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Evaluation of certain Egyptian Heterorhabditids Isolates as Molluscicidal Nematodes for the Control of *Deroceras reticulatum* and *D. leave* Slugs under Laboratory Conditions

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ABSTRACT

:

Deroceras laeve Müller and *D. reticulatum* Müller are serious slugs of field crops in Egypt. Chemical control using metaldehyde baits is still the main control tactic. In 2018, a survey of terrestrial slugs at Belbies district, Sharkia Governorate, Egypt failed to find the molluscicidal nematode, *Phasmarhabditis hermaphrodita*. Dead slugs produced only free-living or non pathogenic nematodes belong to Family Rhabditidae. Two imported EPNs species; *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP88 strain) were screened for molluscicidal activity against *D. laeve* and *D. reticulatum* compared with three Egyptian *Heterorhabditis* isolates, Ht strain, Ar-4 strain and Serag1 strain. *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain), showed promising results by killing territorial slugs. *S. carpocapsae* (All strain) resulted in the greatest percentage mortalities 100% in *D. laeve* and *D.reticulatum* after 14 days at a concentration of 2000IJs/cm whereas, the application of *H. bacteriophora* (HP88 strain) induced percentages mortality 50 and 66.67% with *D. laeve* and *D. reticulatum*, respectively. The Egyptian strain *H. bacteriophora* showed less molluscicidal activity against the two tested slugs. After 14 days, *D. reticulatum* mortalities were 36.67, 40.00 and 46.33 % by the application of *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain), respectively. Whereas, the percentage mortalities were 20.00, 23.33 and 26.67 %; 10.00, 13.33 and 13.33% in *D. leave* at low concentrations (1000 IJs/cm2 & 500 IJs/cm2), respectively. Egyptian isolates showed less encourage usage as a specific biological control agent against *D. laeve* and *D. reticulatum* compared to *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain). Finally, results of this study indicate the need to an extensive survey in all Egypt to detect and isolate the slug parasitic nematode, *P. hermaphrodita* as it was recorded in Dakahlia governorate infesting different snails and slug species and research should proceed to find virulent isolates from EPNs to control terrestrial slugs.

Keywords: Survey, Biological control**,** *Deroceras laeve*, *D.reticulatum*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*,

INTRODUCTION

Slugs belong to Mollusca, Gastropoda cause considerable damage to field crops, vegetables, fruit trees, ornamental plants, landscapes, nurseries and greenhouses (Kaya, 2001). Slugs may also pose a health threat to humans, pets, and wildlife by

serving as intermediate hosts for many vertebrate parasites (e.g., lungworm) (South, 1992).

In Egypt, land gastropods could be considered as dangerous crop pests and cause considerable damage to the majority of economic crops. The increased pest status of these species differed from place to another, depending on food supply and weather conditions especially in moist areas where they find the optimum conditions for rapid multiplication (Kassab and Daoud,1964; El-Okda, 1980 and El-Deeb et al.,1996, 2003). Theenhaus and Scheu (1996) showed that many of slug species do not pose a risk to plant, human or veterinary health; indeed, they can play a role in nutrient cycling and ecological diversity. On the other hand, numerous slug species cause considerable amounts of economic damage in field crops especially in damp, mild climates (Barker, 2002).

Current control options include chemical, cultural and biological approaches. Molluscicides based on baits containing metaldehyde and methiocarbas active ingredients dominate chemical control products (Glen and Moens, 2002&Abd El-Aal, 2007), have proven to be effective when properly used. These materials are the most widely used for slug control. However, under cool and wet conditions when slugs are most active and troublesome, the slugs can often recover (Ohlendorff, 1998). In addition, these chemical baits are toxic, posing health concerns for children, pets, and wildlife. Furthermore, there are environmental objections to the use of molluscicidal chemicals. Consequently, an integrated strategy is usually required and new developments will be crucial to maintain and improve the existing levels of control.

With respect to pest slug management, although natural enemies of mollusks have been known for many years (Runham and Hunter, 1970), their wide-scale potential as a control solution has not been subject to intensive research activity until the last 30–50 years, in large part because of the dominance of the agrochemical industry. The parasitic nematode *Phasmarhabditis hermaphrodita* (Schneider) is the most widely established, commercially available slug biocontrol agents in Europe and is currently sold as the proprietary product Nemaslug1(Wilson et al.,1993). Nemaslug1 is effective against adults and juveniles of nine pest slug species and solely juveniles of two species (Rae et al., 2007). Notable exceptions include *Limax maximus* and the *Arion* slugs, *A. hortensis* and *Arion subfuscus,* which are not susceptible.

