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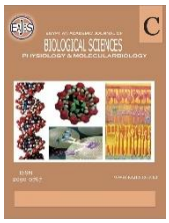
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(1) pp403-415 (2024)*
DOI: 10.21608/EAJBSC.2024.359469

Egypt. Acad. J. Biolog. Sci., 16(1):403-415(2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Histopathological and Protein PatternAlterations of *Eobania vermiculata* (Müller) Infected with Snail Parasitic Nematode, *Phasmarhabditis eagyptiaca* Azzam2023

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ARTICLE INFO
Article History
Received:7/5/2024
Accepted:10/6/2024
Available:14/6/2024

Keywords:
Phasmarhabditis eagyptiaca, *Eobania*, Snails, Histopathological, Protein, SDS-PAGE.

ABSTRACT

The land snails cause damage to their host plants, by feeding on leaves, blooms, flowers, fruits, trunks, limbs and barks. Thus, necessary to control these pests.

Phasmarhabditis eagyptiaca Azzam 2023 is a new species of snail parasitic nematode, which was recorded and described with investigate its capability to infect some snails and slugs.

This investigation aims to prove the parasitization of the *P. eagyptiaca* nematode on *Eobania vermiculata* (Müller, 1774) and clear its effect on snails consequently suitability of the nematodes for application to control gastropods, also to differentiate between the two strains of *E. vermiculata*.Two strains of *Eobania* snails were infected with the snail parasitic nematodes *P. eagyptiaca*. The differences between infected and non-infected snails in histological and protein pattern alterations were investigated.Immunoreactivity as fibroblast region and splitting necrosis and degeneration regions were observed in the head foot, ovary, testis, and digestive gland in the two strains of infected *E. vermiculata*, in addition to changes in protein patterns. All these alterations lead to snail death.

The histopathological alterations in *E vermiculata* snails infected with snail parasitic nematode *P eagyptiaca* confirm the parasitic relation between them. Although the immune defense from snails against the parasitic nematodes, the nematodes could kill the snails. This indicates the suitability of this nematode for land gastropods biocontrol.

INTRODUCTION

Nematodes and terrestrial gastropods associations range from accidental phoresis to parasitic or pathogenic relationships **(**Grewal *et al.*, 2003; Nermut' and Půža, 2017). Azzam and El-Abd, 2021) recorded *Phasmarhabditis* sp. from eggs of *E. vermiculata* for the first time. They also studied the infectivity of this nematode for eggs, juvenile and mature snails, *E. vermiculata* and *Limax flavus* L. slugs, and also adult and eggs of the non-local species, *Achatina fulica* Bowdich

Azzam *et al*., (2000) studied the symptomatolgical and histopathological alterations of the land snail *E. vermiculata* infected with the snail parasitic nematode, *Rhabditis* sp., and found that the nematode affected the snail movement, and feeding in addition to cellular reaction especially in the snail head foot region.

The symptomatolgical and histopathological studies on *E. vermiculata* and the cutworm *Agrotis ipsilon* Hufengel infected with the snail parasitic nematode, *Phasmarhabditis tawfiki* Azzam indicated that this nematode has a direct effect on movements and feeding of both experimentally infected pests in addition to cellular reaction formed around the different developing stage of the nematode before host death (Azzam *et al*., 2005).

Bonfim *et al*., (2020) reported oocytes with granular nucleus and cytoplasm in the process of apoptosis in the snail, *Subulina octona* after infection by *Paratanaisiabragai*. They referred the decrease in the reproductive activity of snails in response to parasitic infections to either direct mechanisms, such as the destruction of gonadal tissues of the host during intramolluscan larval development of the parasite, or indirectly, due to interferences with the neuroendocrine system (NES) or absorption, by the parasite, of circulating nutrients in the hemolymph required for the development of the snail. Infection of *Biomphalaria glabrata* with *Angiostrongylus cantoniensis* showed a decrease in total protein concentration and an increase in nitrogen excretion products, as well as in the aminotransferases activity of the host snail hemolymph (Tunholi-Alves, *et al*., 2011)

Azzam (2004) reported nematodes invading their hosts (snails and slugs) from their natural opening. After nematodes find a susceptible host, they enter its hemocoel and then release their symbiotic bacteria that are known to implement a variety of infection-facilitating processes (Lu, *et al*., 2017 and Chang, *et al*., 2019)

The amino acid deamination resulted in the formation of byproducts that are essential to the parasites' development, such as arginine (Tunholi-Alves, *et al*., 2011; Tunholi, *et al*, 2011).These alterations can change the glycogenesis, gluconeogenesis, and glycolysis rates in the snail (Mello-Silva,.*et al*., 2010). The snails infected with larvae of dicrocoeliidtrematodes showed a significant increase in phenoloxidase activity when compared with non-infected snails (; Unlu, and Ekici, 2021). Desouky and Busais(2012) differentiate between *E. vermiculata* shell color (dark and light) from Egypt and Saudi Arabia by mitochondrial markers. The SDS-PAGE technique is commonly employed in biological analysis to determine shifts in protein bands. These bands might be proteins or enzymes. Bio-stress, due to hormonal changes could cause protein synthesis and enzymatic shifts (Ghasempour and Maleki 2003),*P. eagyptiaca* was first recorded and described morphologically and phylogenetically as a new species and its capability to kill some snails and slugs was investigated (Azzam,2023).

