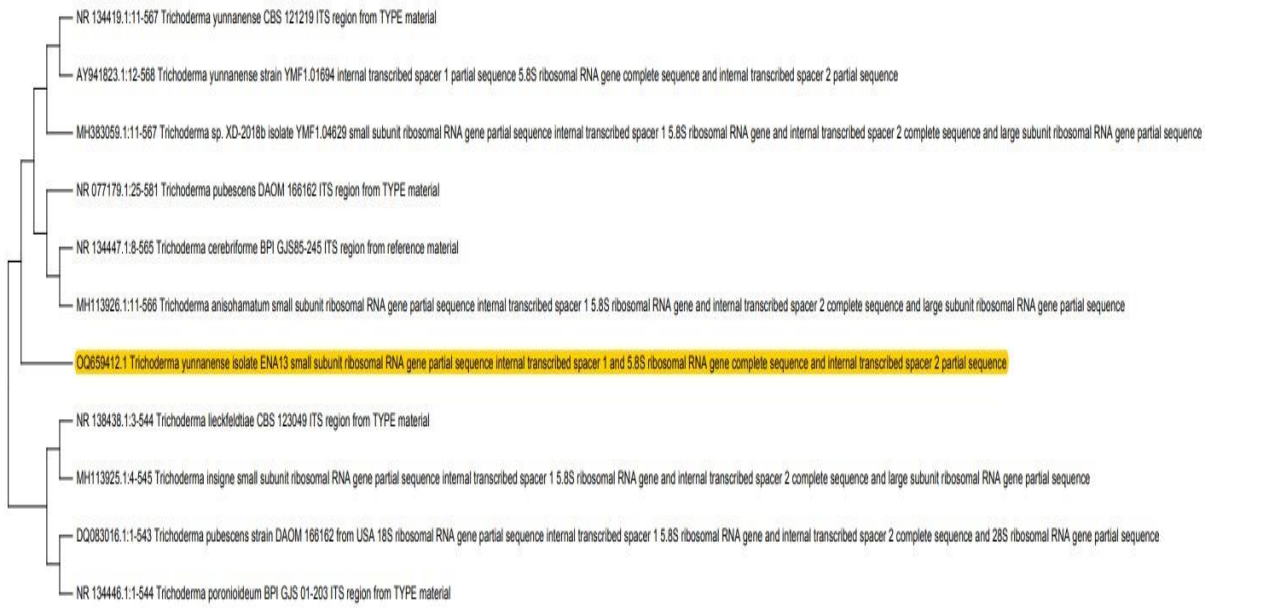

Figure (2): Phylogenetic dendrogram of several fungal isolates accessions detected via average correlation cluster analysis based on 18S rRNA partial sequence.

T. yunnanense CBS 121219 ITS region; from TYPE material Sequence ID: NR_134419.1Length: 599 Number of Matches: 1. See 1 more title(s) See all Identical Proteins (IPG) Range 1: 11 to 567GenBankGraphics 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.

CTCCGTTATGGGGTTCCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATG 61 ||||||| || | ||
|||||||||||||||||||||||||||||||||||||||| Sbjct 11 CTCCGTT--
GGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATG 68 Query 62
TGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGA 121
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 69
TGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGA 128 Query
122 ACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATT 181
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 129
ACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATT 188 Query
182 TCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGG 241
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 189
TCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGG 248 Query
242 TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG 301
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 249
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG 308 Query
302 TGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT 361
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 309
TGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT 368 Query 362
CCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGATCGGCGTTGGGGATCGGGACCCC 421
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 369
CCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGATCGGCGTTGGGGATCGGGACCCC 428 Query
422 TCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGT 481
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 429
TCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGT 488 Query
482 AGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTTC 541
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| Sbjct 489
AGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTTC 548 Query
542 TGAAATG-TGACCTCGGAT 559 ||||||| |||||||||| Sbjct 549 TGAAATGTTGACCTCGGAT 567

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

TGGGGAATTCTACCTGATCCGAGGTCAACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGACGCGCCGC
GCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGG
GCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGAGGGTTGAAATGA
CGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGA
ATTC

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

2. Biochemical studies:

The composition and operation of pest tissues are supported by carbohydrates. Amylase, trehalase, and invertase enzymes have a role in the digestion and utilization of carbohydrates by pests, controlling the metabolism of carbohydrates primarily Wyatt (1967). Enzymes like amylase and invertase are crucial for breaking down and using carbs as energy Naveed *et al.* (2009). According to the data in Tables (1 and 2) *T. yunnanense*, a fungus, causing alterations in the activity of the enzymes amylase and invertase in the adults of the *M. cartusiana* snail and *M. persicae* insect as compared to the control utilizing the dipping technique.