Although *P. hermaphrodita* was discovered almost 155 years ago (Schneider, 1859), it was not until it was isolated again in the UK in 1988 that its potential as a slug biocontrol agent was recognized (Wilson et al., 1993), and it was later shown to be associated symbiotically with the bacterium *Moraxella osloensis* (Bevre &Henrikson) (Tan and Grewal, 2001; 2002; 2003). A number of snail species has been shown to be susceptible to *P. hermaphrodita* (Rae et al., 2007), which reflects the similarities in their anatomy and biology. Despite the known parasitic relationships between a range of slug and nematode species (Morand et al., 2004), *P. hermaphrodita* was the first nematode actually shown to kill slugs (Wilson et al., 1993; Pieterse et al., 2017 & Nermut and Puza, 2017 ; Askary et al., 2017).

Certain strains of the entomopathogenic nematodes, *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora* have also been tested in bioassays, but positive results reported by Jaworska (1993) could not be replicated elsewhere (Wilson et al., 1994). Host range experiments have shown that *P. hermaphrodita* is effective against a wide variety of economically important pest slug and snail species (Coupland, 1995; Wilson and Gaugler, 2000; Glen and Coupland, 2017;Tandingan et al.,2017).

Therefore, the aim of present study is to conduct a survey for molluscicidal

nematodes at Belbis district, Zagazig, Egypt and evaluate the efficacy of three local strains of entomopathogenic nematode, *Heterorhabditis bacteriophora* (Ar-4, Serag1and Ht strains) isolated from EL-Arish and Giza Governorates, Egypt and two imported nematode species, *Steinernema carpocapsae* (All strain) and *H.bacteriophora* (HP88strain) for the control of two terrestrial slugs (*Deroceras leave* Müller,1774 and *D. reticulatum* Müller,1774) under laboratory conditions.

MATERIALS AND METHODS

1- Survey studies

A survey study for collecting molluscicidal nematodes was conducted throughout slug-prone areas (fruit trees, mandarin, navel orange, guava orchards, mango trees and ornamental plants, hibiscus, magnolia, rose trees) of Basateen Serag El-Dein locality, Belbies district. Slug collection was accomplished mainly through sight inspection of groundcover. Refuge traps were used to collect current slug species (Abd El-Aal,2001). It is simply consisted of lengths (15 cm x 20 cm) of damp wood placed adjacent to slug infested areas. The slug species collected are the field (gray garden) slug, *Deroceras laeve* Müller and *D. reticulatum* Müller.

The collected slugs were kept in 640 cc plastic containers with moist cotton cloth inside coolers maintained below 20°C, until transported back to Agricultural Zoology Laboratory in Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt where this work was conducted. Captured slugs were placed in large plastic containers (30 x 25 x 13 cm) lined with moist sterile sandy soil, provided daily with lettuce as food, and observed for signs of nematode infection (swollen mantle) for a period of more than two weeks. Declining slugs were placed in White traps (Kaya and Stock 1997) to collect any emerging dauer juvenile nematodes. The healthy slugs were maintained in the plastic containers for future use in bioassays. Nematodes isolated from the survey were tested with Koch's Postulates to determine pathogenicity.

2- Isolation, rearing and preparing of native entomopathogenic nematodes (EPNs):

EPNs were collected, reared and prepared as described by EL-Ashry et al. (2018). The original method described by Dutky et al.(1964) was used with some modifications, where the dead larvae were placed on a special fabric muslin cloth to avoid the disintegration of the filter papers. Nematode juveniles were maintained in 0.1% formaldehyde until identification. Three isolates designated as *H.bacteriophora* (Ar-4strain) from EL-Arish, *H. bacteriophora* (Serag1strain) from Belbies and *H.bacteriophora* (Htstrain) from Giza were extracted from soil samples.

