

EFFECT OF THE SNAIL PARASITIC NEMATODE *Phasmarhabditis tawfiki* AZZAM ON SOME SNAILS AND INSECTS IN DIFFERENT TYPES OF SOILS UNDER THE GREEN HOUSE CONDITIONS

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ABSTRACT

The capability of *Phasmarhabditis tawfiki* Azzam to control the snails *Eobania vermiculata* (Müller) and *Theba pisana* (Müller) and the insect larvae of *Agrotis ipsilon* (Hüfnagel) and *Spodoptera littoralis* (Boisd.) was investigated under greenhouse conditions, using three types of soils (clay, mixture of sand and clay, and sand), using an infective dose of about 200 I.S per snail or larva for four weeks .

The mortality rate of *Eobania vermiculata* (Müller), *Theba pisana* (Müller), *Agrotis ipsilon* (Hüf.) and *Spodoptera littoralis* (Boisd.) exposed to infection with *P. tawfiki* was 88.94, 83.75, 93.75 and 71.25 %, respectively in the clay soil. In the mixture of sand and clay soil, these rates were 67.5, 72.5, 70 and 60 % ,while in the sand soil they recorded, 53.75, 63.75, 62.5 and 52.5 %, respectively.

The highest number of recovered nematodes (14579.09 I.S./snail) was reported for the snail *E. vermiculata* infected in the clay soil. Reversely, the lowest number(2560 I.S./larva) was associated with larvae *S. littoralis* in the sandy soil. *T. pisana* and *A. ipsilon* recorded 9807.17 I.S./snail and 6803.28 I.S./larva respectively in the clay soil.

Rates of individuals recovered nematodes in the clay soil were 89.94, 85.04, 82.79 and 79.01 % for *E. vermiculata*, *T. pisana*, *A. ipsilon* and *S. littoralis*, respectively.

Key words : *Agrotis ipsilon*, *Eobania vermiculata*, insects, *Phasmarhabditis tawfiki* Azzam, snails, *Spodoptera littoralis*, *Theba pisana* .

1- INTRODUCTION

Terrestrial snails are very prevalent animals in Egypt causing damage to all plant parts, from root to fruit of more than 47 species from ornamental plants, orchard crops, vegetables, field crops and medicinal plants including very economically important plants such as wheat, citrus, and potato (Azzam, 1995).The greasy cutworm, *Agrotis ipsilon* attacks about 50 plant species including wheat, cotton and potato (Amin and Abdin, 1997 and El Malki *et al.*, 1998). *Spodoptera littoralis* attacks several plants including cotton (Khattab, 1988). Wilson *et al.* (1994) investigated the effect of *Phasmarhabditis hermaphrodita* (Schneider) on slug populations and their damage to winter wheat under field conditions. They found that seedling survival increased and slug damage to seedlings declined linearly with increasing log nematode dose. Wilson *et al.* (1996) studied the effect of soil incorporation of nematodes on the

efficacy of *Ph. hermaphrodita* and found that nematode application reduced slug damage to plants in plots where nematodes were incorporated into the soil but not when they were left on the surface as a drench.

Ph. tawfiki Azzam was first recorded and described by Azzam (2003). The capability of this nematodes to infect and kill some terrestrial snails and slugs, aquatic snails and some insect larvae was investigated by Azzam and Tawfik (2003). The biology of this nematodes was also studied by Azzam (2004a). She found that the infective dauer larvae (third stage inside the retained second stage cuticle) move through the thin film of water in the soil searching for their hosts. Once they have found their hosts, the nematodes invade them through their natural openings and develop inside the host into hermaphroditic females and sometimes reproduce very few males. The same authoress (2004b) studied the

effect of soil type on the capability of *Ph. tawfiki* Azzam to infect snails under laboratory and semi-field conditions and she mentioned that this parasitic nematodes, recorded a higher mortality of snails in clay than in sand or a mixture of both. The present contribution aimed to investigate the potential of *Ph. tawfiki* to control some harmful snails and insects under the greenhouse conditions and to check the possible role of this nematode as a biocontrol agent of these harmful pests in the field.

2. MATERIALS AND METHODS

The snail parasitic nematode *Ph. tawfiki* Azzam was obtained from the progeny of the original colony which was isolated from *Eobania vermiculata* Müller and *L. flavus* Linnaeus by Azzam (2003) using the same technique previously described by Azzam (1998). *E.obania vermiculata* Müller was reared by the technique previously described by Azzam (1995). *A. ipsilon* (Hüf.) and *S. littoralis* (Boisd.) were reared by the method described by Khattab (2003).

Three types of soils were tested *i.e.*, clay, sand and a mixture of them (1:1). The soil was first sterilized at 160-180 °C for 2-4 hours. The sterilized soil was placed into plastic pots (20 cm inner diameter), sterilized by 10% HCl acid or 90% ethanol alcohol, then well washed by distilled water. Four pots were used for each type of soil for each pest. Each pot was filled with soil to 5 cm height. One nursery of lettuce plant *Lactuca sativa* was planted in the middle of each pot as a food source for the snails or insect larvae.

The pots were irrigated without submersion (inundation). Twenty laboratory bred individuals of *E.vermiculata*, *Theba pisana*, recent fifth larval stage of *A. ipsilon* or *S. littoralis* were placed in each pot.. About 4000 I.J. from the parasitic nematode *Ph. tawfiki* were added to each pot in the treatment.