The activity of amylase and invertase was decreased because of all treatments 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 (-50.12, - 40.30, -24.96 %) and (-48.01, -33.40, -12.73 %) of amylase enzyme (-29.73, - 39.94, -51.42 %) and (-25.48, -38.91, -54.16 %) of invertase enzyme of tested snail and aphid, respectively. While concentration 10^6 gave (-27.87, - 15.55, -6.78 %) and (-31.76, -24.68, -5.01 %) of amylase enzyme (-2.76, - 12.49, -22.12 %) and (-15.76, -26.29, -39.44 %) of invertase enzyme of tested snail and aphid, respectively.

Results showed a highly significant difference between the two concentrations of snails and aphids over time, except for seven days for the insect's amylase enzyme. Previous data agreed with those obtained by Khedr *et al.* (2005) discovered that five insect growth regulators (IGRs) had different biochemical effects on *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) insect larvae in their second and fourth instars when tested under laboratory conditions. After 2 and 5 days, the tested IGRs significantly boosted the activity of the trehalase, invertase, and amylase enzymes in both the 2nd and 4th instars.

Omar *et al.* (2006) studied the biochemical impacts of Chinmix, Spintor and Biorepel components on larvae of pink and spiny bollworms *P. gossypiella*, as compared to control. In comparison to the control, Farag (2012) found that all castor oil treatments of the *M. cartusiana* snail reduced the activity of the enzymes, amylase and invertase. Khaleil *et al*. (2016) reported that the biochemical analysis of adult cotton aphid treated with fungus, *Trichoderma hamatum* studied different quantitative changes in the amylase and invertase enzymes relative activities as compared to control. Farag and Sabry (2017) showed that *M. cartusiana* adults treated with used frying soybean oil have decreased amylase, invertase, and trehalase enzymes activity compared to control.

Table (1): Activities of the enzymes (Amylase and invertase) change in adults of the snail *Monacha cartusiana* after treatment with the fungus *Trichoderma yunnanense* compared with control using dipping technique.

Tested Fungus	Conc. (spore/ml)		Amylase			Invertase		
			1 day	3 days	7 days	1 day	3 days	7 days
Trichoderma yunnanense	10^6	SA	23.27^b	26.82^b	31.63^a	38.47^a	33.92^b	31.05^b
		RA%	(-27.87)	(-15.55)	(-6.78)	(- 2.76)	(- 12.49)	(- 22.12)
	10^8	SA	16.09^c	18.96^c	25.46^b	27.80^b	23.28^c	19.37^c
		RA%	(- 50.12)	(- 40.30)	(- 24.96)	(- 29.73)	(- 39.94)	(- 51.42)
Control		SA	32.26^a	31.76^a	33.93^a	39.56^a	38.76^a	39.87^a
P			0.0001 ***	0.0001 ***	0.0005 ***	0.0001 ***	0.0001 ***	0.0001 ***
L.S.D.$_{0.05}$			2.58	1.64	2.60	2.59	2.56	1.21

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

Table (2): Activities of the enzymes (amylase and invertase) change in adults of *Myzus persicae* insect treated with fungus, *Trichoderma yunnanense* compared with control using dipping technique.

Tested fungus	Conc. (Spore/ml)		Amylase			Invertase		
			1 day	3 days	7 days	1 day	3 days	7 days
Trichoderma yunnanense	10^6	SA	15.79^b	18.65^b	21.41	23.84^b	19.74^b	15.71^b
		RA%	(-31.76)	(-24.68)	(-5.01)	(- 15.76)	(- 26.29)	(- 39.44)
	10^8	SA	12.03^b	16.49^b	19.67	21.09^c	16.36^b	11.89^c
		RA%	(- 48.01)	(- 33.40)	(- 12.73)	(- 25.48)	(- 38.91)	(- 54.16)
Control		SA	23.14^a	24.76^a	22.54	28.30^a	26.78^a	25.94^a
P			0.0014 **	0.0009 ***	0.0899^{ns}	0.0003 ***	0.0054 **	0.0001 ***
L.S.D.$_{0.05}$			**3.99**	**2.83**	**2.60**	**1.99**	**4.90**	**3.27**