3- Molluscicidal activity of native and imported entomopathogenic nematodes:

EPNs were screened for molluscicidal activity against two common pest slug species, *D. reticulatum* and *D. laeve*. The following experimental design was used for all bioassays. Plastic boxes (10 x15x 6cm) were lined on the inside walls with copper tape to prevent the slugs from moving to the lid and still in contact with the nematodes. Five small ventilation holes were made in the lids, and the plastic cages were filled with 10 g of sterile sand. Nematode infective juvenile (IJ) suspensions were concentrated to the desired concentrations (2000 IJs/cm2, 1000 IJs/cm2and 500

IJs/cm2) and applied directly to the soil surface in 1.5 ml of water. To insure optimum pathogenicity, only less than 2 weeks old IJ cultures were used in the bioassays. The control treatment consisted of 1.5 ml of distilled water. Every plastic cage contained a single, healthy slug. Slugs were fed daily on one lettuce disc (3.5 cm diameter) and replaced every 48 h. Moisture was adjusted as needed. The experiment was maintained at laboratory temperature (22º ± 3C), and slug mortality was monitored daily for two weeks. Slug cadavers were placed in White traps to recover any emerging nematodes.

Each screening or treatment consisted of three replicates consisting of 10 slugs for each nematode species or strain. Mortality percentages were calculated according the following formula:

$$\text{Mortality}(\%) = \frac{\text{Number of dead slugs}}{\text{Total number of slugs}} \times 100$$

4- Statistical analysis:

The experiments were carried out in a completely randomized design with 3 replications for each treatment. Data were subjected to analysis of variance (ANOVA) using MSTAT version 4 (1987).Means were compared by Duncan's multiple range test (Duncan, 1955) at $P \leq 0.05$

RESULTS AND DISCUSSION

1- Survey of terrestrial slugs and molluscicidal nematodes at Belbis district, Sharkia Governorate:

An extensive survey was carried out during two successive years started from January, 2016 to December, 2017 to obtain basic information's concerning occurrence of terrestrial slugs in 35 localities (villages) at Belbis district (county) of Sharkia Governorate . The two gray field slug species, *Deroceras reticulatum* and *D.laeve* were collected at some given site, but, *D. reticulatum* was usually the dominant one. Collected slugs were observed carefully for signs of nematode infection (swollen mantle). Slug cadavers placed on White traps and Koch's Postulates were followed. Isolated nematodes were non-pathogenic. Survey of terrestrial slugs at Belbis district failed to find the molluscicidal nematode, *P. hermaphrodita*. The slug parasitic nematode, *P. hermaphrodita* was recorded in Egypt in Dakahlia governorate associated with different snail and slug species i.e. *Monacha cantiana, Eobania vermiculata, Helix aspersa* and *Lehmania marginata*. (Genena et al., 2011).

2- Pathogenicity of imported EPNs and local isolates of Egyptian *Heterorhabditis bacteriophora* isolates against *Deroceras leave* and *D. reticulatum*:

Three Egyptian *Heterorhabditis* isolates were screened for molluscicidal activity against *D. leave* compared with two imported species, *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) after different periods of exposure. Both *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain), at the highest concentration (2000 IJs/cm2) showed promising results by killing 100% and 50 % of territorial slug, *D. leave* after 14 days of exposure, respectively. (Table1). At low application rates, 1000IJs/cm2and 500IJs/cm2, percentage mortalities decreased to 70.00, 43.33; 43.33, 26.67 % with *S. carpoapsae* (All strain) and *H. bacteriophora*

(HP88 strain), after 14 days of exposure, respectively. Whereas, the three strains of Egyptian *H. bacteriophora*, *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain) showed less effectiveness as molluscicidal activity against *D. laeve* slug. At concentration of 2000 IJs/cm2, percentage mortalities in *D. leave* ranged from 26.33 to 30.00 % by the application of *H. bacteriophora* (Serag1 strain) , *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain).

Table 1. Molluscicidal activity of Egyptian *Heterorhabditis* strains against the gray slug *Deroceras laeve* compared with *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP88 strain) after different periods of exposure.