The present investigation aims to differentiate between the two strains of Egyptian *E. vermiculata* and to prove the parasitization of *P. eagyptiaca* nematode and its effect on snails consequently suitability of the nematodes for application to control gastropods.

MATERIALS AND METHODS

The nematode, *P. eagyptiaca* Azzam2023 (Nematoda: Rhabditidae) was supplied from the offspring of the original isolation, which was isolated from *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) by (Azzam, 2023).

Rearing of *E .vermiculata* was conducted in sterilized plastic cages with ethanol then substrated with sterilized clay and, moistened with distilled water. After the eggs are lying, the eggs are gently washed with distilled water and transferred to sterilized clay in Petri dishes, then placed in a dark place until hatching.

After hatching the new individuals were transferred gently to new sterilized cages as mentioned above, then provided with a source of food (lettuce leaves that were washed with a strong stream of tape water to remove associated organisms and then washed with distilled water (Azzam, 2023).

Histopathological Studies:

For histopathological studies two Petri dishes each containing five snails were exposed to concentrations of 125 I.S./snail and another dish contained snails only without adding nematodes as a control.

After 24-48 and 72 hrs, the shells of some infected and non-infected snails were gently crushed and removed without anesthesia. The soft parts of the snail were immediately fixed in Bouin's solution. After 24 hrs the samples were washed in ethanol 70% and dehydrated through ascending grades of ethanol (10, 30, 50, 70, 90, and 96%), Then cleared in terpineol washed in benzene embedded in paraffin-wax and stained with hematoxylin and eosin.The sections were mounted permanently and examined and photographed using a light microscope (Olympus BX53, Tokyo, Japan) connected to a digital camera (Olympus PEN Lite, Tokyo, Japan).

Protein Extraction:

To prepare protein extract soft parts of the snail were homogenate-using glass in 2:1 w/v of extraction buffer (0.06 M Tris-HCL (Tris (hydroxymethyl) aminomethane hydrochloride) pH=8.8, 0.002 M EDTA (Ethylenediaminetetraacetic acid) pH=8.0). The samples were centrifuged (Centrifuge 2K15, Sigma, Germany) at 14000 rpm for 10 min at 4 ºC and kept frozen.

Sample Preparation:

Samples were prepared by adding buffer 2 (0.125 M Tris-HCl pH 6.8, 20 % glycerol, 4% SDS, 2% B -mercaptoethanol (B-ME) 0.02 % bromphenol blue) 2:1 v/v. and boiling 10 min in water bath (MAS Medica& Scientific Equipment, Egypt) 100 ºC.

The Protein fractionations and protein marker were loaded to 12.5% polyacrylamide gels according to Laemmli (1970) and achieved on mini vertical slab gel by electrophoresis (Cleaver, UK). After separation, slab gel was stained with commassie brilliant blue. The gel was photographed by Gel Doc Bio-Rad System (California, USA) (Gel Pro analyzer V.3,). Molecular weights of each band and comparing the presence and absence of each band among strains were conducted by the Total Lab program version 1.10 software based on protein marker Data was imported in multi-variant statistical package (MVSP) Version 3.1 available at https: //www. freedownloadcenter.com/windows/mvsp-a, to find the similarity matrix and dendrogram.

RESULTS

It was noticed that the collected *E. vermiculata* snails include two strains (Fig 1). The first, strain 1(Dark soft part) (S1) (Fig.2) had a dark color animal soft part while the second, strain 2(Pale soft part) (S2) (Fig. 3) had a pale color. The soft part color did not relate to the color of the shell. The pale color soft part sometimes has a pale color or dark color shell, and vice versa the soft part of animals may be a dark or pale color shell. Therefore, the investigation was applied to the different two strains.

Fig.1: The two strains of *E. vermiculata* snail, S1 (dark soft body) and S2(pale soft body).

Fig. 2: *E. vermiculata* snail, S1 (dark soft body).

Fig.3:*E.vermiculata* snail S2(pale soft body).