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

3. Histological studies

3.1. Histological observation in the digestive gland (Hepatopancreas) of *Monacha cartusiana* snail:

The digestive gland of normal snails Figure (4) is a lobed part of the alimentary canal. This gland has several tubules in each of its lobes. Each digestive gland tubule is surrounded by a thin layer of loose connective tissue which fills the intertubular spaces and contains few muscle fibers. The lumen or ductless tubules open into a collecting duct which opens into the stomach from one side and into the intestine from another side. Primary secondary lobe (Acini) is present. Each acinus consists of a single layer of hepatic and pancreatic cells (Hence the name hepatopancreas). Lumen opens into collecting ducts which pour their digestive enzymes into pyloric stomach, when compared to snails exposed to fungus, *T. yunnanense* at a concentration of 10^8 spore/ml after five days of treatment.

Figure (5) showed that the lumen of the tubules appeared more branched shape than those of normal. The digestive cells became swollen and more vacuolated. The outer tubular membranes of some tubules are damaged. The shapes of all cell types became more irregular, and the cell membranes were impaired in some digestive gland tubular epithelium. Snails stopped nourishment and wasted their appetite with very sluggish movement. The results agreed with those acquired by El-Said (2009) indicated that following oral administration of the three doses of Protecto, Biovar pesticides and *Aspergillus flavus* fungus against *M. cartusiana* the general architecture of the digestive glands wasted its normal appearance.

The damaged acini in the treated snails contained narrowing in acinar lumen, atrophied acini and acini became study to each other with confused architecture acini, evanescence of glandular and calcium cells. Farag (2012) reported that following oral administration of castor oil compared to methomyl pesticide on *M. cartusiana* and *E. vermiculata* snails, the general architecture of the digestive glands wasted its normal appearance compared to control.

El-Said (2017) showed the effect of isolated *Streptomyces heliomycini* and Gastrotox pesticide on the histology of the digestive gland of *M. cartusiana* snail. Data examined different alterations in the digestive gland compared with the control.

3.2. Histological observation of *the green peach aphid* insect *Myzus persicae*:

After 5 days at a concentration of 10^8 spore/ml, the adults of *M. persicae*, both treated and untreated, underwent histological examination. This study's microscopic analysis of adult *M. persicae* revealed that the *T. yunnanense* spore suspension significantly deformed and disintegrated the aphid's body and tissues. It discovered the growth and colonisation of the fungus inside the insect. The examination using a light microscope showed different histological alterations between the treated and untreated adult peach aphids Figures (6 and 7).

An adult untreated peach aphid was depicted in Figure (6) as having a typical, undamaged cuticle that clearly distinguished between layers called the

epicuticle and the endocuticle. Additionally observed were normal adipose tissue, normal intestinal epithelial cells encircling the lumen, a normal salivary gland in its entirety, encircled by its membrane, and normal ovarian tissue containing an embryo. The basement membrane that encompassed all aphid organs in the haemcoel of the aphid as well showed normal and intact.

Furthermore, as seen in Figure (7), the body of the treated adult aphid exhibited several deformities. The basement membrane that surrounded the internal organelles of the green peach aphid and clearly appeared in the treated adult *M. persicae* reported disintegrating and disappearing. Similar findings reported by many researchers (Quesada *et al.*, 2006; Schneider *et al.*, 2013 and Gabarty *et al.*, 2014) used light microscopy to reveal numerous histological alterations in numerous aphids because of infection with various entomopathogenic fungi.

Hussein *et al.* (2012) and Schneider *et al.* (2013) indicated that the cuticle is disorganized, not differentiated within its epicuticle and endocuticle layers, and has developed black spots, mentioning a direct fungus attack on the insect defense system. Numerous cuticle abnormalities were observed after *S. littoralis* larvae were treated with five different strains of entomopathogenic fungi (Amer *et al.*, 2008). According to Alves (1998) and Schneider *et al.* (2013), the mycelium growth and the beginning of the fungus' sporulation process likely caused physical damage to the host's cuticle. It also may have caused biochemical degradation. Gunnarsson (1988) demonstrated that the host's innate defense system is activated once it recognizes the invading fungus by changes in the cuticle basement membrane's characteristics.