Nematode species	**Conc.**	***Deroceras leave***		
		Mean percent mortality %		
		6 days	**10 days**	**14 days**
S. carpocapsae (All strain)	2000 IJs/ cm^2	56.67 a	83.33 a	100.0 a
	1000 IJs/cm^2	36.67 a	60.00 a	70.00 a
	500 IJs/cm^2	6.67 de	16.67 c	43.33 b
H. bacteriophora (HP88 strain)	2000 IJs/ cm^2	26.67 b	43.33 b	50.00 b
	1000 IJs/ cm^2	20.00 b	30.00 b	43.33 b
	500 IJs/ cm^2	6.67 de	16.67 c	26.67 bc
H. bacteriophora (Serag1 strain)	2000 IJs/ cm^2	6.67 de	16.67d	26.33 cd
	1000 IJs/ cm^2	6.67 de	10.00 cd	20.00 c
	500 IJs/ cm^2	0.00 f	10.00 cd	10.00 d
H. bacteriophora (Ht strain)	2000 IJs/ cm^2	6.67 de	13.33 d	26.67 bc
	1000 IJs/ cm^2	6.67 de	13.33 c	23.33 cd
	500 IJs/ cm^2	0.00 f	10.00 cd	13.33 d
H. bacteriophora (Ar-4 strain)	2000 IJs/ cm^2	13.33 c	20.00cd	30.00 bc
	1000 IJs/ cm^2	10.00 cd	16.67 c	26.67 c
	500 IJs/ cm^2	3.33 de	13.33 c	13.33 d

Each value is a mean of three replicates with 10 slugs in each replicate.
Tested slugs were observed daily for mortality but only table contains data of 6,10 and 14 days.
The same letter(s) in columns indicates no significant differences at P≤ 0.05according to Duncan'smultiple range test.

Minimum percentage mortalities were obtained when Egyptian *H. bacteriophora* strains were used at low concentrations**,** 500IJs/cm^2 and 1000IJs/cm^2**.** Percentage mortalities in *D. leave* ranged from 10.0 to 20.0; 13.33 to 23.33; 13.33 to 26.67% by the application of *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain) after 14 days of exposure, consequently. After 6 days, percentage mortalities in *D. leave* ranged from 6.67 to 13.33 % by the application of *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain) , consequently. Whereas, in imported EPNs species, *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain), percentage mortalities values were 56.67 and 26.67%, respectively.

Data in Table (2) showed that exotic nematode species, *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) were more aggressive killer to *D. reticulatum* than native strains of *H. bacteriophora.* Data revealed that *S. carpocapsae* (All strain) and *H.bacteriophora* (HP88 strain) after 14 days of application, recorded percentage mortalities in *D. reticulatum* reached 100 and 66.67 %, respectively.

Moreover, *H.bacteriophora* (HP88 strain) exhibited lower percentage mortalities, 56.67 and 43.33% at a concentration of 1000 IJs/cm2 and 500 IJs/cm2, respectively. Percentage mortalities resulted from usage Egyptian isolates were the least at three tested rates 2000, 1000 and 500 IJs/cm.2

Table 2. Molluscicidal activity of Egyptian *Heterorhabditis* strains against the gray slug *Deroceras reticulatum* compared with *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP88 strain) after different periods of exposure.

Nematode species	**Conc.**	***Deroceras reticulatum***		
		Mean percent mortality %		
		6 days	**10 days**	**14 days**
S. carpoapsae (All strain)	2000 IJs/ cm^2	66.67 a	90.00 a	100.0 a
	1000 IJs/cm^2	63.33a	80.00 a	90.00 a
	500 IJs/cm^2	33.33 b	40.00 b	60.00 b
H. bacteriophora (HP88 strain)	2000 IJs/ cm^2	50.00 b	56.67 b	66.67 b
	1000 IJs/ cm^2	33.33 b	43.33 b	56.67 b
	500 IJs/ cm^2	23.33bc	40.00 b	43.33 c
H. bacteriophora (Serag1 strain)	2000 IJs/ cm^2	23.33 c	26.67 c	36.67c
	1000 IJs/ cm^2	16.67c	26.67bc	26.67d
	500 IJs/ cm^2	16.67 d	20.00 c	23.33 d
H. bacteriophora (Ht strain)	2000 IJs/ cm^2	16.67 c	23.67 c	40.00 c
	1000 IJs/ cm^2	16.67 d	20.00 c	33.33 cd
	500 IJs/ cm^2	13.33 d	16.67 c	26.67 d
H. bacteriophora (Ar-4 strain)	2000 IJs/ cm^2	20.00 c	30.00 c	46.33 c
	1000 IJs/ cm^2	16.67 d	23.33 c	33.33 cd
	500 IJs/ cm^2	13.33 d	20.00 c	26.67 d

Each value is a mean of three replicates with 10 slugs in each replicate.
Tested slugs were observed daily for mortality but only table contains data of 6, 10 and 14days.
The same letter(s) in columns indicates no significant differences at P≤ 0.05 according to Duncan's multiple range test.