Histopathological Examination:

Histology of the infected two strains of *E. vermiculata* snails (S1 and S2) showed an immunic defense reaction against nematodes. These reactions are represented by fibroblasts formed around the developing larvae in the snail head foot tissues, large and small necrotic granuloma and degeneration of mesoepithelium (Figs. 4&5) compared with control (Figs.6 &7) that showed normal intact covering epithelial layer, distribution of pigment cells and well-organized muscular tissue. The infected snail showed also some changes in the male gonad, i.e. sperm duct and prostate tubules, lumen of prostate tubules dilated, degenerative sperm, lytic storage tissues, degenerative spermatid and spermatogonia (Figs. 8&9). The histological structure of the uninfected male reproductive system showed, glandular tubules interspersed with connective tissues of onormal sperm, spermatids and spermatogonia (Figs. 10&11). Also in the female gonad of infected snails, partial destruction, degeneration of oocytes and necrosis of both oocyte and oogonia, atretic follicle, and unregular oocytes were observed (Figs. 12&13). The normal hermaphrodite gland of the adult of *E. vermiculata* is comprised of many separated acini by thin vascular connective tissue where complete development eggs are located at the circumference of the acini showing normal vetillogenic oocytes and oogonia (Figs. 14 &15).

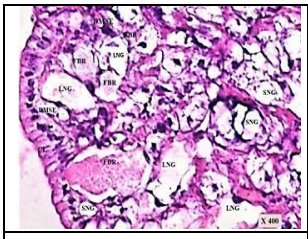

Fig. 4: Section through the foot of infected snail (S1) showing fibroblast reaction was formed around the developing larvae, large and small necrotic granuloma degeneration of mesoepithelium. H&E X 400

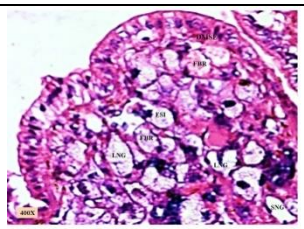

Fig 5: Section through the foot of infected snail (S2) showing fibroblast reaction was formed around the developing larvae, large and small necrotic granuloma degeneration of mesoepithelium. H&E X 400

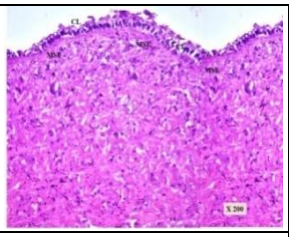

Fig. 6: Section through the foot of non-infected snail (S1) showing normal cuticle and mesoepithelial layer, distribution of pigment cells and well-organized muscular tissues H&E X 200

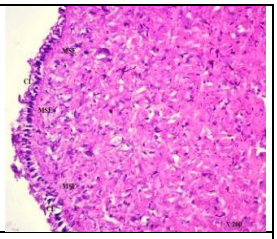

Fig. 7: Section through the foot of non-infected snail(S2) showing normal cuticle and mesoepithelial layer, distribution of pigment cells and well-organized muscular tissues H&E X 200

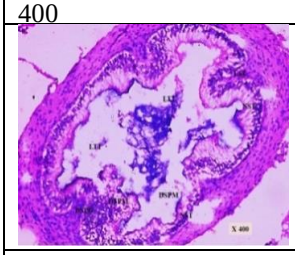

Fig. 8: Section through the testicular part of the gonads of infected snail(S1) showing lytic testis and necrotic storage tissues, degenerative sperm, spermatids and spermatogonia. H&E X 400

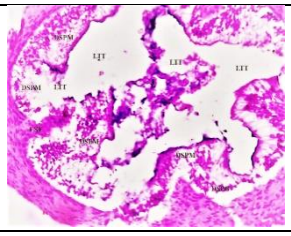

Fig. 9: Section through the testicular part of the gonads of infected snail(S2) showing lytic testis and necrotic storage tissues, degenerative sperm, spermatids and spermatogonia. H&E X 400

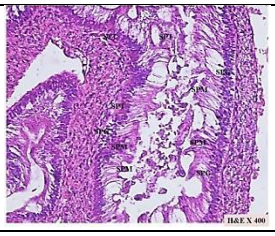

Fig.10: Section through the testicular part of the gonads of a non-infected snail (S1) showing normal testicular tissues, normal sperms, spermatids and spermatogonia. H&E X 400

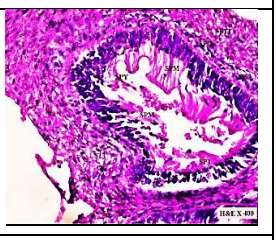

Fig.11: Section through the testicular part of the gonads of non-infected snail(S2) showing normal testicular tissues, normal sperms, spermatids and spermatogonia. H&E X