The salivary gland of adult *M. persicae* under a light microscope lost its protective barrier and appear because of and distorted because of *T. yunnanense* treatment, the hind intestine epithelial cells of the treated insect's observations revealed deformation Figure (7). According to Schneider *et al.* (2013) and Alves (1998), the alteration in the host's cuticle is most likely caused by physical damage brought on by the mycelium growth and the start of the fungus's sporulation process. It may also be the result of biochemical degradation. El-Banna *et al.* (2012) investigated a variety of histological alterations brought on by bacterial and viral infection of cotton leaf worm larvae. Sowjanya *et al.* (2008) examined the ultrastructural responses of crude destruxin produced via *Metarhizium* sp. on the salivary gland.

The objectives of studies on fungus, *T. yunnanense* is to safe method for controlling of adults of the glassy clover snail, *M. cartusiana* and *the green peach aphid* insect, *M. persicae* which studied of changes in activity of amylase and invertase enzymes and histological studies in tested snail and aphid using dipping technique compared to control.

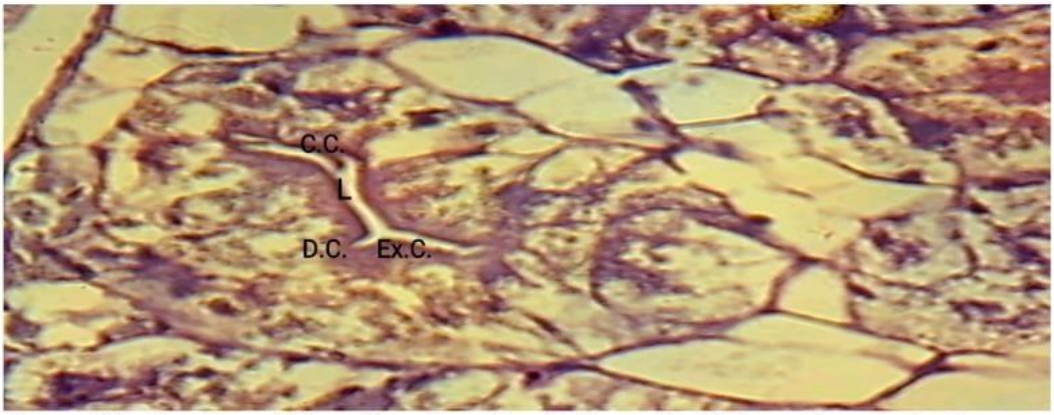

Figure (4): Section of the digestive gland of adult of *Monacha cartusiana* (normal snail) showing the lumina (L) of the tubules open into a collecting duct which opens into the stomach from one side and into the intestine from another side, digestive cells (D.C.), excretory cells (Ex.C.) and calcium cells (C.C.). (X100).

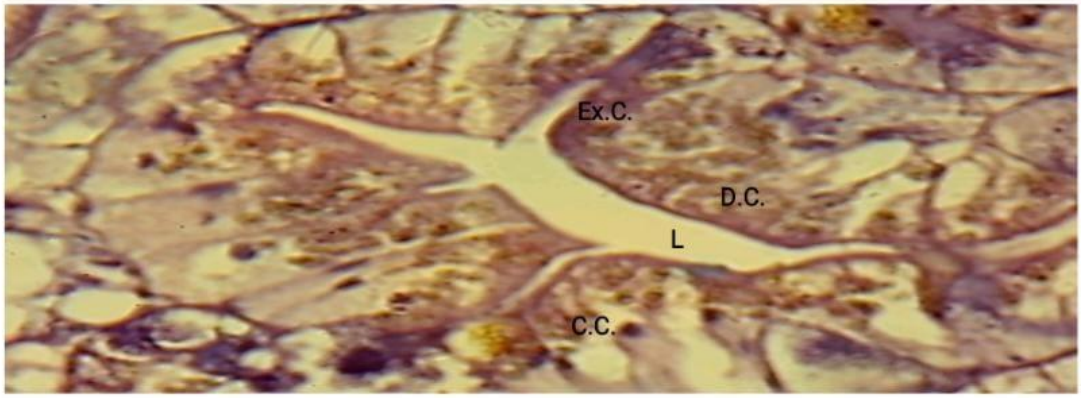

Figure (5): Section of the digestive gland of adult of *Monacha cartusiana* exposed to fungus, *Trichoderma yunnanense* at 10^8 spore/ml after five days compared with control using dipping technique showing changes of excretory cells (Ex.C.),calcium cells (C.C.),dilatation of tubule with a wide lumen, infiltration within the intertubular connective tissue digestive cells (D.C.), and the lumina (L) of the tubules appeared branched shape than those of the normal. (X100).