Percentage moralities in *D. reticulatum* were 36.67, 26.67; 40.00, 33.33 and 46.33, 33.33% at a concentration of 2000 IJs/cm^2 and 1000 IJs/cm^2 with *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain), respectively. At a concentration of 500 IJs/cm^2, percentage mortalities decreased to reach 23.33, 26.67 and 26.67 % after 14 days of application in *D. reticulatum* , consequently.

Efficacy of two imported nematode species, *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) compared to Egyptian *Heterorhabditis* strains as molluscicidal activity against *D. leave* and *D. reticulatum* slugs under laboratory conditions clearly showed that *D. reticulatum* was more susceptible than *D. laeve* and ineffectiveness of Egyptian strains (Table 3). At highest inoculum level (2000 IJs/ $cm^{2)}$, *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) induced maximum mean percentage mortalities with values amounted to 71.11, 94.44 and 44.45, 57.78% in *D. laeve* and *D.reticulatum* , respectively. Egyptian heterorhabditis, *H. bacteriophora* (Serag1 strain), *H. bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain), ranked the next and induced mean percentage mortalities in *D. leave* amounted to 18.89, 15.67 and 21.11% consequently.

Whereas, in *D. reticulatum* percentage mortalities were 28.89, 26.78 and 32.11% with the same mentioned nematode strains. Whereas, tested entomogenous nematodes at concentrations of 1000 and 500 IJs/cm2 showed lower mean percent mortality against *D.leave* and *D. reticulatum*, nevertheless imported EPNs nematodes were more effective than Egyptian strains (Table 3).

Table 3. Low susceptibility of two collected slugs, *Deroceras laeve* and *D.reticulatum* to Egyptian *Heterorhabditis* strains as compared with imported EPNs, *S. carpoapsae* (All strain) and *H. bacteriophora* (HP88 strain).

Nematode species	**Concentrations**	***D. leave***	***D. reticulatum***
		% Mortality Mean	% Mortality Mean
S. carpocapsae (All strain)	2000 IJs/ cm^2	71.11	94.44
	1000 IJs/cm^2	60.00	73.33
	500 IJs/cm^2	26.67	44.45
H. bacteriophora (HP88 strain)	2000 IJs/ cm^2	44.45	57.78
	1000 IJs/ cm^2	32.22	44.44
	500 IJs/ cm^2	23.33	35.55
H. bacteriophora (Serag1 strain)	2000 IJs/ cm^2	18.89	28.89
	1000 IJs/ cm^2	16.67	23.34
	500 IJs/ cm^2	12.22	20.00
H. bacteriophora (Ht strain)	2000 IJs/ cm^2	15.67	26.78
	1000 IJs/ cm^2	14.45	23.33
	500 IJs/ cm^2	10.00	18.89
H. bacteriophora (Ar-4 strain)	2000 IJs/ cm^2	21.11	32.11
	1000 IJs/ cm^2	16.67	23.33
	500 IJs/ cm^2	12.22	21.11

The estimation of the results of the molluscicidal activity of Egyptian isolates namely *H.bacteriophora* (Serag1 strain), *H.bacteriophora* (Ht strain) and *H. bacteriophora* (Ar-4 strain) based on percentage mortalities when compared to *S. carpocapsae* (All strain) and *H. bacteriophora* (HP88 strain) at the three concentrations (2000, 1000 and 500 IJs/cm^2) showed less encourage usage as a specific biological control agent against *D. leave* and *D. reticulatum*.