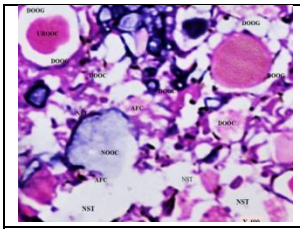	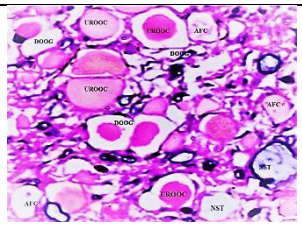	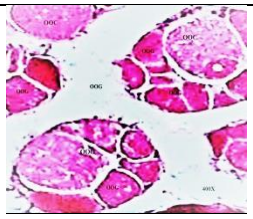	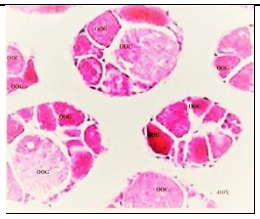
Fig. 12: Section through the ovarian part of the gonads of the infected snail (S1) showing, degenerative oocyte and oogonia, necrotic storage tissues, atretic follicle, necrotic and unregular oocyte. H&E X 400	**Fig. 13:** Section through the ovarian part of the gonads of the infected snail (S2) showing, degenerative oocyte and oogonia, necrotic storage tissues, atretic follicle, necrotic and unregular oocyte. H&E X 400	**Fig 14:** Section through the ovarian part of the gonads of a non-infected snail (S1) showing normal vitellogenic oocytes and oogonia. H&E X 400	**Fig. 15:** Section through the ovarian part of the gonads of a non-infected snail (S2) showing normal vitellogenic oocytes and oogonia. H&E X 400
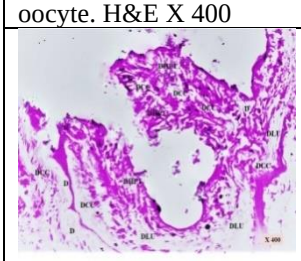	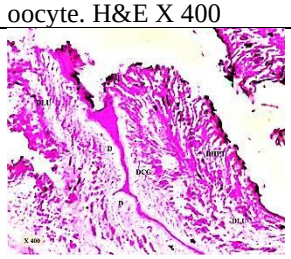	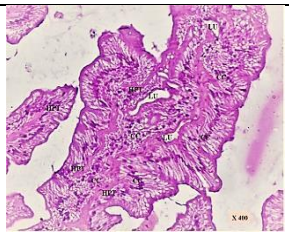	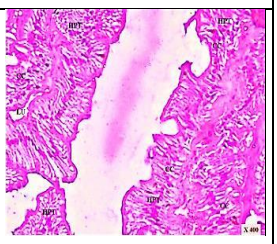
Fig 16: Section through the hepatopancreas of the infected snails (S1) showing dilated lumen, degenerative crypt cells and Hepatopancreatic tubules and degeneration tissues. H&E X 400	**Fig. 17:** Section through the hepatopancreas of the infected snails (S2) showing dilated lumen, degenerative crypt cells and Hepatopancreatic tubules and degeneration tissues. H&E X 400	**Fig 18:** Section through the hepatopancreas of the non-infected snails (S1) showing normal hepatopancreatic tubules, narrow lumen, and normal crypt cells. H&E X 400	**Fig. 19:** Section through the hepatopancreas of the non-infected snails (S2) showing normal hepatopancreatic tubules, narrow lumen, and normal crypt cells. H&E X 400

The digestive gland of the infected *E. vermiculata* S1 &S2 was destructed as a result of infection by a nematode, the lumen tubules were dilated, and the mesoepithelium and crypt cells and tissues degenerated (Figs. 16&17). While the epithelium of the digestive gland is located in the thin membrane basement, normal hepatopancreatic tubules, narrow lumen, and normal crypt cells in the non-infected snail, (Figs. 18&19).

Proteins Electrophoresis:

Comparative between the Two Strains of *E. vermiculata* Snail:

Evaluation of protein profiling by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the two snail strains S1 and S2, showed different banding patterns. According to the locomotion of proteins on the gel, (Fig. 20) many changes in protein ideals were observed, (26) total bands; 10 of them are monomorphic bands with molecular weights MW; (362, 210, 191, 142, 120, 96, 47, 25, 16 and 3) kilodalton (kDa), respectively. While (16) bands (62%) polymorphic with unique bands or unique bands were revealed. 9 bands in S1 with MW (289, 264, 223, 72, 37, 31, 14, 11, and 9) kDa,.While 7 bands S2 were (291, 71, 64, 41, 33, 13 and 10) kDa as shown in Table (1) and Figure (21).

Table 1: Identification of the two strains of *E. vermiculata* using SDS-PAGE.