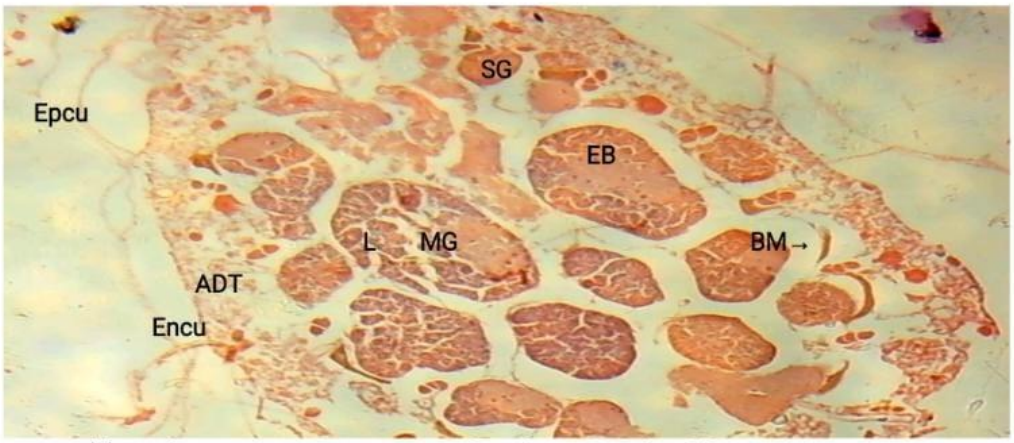

Figure (6): Light microscope micrographs of untreated adult *Myzus persicae* insect. epicuticle (Epcu), endocuticle (Encu), embryo(EB), lumen(L), mid gut (MG), salivary gland (SG), basement membrane (BM) and adipose tissue (ADT) (X100).

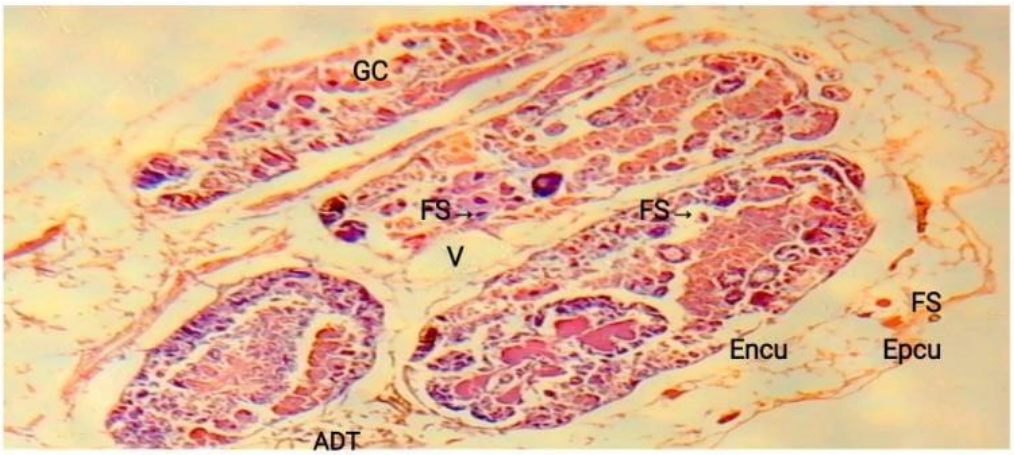

Figure (7): Light microscope micrographs of adult *Myzus persicae* insect treated with fungus, *Trichoderma yunnanense* (10^8 spores/ml) spore suspension after 5 days post-treatment. epicuticle (Epcu), endocuticle (Encu), adipose tissue (ADT), gut cavity (GC), fungus spore (FS) and vacuole (V) (X100).

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