Terrestrial gastropods species like *Deroceras* spp. caused damage to important agricultural and horticultural crops due to feeding or contamination with faeces and slime (Godan, 1983, 1999 and South, 1992), leading to deterioration in the quality of the crops and economic loss. *D. reticulatum* distribute not only in temperate climates such as Egypt but also in in Europe. Obtained results confirmed those reported by many authors who detected molluscicidal activity of *Steinernema* and *Heterorhabditis* species infecting slugs and snails (Jaworska, 1993; Wilson and Gaugler, 2000 and Genena, 2008). Most of these studies were conducted on *S.carpocapsae* (All Polish isolates),*S.feltiae* and *H.bacteriphora* infected terrestrial slugs, *D. agreste* and *D. reticulatum*(Jaworska,1993).Likewise , suppressive effects of EPNs have been demonstrated on other slugs, *D. reticulatum* or *Limax marginatus* under laboratory conditions like *H. bacteriophora*, *H. marelatus*, *S. carpocapsae*, *S. glaseri*, *S. kushidai*, *S. longicaudum*, *S. oregonense*, *S. riobrave* and *S. Siamkayai* (Kaya, 2001)

at concentration of 1000 IJs/cm^2 and 500 IJs/cm^2. EPNs of the *Steinernema* and *Heterorhabditis* genera live in the soil and are natural parasites of insects found in this ecosystem, as well as having a symbiotic relationship with bacteria of the *Xenorhabdus* and *Photorhabdus* genera, respectively (Forst and Clarke , 2002).Tan and Grewel (2002 & 2003) revealed that *P. hermaphrodita* associated symbiotically with the bacterium *Moraxella osloensis* and kills slugs using an endotoxin (Bevre & Henrikson). Glen and Coupland (2017) mentioned that slug parasitic nematode , *P. hermaphrodita* was effective as biocontrol agent.

Our results showed that, in most cases imported EPNs (*S.carpocapsae* All strain and *H. bacteriophora* H88 strain) belonging to steinernematids and heterorhabditids were more effective than local isolates of *H. bacteriophora* (Serag1 strain, Ht strain and Ar-4 strain) against terrestrial slugs, *D. reticulatum* than *D. leave* even at high or low concentrations (2000 IJs/cm^2, 500 IJs/cm^2). *D. reticulatum* was more susceptible to local isolates than *D. leave.*

In conclusion, more efforts should be done to isolate an efficacious nematode species or to find *P. hermaphrodita* in Egypt for use against slug pests.

REFERENCES

Abd El-Aal, E. M. (2001). Studies on certain land snails at Sharkia Governorate. M. Sc. Thesis, Fac. Agric., Zagazig Univ. 160 pp.

Abd El-Aal, E. M. (2007). Ecological, biological and control studies on certain land snail species in Sharkia Governorate. PhD. Thesis, Fac. Agric., Zagazig Univ. , 188 pp.

Askary, T.H., Nermut, J., Ahmad, M.J. and Ganai, M.A. (2017) Future thrusts in expanding the use of entomopathogenic and Slug Parasitic Nematodes in agriculture. In: Biocontrol Agents*:* Entomopathogenic and Slug Parasitic Nematodes.

Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J. (eds), Wallingford, CAB International, UK, pp. 620-628.

Barker, G.M. (2002). Gastropods as pests in New Zealand pastoral agriculture, with emphasis on Agriolimacidae, Arionidae and Milacidae. In: Barker GM, editor. Molluscs as Crop Pests. CABI Publishing, Wallingford, Oxford, UK; p. 361–423.

Coupland, J. B. (1995). Susceptibility of helicid snails to isolate of the nematode *Phasmarhabditis hermaphrodita* from southern France. J. Invertebr. Pathol., 66 :207-208.

Dutky, S.; Thompson, J. and Cantwell, G.(1964). A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol., 6: 417–422.

EL-Ashry, R.M.; El-Sheikh, M.F.M.; Azazy, A. M. and Arafa, Olfat ,E. (2018). Field control of *Synanthedon myopaeformis* Borkh and *Zeuzera pyrina* L. using entomopathogenic nematodes, insecticides and microbial agents. Egypt. J. Agronematol.,17:(2)121-131.

El-Deeb, H.I.; Ghamry, E. M.; El-Hawashy, N. and Essa, N. (1996). Relative abundance of some land snails in certain Governorates of Egypt. J. Agric. Sci. Mansoura Univ., 21 (8): 2922-2933.