No. of protein Bands.	MW	S1	S2	Polymorphism
1	362 kDa	+	+	Monomorphic
2	291 kDa	-	+	Polymorphic
3	289 kDa	+	-	Polymorphic
4	264 kDa	+	-	Polymorphic
5	223 kDa	+	-	Polymorphic
6	210 kDa	+	+	Monomorphic
7	191 kDa	+	+	Monomorphic
8	142 kDa	+	+	Monomorphic
9	120 kDa	+	+	Monomorphic
10	96 kDa	+	+	Monomorphic
11	72 kDa	+	-	Polymorphic
12	71 kDa	-	+	Polymorphic
13	64 kDa	-	+	Polymorphic
14	47 kDa	+	+	Monomorphic
15	41 kDa	-	+	Polymorphic
16	37 kDa	+	-	Polymorphic
17	33 kDa	-	+	polymorphic
18	31 kDa	+	-	Polymorphic
19	25 kDa	+	+	Monomorphic
20	16 kDa	+	+	Monomorphic
21	14 kDa	+	-	Polymorphic
22	13 kDa	-	+	Polymorphic
23	11 kDa	+	-	Polymorphic
24	10 kDa	-	+	Polymorphic
25	9 kDa	+	-	Polymorphic
26	3 kDa	+	+	Monomorphic
Total bands		**19**	**17**	**26 bands**
Monomorphic		**10**	**10**	
Polymorphic with unique bands or unique bands		**9**	**7**	
Polymorphism%		**62%**		

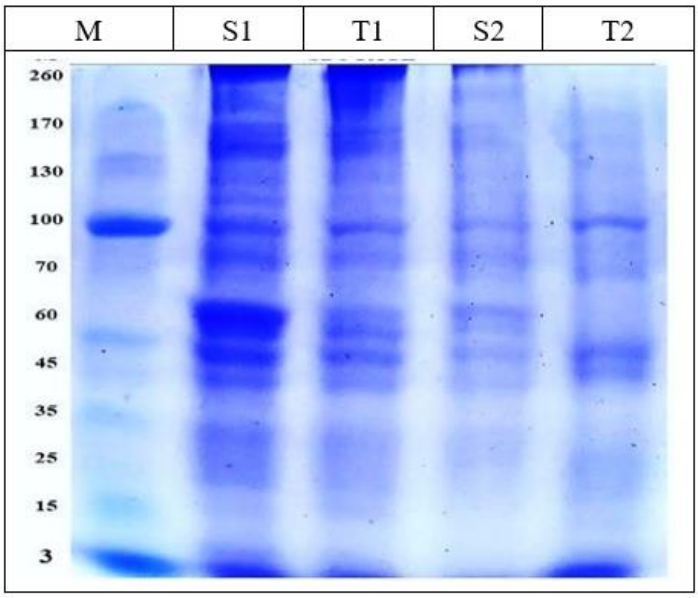

Fig.20 : Sodium dodocylesulphate polyacrylamide gel electrophoresis (SDS-PAGE)12.5 % acrylamide of 4 samples of snail control and treatment. compared with protein marker S1Strain 1(Dark soft part) of *E. vermiculata* un infected (snail1 control), (T1) Infected strain 1(Dark soft part) of *E. vermiculata* , S2: Strain 2(Pale soft part) of *E. vermiculata* un infected (snail control) and T2 Infected strain 2(Pale soft part) of *E. vermiculata***.**

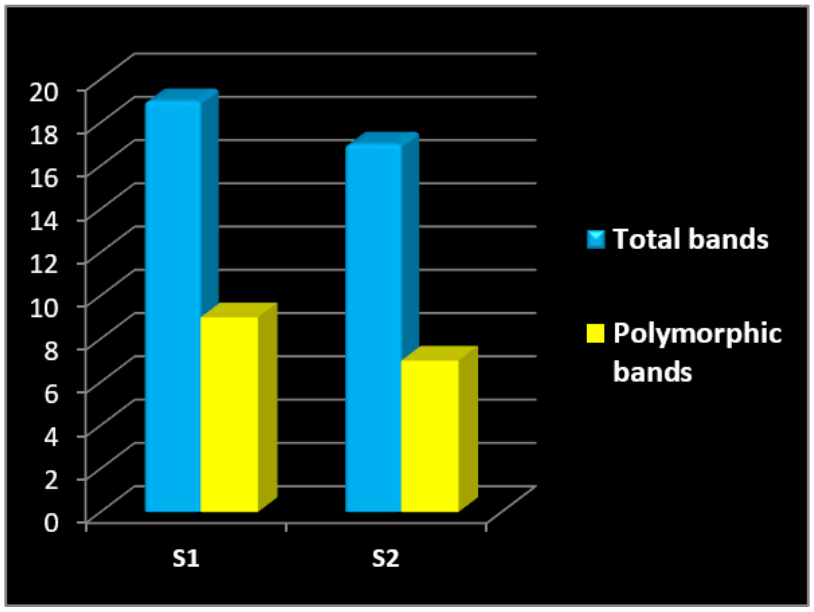

Fig.21:The relationship between total bands and polymorphic with unique bands or unique of SDS PAGE used to differentiate between two strains of snails**.**

Comparative between Infected and Non-Infected Two *E. vermiculata* Strains

Recognition of protein profiling of the two snail strains, S1 and S2,showed different banding ideals between control and treatments (T1, T2) in both snails strains (S1, and S2)(Table 2 and Fig. 22). According to the locomotion of proteins on the gel, many changes in protein ideals were observed in snails infected with *P. eagyptiaca* where 32 total bands appeared; three of them were monomorphic bands with molecular weights MW; (191, 96 and 3) kDa, respectively represented with 9%. While (29) polymorphic with unique bands were revealed with (91%). The highest No. of total and polymorphic with unique bands was revealed in the sample (S1) with 19 and 16 bands respectively. On the

other hand, T2 was the lowest total and polymorphic with unique bands (14 and 11) band respectively as shown in Table (2). Identification of snail samples using SDS-PAGE protein profiling has revealed many alterations in protein patterns from snails treated with *P. eagyptiaca* (T1 and T2) and control, S1 and S2. (Figs. 20 and 22)

Table 2: Protein identification of the two *E. vermiculata* strains infected with *P. eagyptiaca* compared with non-infected one susing SDS-PAGE

No. of protein bands	MW	S1	T1	S2	T2	Polymorphism
1	362 kDa	+	+	+	-	Polymorphic
2	339 kDa	-	-	-	+	Unique
3	295 kDa	-	-	-	+	Unique
4	291 kDa	-	-	+	-	Unique
5	289 kDa	+	-	-	-	Unique
6	264 kDa	+	-	-	-	Unique
7	223 kDa	+	-	-	+	Polymorphic
8	210 kDa	+	+	+	-	Polymorphic
9	191 kDa	+	+	+	+	Monomorphic
10	157 kDa	-	-	-	+	Unique
11	142 kDa	+	+	+	-	Polymorphic
12	120 kDa	+	+	+	-	Polymorphic
13	96 kDa	+	+	+	+	Monomorphic
14	80 kDa	-	-	-	+	Unique
15	72 kDa	+	+	-	-	Polymorphic
16	71 kDa	-	-	+	-	Unique
17	64 kDa	-	-	+	+	Polymorphic
18	47 kDa	+	+	+	-	Polymorphic
19	41 kDa	-	+	+	-	Polymorphic
20	37 kDa	+	-	-	-	Unique
21	33 kDa	-	-	+	+	polymorphic
22	31 kDa	+	+	-	-	Polymorphic
23	28 kDa	-	-	-	+	Unique
24	25 kDa	+	+	+	-	Polymorphic
25	16 kDa	+	+	+	-	Polymorphic
26	14 kDa	+	-	-	-	Unique
27	13 kDa	-	-	+	+	Polymorphic
28	11 kDa	+	+	-	-	Polymorphic
39	10 kDa	-	-	+	+	Polymorphic
30	9 kDa	+	+	-	-	Polymorphic
31	7kDa	-	-	-	+	Unique
32	3 kDa	+	+	+	+	mono
Total bands		**19**	**15**	**17**	**14**	32
Mono		**3**	**3**	**3**	**3**	3
Poly		**12**	**12**	**12**	**5**	17
Unique Bands		**4**	**0**	**2**	**6**	12
Pol+ Unique		**16**	**12**	**14**	**11**	29
Polymorphism%						91%

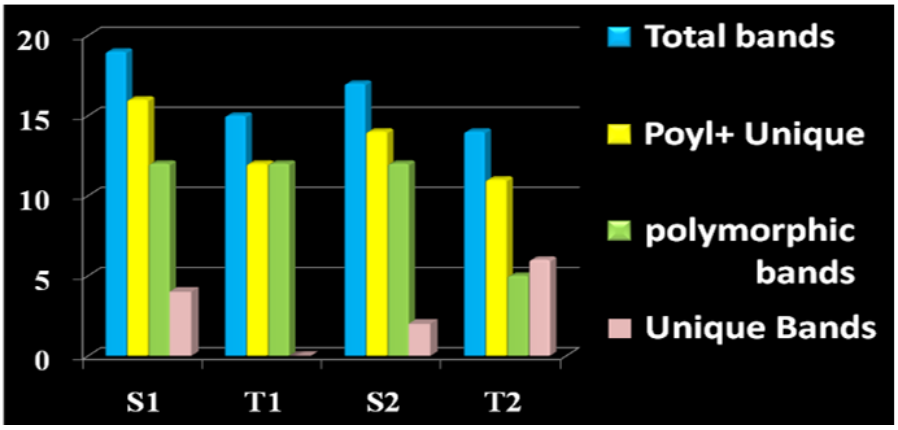

Fig. 22 : The relationship between total, polymorphic with unique band, polymorphic and uniquebands of SDS PAGE used to differentiate between S1, S2 control and T1, T2 treatments