El-Deeb, H.I.; Sobeiha, A. K.; Fouad Maha, M. and Asran Fawkyia, D.A. (2003). Utilization of some mechanical and cultural measures against terrestrial snails. Zagazig J. Agric. Res., 30 (6) : 2371-2383.

El-Okda, M.M. (1980). Land snails of economic importance on vegetable crops at Alexandria and neighbouring regions. Agric. Res. Rev., 58 (1): 79-85.

Forst, S. and Clarke, D. (2002).Bacteria-nematode symbiosis, CABI Publishing, Oxon, UK, 55-77.

Genena, Marwa A.M. (2008) Advanced studies on chemical and biorational control of certain terrestrial gastropods. M.Sc. Fac. Agric. Mansoura Univ. pp.113.

Genena , Marwa A.M.; Mostafa, Fatma A.M. ; Fouly, A.H. and Yousef, A.A.(2011). First record for the slug parasitic nematode, *Phasmarhabditis hermaphrodita* (Schneider) in Egypt. Arch. Phytopathol.Plant Protect. 44(4):340-345.

Glen, D.M. and Coupland, J. (2017) The discovery and commercialization of a slug parasitic nematode. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J. (eds), Wallingford, CAB International, UK, pp. 548-559.

Glen. D.M. and Moens, R. (2002). Agriolimacidae, Arionidae and Milacidae as pests in West European cereals. In: Barker GM, editor. Molluscs as Crop Pests. CABI Publishing, Wallingford, UK; p. 468.

Godan, D. (1983). Pest slugs and snails: biology and control. Springer-Verlag, Berlin.

Godan, D. (1999). Molluscs: their significance for science, medicine, commerce and culture. Blackwell Wissenschafts -Verlag, Berlin. http:// www. ipm. ucdavis. edu/PMG/PESTNOTES/pn7427.html.

Jaworska, M. (1993). Laboratory infection of slugs (Gastropoda: Pulmonata) with entomopathogenic nematodes (Rhabditida: Nematoda). J. Invertebrate Pathol. 61(2):223–224.

Kassab, A. and Daoud, H. (1964). Notes on the biology and control of land snails of economic importance in the U. A. R. J. Agric. Res. Rev. Cairo, 42: 77-98.

Kaya, H. K., and Stock, S. P. (1997). Techniques in insect nematology. Pp. 281-324. In L. A. Lacey (ed.). Manual of Techniques in Insect Pathology. Academic Press, San Diego, CA**.**

Kaya, H.K. (2001). Molluscicidal nematodes for biological control of pest slugs. Slosson Report. pp. 1–5.

Mc Donnell RJ, Lutz MS, Howe DK and Denver DR (2018) First Report of the Gastropod-Killing Nematode, *Phasmarhabditis hermaphrodita*, in Oregon, U.S.A. J. Nematol. 50(1):77-78. doi: 10.21307/jofnem-2018-014.

Morand, S; Wilson, M.J. and Glen, D.M. (2004). Nematodes (nematoda) parasitic in terrestrial gastropods. In: Barker GM, editor. Natural Enemies of Terrestrial Molluscs. CABI Publishing,Oxford; p. 525–557.

Nermut, J. and Puza, V. (2017) Slug parasitic nematodes: biology, parasitism, production and application. In: Biocontrol Agents*:* Entomopathogenic and Slug Parasitic Nematodes. Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J.(eds), Wallingford, CAB International, UK, pp. 533-547.

Ohlendorff, B. (1998). Slugs and snails. In: UC Pest Management Guidelines, Pest notes: Univ. Calif. Agric. Nat. Resources Publication 28.

Rae, R.; Robertson, J.F. and Wilson, M.J. (2005).Susceptibility of indigenous UK earthworms and an invasive pest flatworm to the slug parasitic nematode *Phasmarhabditis hermaphrodita*. Biocontrol, Sci. Technol.:15(6):623–626.

Rae, R.; Verdun, C.; Grewal, P.S.; Robertson, J.F. and Wilson, M.J. (2007). Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita*-progress and prospects. Pest Manage. Sci.;63:1153–1164.

Schneider, A. (1859). Über, eine Nematodenlarvae und gewisseVerscheidenheiten in den Geschlechtsorganen der Nematoden. Zeitschrift fur Wissenschaft Zoologie;10:176–178.

South, A. (1992).Terrestrial slugs. Biology, ecology and control. Chapman & Hall,

London.