Proximity Matrix Analysis (Genetic Similarity):-

As seen in Table (3**),** pairwise comparisons between the two snail strains infected and non-infected, showed that genetic similarity ranged from (0.190 to 0.800). The biggest value (0.800) was recorded between (S1 and T1) while the lowest (0.190) was recorded between (T1 and T2), respectively. While (T1 and S2) (0.750), (S1 and S2) (0.593), (S2 and T2) (0.261) and (0.250) between (S1 and T2) respectively. As seen in Figure (23), cluster analysis divided all results into two main clusters. The first one is inclusive (T2) only while cluster two comprises two sub-clusters. The first sub-cluster includes (S2), while, the second sub-cluster is divided into two sub-sub clusters. The first one included (T1), while the second two included one group (S1).

Table 3: Genetic similarity of the two *E. vermiculata* strains (control and infected with *P. eagyptiaca*) 4 genotypes using SDS-PAGE.

	S1	T1	S2	T2
S1	1.000			
T1	0.800	1.000		
S2	0.593	0.750	1.000	
T2	0.250	0.190	0.261	1.000

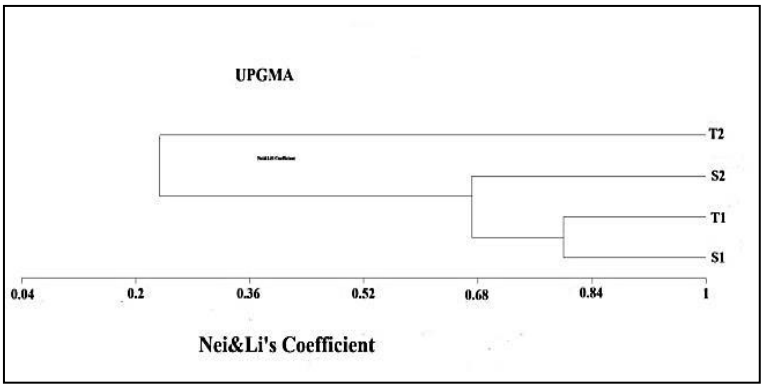

Fig. 23. Dendrogram representing the genetic relationship among the 4 snail genotypes using UPGMA cluster analysis of Nei-Lis similarity coefficient generated from the SDS PAGE

DISCUSSION

In the present investigation, the nematodes caused histopathological effects on different parts of the infected snails, which have more effect on the strain S2 than S1. These changes were fibroblast reactions formed around the developing larvae and large and small necrotic granuloma degeneration of mesoepithelium. Lytic testis and necrotic storage tissues, degenerative sperm, spermatids and spermatogonia also degenerative oocytes and oogonia, necrotic storage tissues, atretic follicles, necrotic and unregular oocytes. lastly caused dilated Hepatopancreatic tubules and lumen, degenerative crypt cells and tissue degeneration. This destruction in different parts may be due to direct mechanisms resulting from nematodes' invasion of the snail body or to metabolic and other excretory materials during nematodes' development or immune cellular defense reaction.

Similar observations were observed when exposure of *Lymnaea luteola* to Paraquat (Kanapala, and Arasada,. (2013).Azzam, *et al.,* (2000) found that *Rhabditis* sp. affects the infected snail *E. vermiculata* movement and feeding and composes cellular reaction especially, in the snail head foot region. While Azzam *et al.* (2005) reported that the snail parasitic nematode *P. tawfiki* has a direct effect on the movements and feeding of both experimentally infected pests *E. vermiculate* and *A. ipsilon* in addition to cellular reactions formed around the different developing stage of the nematode inside the host before death.Giannelli, *et al*., (2015) recorded that the larvae of *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* caused fibromuscular tissue of the foot of infected *Helix aspersa* snails, and small necrotic granulomas fibroblast-like encapsulations, large necrotic granulomas were formed in different organs. The immunoreactivity to nematode larvae included: non-vacuolated or vacuolated small necrotic granulomas and large necrotic granulomas.Colella *et al* . (2015) detected L3 of *Aelurostrongylusa bstrusus* in experimentally infected *Helix aspersa*, surrounded by fibro-muscular tissue, in each of the foot, intestine and kidney parenchyma.Bonfim *et al*., (2020) reported oocytes with granular cytoplasm and nucleus in the process of apoptosis and destruction of gonadal tissues in the snail *S. octona* after infection by *Paratanaisia bragai*. They refer these changes to either direct mechanisms during intramolluscan larval development of the parasite, or indirectly, due to interferences with neuroendocrine system (NES) or absorption of nutrients in the snail hemolymph by the parasite, (Tunholi-Alves, *et al*., 2011 and Tunholi, *et al.,* 2011).Bighiu *et al*., (2017) reported haemocyte infiltration in the foot, autolysis of the digestive gland tubules, granulocytoma, and lysis in the kidney, of *Theodoxus fluviatilis* infected by trematode larvae. Ziegler *et al*. (2021) reported irregularly shaped apices of the tubules, compartmentation of the digestive cells and nuclei of crypt cells and dilated lumina of the tubules in all snail *Planorbarius corneus* treated with an antidepressant. Nantarat *et al*., (2019) compared two types of snail protein and found that protein ranged from 105 to 17 kDa in the mucus of *Lissachatina fulica* (Ferussac,1821) (Mollusca, Gastropoda, Stylommatophra, Achatinidae) were 35, 48, 55, >70 and >100 kDa, while those of *P. canaliculata* were 17, 48, >70 and >100 kDa. Also, the results agreed with Keremedchiev *et al.* (2021) who used SDS-PAGE and detected protein bands ranging from 25 to 250. Proximity matrix analysis showed low similarity either between infected and un-infected snails or the two strains of snails. The morphometric characteristics of the dark and light shells of *E. vermiculata* showed significant differences between the populations of dark and light shells of *E. vermiculata* from Saudi Arabia and Egypt in morphological characteristics represented two separate groups that could be considered two separate subspecies Desouky and Busais (2012).

CONCLUSION:

The present study proved that *Phasmarhabditis eagyptiaca* Azzam 2023 has a great effect on *E. vermiculata* snails either S1 (dark soft body) or S2 (pale soft body), causing fibroblast region and splitting necrosis and degeneration regions between the head foot, ovary, testis and digestive gland in addition to many alterations in protein patterns. These changes affect the vitality of the snails leading to death. Thus, these parasitic nematodes could be considered an environmentally friendly biocontrol agent for these snails.

LIST OF ABBREVIATIONS:

*P. eagyptiaca***:** *Phasmarhabditis eagyptiaca* Azzam 2023 *P. tawfiki* :*Phasmarhabditis tawfiki* Azzam2003.
P. hermaphrodita :*Phasmarhabditis hermaphrodita* (Schneider, 1859).
D. bonaerensis: *Daubaylia bonaerensis*
H.aspersa : *Helix aspersa* Müller
T .pisana : *Theba pisana* (Müller)
E.vermiculata : *Eobania vermiculata* (Müller)
A. ipsilon : *Agrotis ipsilon* (Hufengel)
CL : Cutical layer
MSE : Mesoepithelium
LNG : Large necrotic Granuloma
SNG : Small necrotic granuloma
FBR : Fibroblast-like reaction
DMSE : Degenerated Mesoepithelium
LST : Lytic storage tissues
DSPM : Degenerative sperm
DSPT : Degenerative spermatid
DSPG : Degenerative spermatogonia
OOC : Oocyte
OOG **:** Oogonia
AFC **:** Atretic follicle
DOOC **:** Degenerative oocyte
ESI **:** Empty space infiltration
LU : Lumen
D : degeneration
N : Necrosis
SPG : Spermatogonia
SPT :Spermatids
SPM :Sperm,
STT : Storage tissue
LTT : Lytic testis
S1 : Strain 1(Dark soft part) of *E. vermiculata*un-infected (snail control)
S2 : Strain 2(Pale soft part) of *E. vermiculata* un-infected (snail control)
T1 : Infected strain 1(Dark soft part) of *E. vermiculata*
T2 : Infected strain 2(Pale soft part) of *E. vermiculata*
SDS-PAGE : Sodium dodocylesulphate polyacrylamide gel electrophoresis
Mono : Monomorphic band
Poly : Polymorphic band
Pol+ Unique : Polymorphic with unique band
kDa : Kilodalton
SDS : Sodium dodecyl sulfate
M : Protein marker (IRIS11 Prestained Protein Ladder) 260-3 kDa.
EDTA : Ethylenediaminetetraacetic acid
Tris-HCL : Tris (hydroxymethyl) aminomethane Hydrochloride.

Declarations:

Ethical Approval: Not applicable

Authors Contributions: KA: Isolation and rearing of nematodes , collection and rearing of snails , Infection of snails , Histology of infected and non-infected snails, contribute in, recording the results, collection of references and wrote manuscript .HF: Extraction of proteinof infected and non-infected snails, Analysis of protein of infected and non-infected snails, contribute in, collection of snails recording the results, collection of references and wrote manuscript.All authors participated in the design of the study, material preparation, animal experiments, data collection, and analysis.

Availability of Data And Materials: The data that support the findings of this study are available from all author upon reasonable request.

Source of Funding: The current research was not funded.

Acknowledgements: Not applicable.

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