Tan, L. and Grewal, P.S. (2001). Pathogenicity of *Moraxella osloensis*, a bacterium associated with the nematode *Phasmarhabditis hermaphrodita* to the slug *Deroceras reticulatum*. Appl. Environ. Microbiol. 67(11):5010–5016.

Tan, L. and Grewal, P.S. (2003). Characterisation of the first molluscicidal lipopolysaccharide from *Moraxella osloensis*. Appl. Environ. Microbiol. 69(6) : 3646–3649.

Tan, L. and Grewel, P.S. (2002). Endotoxin activity of *Moraxella osloensis* against the grey garden slug, *Deroceras reticulatum*. Appl. Environ. Microbiol. 68(8):3943–3947.

Tandingan De Ley, I., MC Donnell, R.J., Paine, T.D. and De Ley, P.(2017) *Phasmarhabditis*: the slug and snail parasitic nematodes in North America. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J. (eds), Wallingford, CAB International, UK, pp. 560-578.

Theenhaus, A. and Scheu, S.(1996). The influence of slug (*Arionrufus*) mucus and cast material addition on microbial biomass, respiration, and nutrient cycling in beech leaf litter. Biology Fertility of Soils 23(1):80–85.

Wilson, M. J., and Gaugler, R. (2000). Terrestrial mollusks. Pp. 787-804. In L. A. Lacey and H. K. Kaya (eds.). Field Manual of Techniques in Invertebrate Pathology. Kluwer, Dordrecht, The Netherlands.

Wilson, M. J.; Glen, D. M. and George, S.K. (1993). The rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs. Biocontr. Sci. Technol. 3: 503-511.

Wilson, M.J.; Glen, D.M.; George, S.K.; Pearce, J.D. and Wiltshire, C.W.(1994). Biological control of slugs in winter wheat using the rhabditid nematode *Phasmarhabditis hermaphrodita*. Annals Appl. Biol. 125(2):377–90.

الملخص العربي

تقييم بعض العزلات المصرية من نيماتودا Heterorhabditids كمبيدات نيماتودية في مكافحة بزاقة الحقل الرمادية *Deroceras reticulatum* وبزاقة المروج *D. leave* تحت ظروف المعمل

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تعد كل من بزاقة الحقل الرمادية *Deroceras reticulatum* وبزاقة المروج*D. laeve* من أخطر بزاقات محاصيل الحقل في مصر ومازالت المكافحة الكيماوية باستخدام طعم الميتالدهيد هي وسيلة المكافحة الرئيسية . وقد أخفق الحصر الذى أجرى في عام 2018 للبزاقات الأرضية في منطقة بلبيس بمحافظة الشرقية في عزل النيماتودا *Phasmarhabditis hermaphrodita* المستخدمة كمبيد نيماتودى لمكافحة البزاقات ونتج عن البزاقات الميتة نيماتودا حرة المعيشة أو غير ممرضة .

ولقد استخدم نوعين من النيماتودا الممرضة للحشرات المستوردة وهما نيماتودا *S. carpocapsae* (السلالة All) ونيماتودا *H. bacteriophora* (السلالة HP88) كمبيدات نيماتودية بالمقارنة مع ثلاثة سلالات مصرية تابعة لنيماتودا *H. bacteriophora* في مكافحة كل من بزاقة الحقل الرمادية *D.reticulatum* وبزاقة المروج *D.laeve* ولقد أظهرت نيماتودا *S. carpocapsae* نتائج واعدة في قتل 100 % من البزاقين *D. & D.laeve reticulatum* بعد 14 يوما من المعاملة مع تركيز 2000 طور معدى/سم^2 بينما قتلت نيماتودا *H. bacteriophora* (السلالة HP88) 50 % ، 66.67 % من البزاقين السابقين على التوالي مع نفس التركيز.

وأخيراً، تشير نَتائِج هذه الدراسة إلى أن هناك حاجة إلى حصر شامل في كل أنحاء مصر لاكتشاف وعزل نيماتودا *P. hermaphrodita* (تم تسجيلها في محافظة الدقهلية) والى المزيد من الدارسات للبحث عن سلالات شديدة السمية من النيماتودا المتطفلة على الحشرات يمكن استخدامها في مكافحة البزاقات الأرضية.