Dead individuals from each pest species were removed during the daily examination, and treated similarly to the technique described by Azzam (1998, 2004b).

Counting the nematodes was carried out according to Azzam, (1999 and 2004b) by taking 5 µl of the nematode suspension using a micropipette after shaking thoroughly. The sample was placed on a micrometer slide and examined under the light microscope. This procedure was repeated four times. The mean number of nematodes was recorded and a volume adjusted to contain 4000 I.S, then added to each pot. Also the same technique was used for

counting the nematodes recovered from dead snails and larvae but the mean number was adjusted as a function of the volume of the recovered nematode suspension. Such checking was made daily until the complete disappearance of emerging nematodes. Experimental pots were covered by Perlon gauze cloth and secured with a rubber band. The pots were checked daily and dead individuals were treated similarly to those of the laboratory experiment. Soil in the pots was sprayed with dechlorinated water if necessary to avoid dryness of the nematodes.

The mortality % of each pest species, the period from infection to death and from death to nematode recovery, the ratios of individuals recovered nematodes and the number of recovered nematodes were recorded during daily examination. Results were statistically analyzed by ANOVA two ways and Duncan's multiple range test.

3. RESULTS AND DISCUSSION

Rates of mortality of the four pest species individuals infected with the snail parasitic nematode *Ph. tawfiki* Azzam, the period from infection to death and from death to nematode recovery, recovered nematodes as individual, and number of recovered nematodes are recorded in Table (1). The table shows that *Ph. tawfiki* caused mortality of *E .vermiculata* reached 88.94 % in the clay soil, 67.5 % in the mixture soil and 53.75 % in the sand soil. These ratios reached 83.75, 72.5, 63.75 %, *T. pisana* in the three types of soil, respectively. These ratios were 93.75 and 70, 62.5 % in *A. ipsilon*, and 71.25,60 and 52.5 % in *S. littoralis* . Statistical analysis showed very highly significant differences between the three types of soils and significant differences between *S. littoralis* and all other pest species in the clay and mixture soils, while significant differences existed between snails and insects larvae in the sand soil.

Recovery of infective stages of the parasitic nematode *Ph. tawfiki* reached 5.94, 78.79 and 82.85 % from dead infected *E. vermiculata* in the clay, mixed clay and sand and sand soil, respectively. These ratios were 85.04,80.43 and 72.29% from *T. pisana* 82.79,82.06 and 71.29 % from *A. ipsilon* and 79.01,71.09 and 71.36 % from *S. littoralis*, respectively.

Statistical analysis showed significant differences between the clay and sand soil, snails and insect larvae in the clay and mixed soils, between *E .vermiculata* and other pest species in the sand soil. Very highly significant differences

Table (1): Effect of *Phasmarhabditis tawfiki* Azzam on different pest species in different types of soil under green house conditions

Type of soil	Pest species	Mortality%	The period (in days)from		% of individuals recovered nematodes	Number of recovered nematodes
			Infection to death Mean \pm S.D (Range)	Death to recovery		
Clay	<i>Eobania vermiculata</i>	88.94 Aa	6.87 \pm 2.65 Aa (3 - 12)	4.80 \pm 1.38 (3 - 8)	85.94Aa	14679.09 \pm 2290.65 Aa (11400 - 18600)
	<i>Theba pisana</i>	83.75 Aa	11.72 \pm 5.77 Ab (4 - 23)	4.88 \pm 1.42 (3 - 8)	85.04Aa	9807.17 \pm 2700.45 Ab (6950 - 13200)
	<i>Agrotis ipsilon</i>	93.75 Aa	6.72 \pm 3.1 Aa (1 - 12)	4.73 \pm 1.12 (3 - 7)	82.79Aab	6803.28 \pm 1290.35 Ac (4900 - 8300)
	<i>Spodoptera littoralis</i>	71.25 Ab	4.77 \pm 2.34 Ac (2 - 11)	4.86 \pm 1.17 (3 - 8)	79.01Ab	3267.05 \pm 514.17 Ad (2300 - 4200)
Mixture of clay and sand	<i>Eobania vermiculata</i>	67.5 Ba	7.55 \pm 2.5 Aa (3 - 13)	5.03 \pm 1.35 (3 - 8)	78.79ABa	12487.03 \pm 2102.08 Ba (9200 - 16200)
	<i>Theba pisana</i>	72.5 Ba	12.29 \pm 5.70 Ab (4 - 22)	5.16 \pm 1.36 (3 - 8)	80.43ABa	9166 \pm 1733.18 B b (6200 - 12200)
	<i>Agrotis ipsilon</i>	70 Ba	8.89 \pm 3.88 Aa (1 - 16)	4.53 \pm 1.07 (3 - 7)	82.06ABb	5280.70 \pm 1294.28 Bc (3500 - 7800)
	<i>Spodoptera littoralis</i>	60 Bb	5.39 \pm 2.63 Ac (2 - 11)	4.85 \pm 1.15 (3 - 7)	71.09ABc	2764.71 \pm 376.49 Bd (2000 - 3400)
Sand	<i>Eobania vermiculata</i>	53.75 Ca	6.91 \pm 2.55 Aa (4 - 12)	5 \pm 1.143 (4 - 7)	82.85Ba	10993.23 \pm 2209.58 Ca (8100 - 14600)
	<i>Theba pisana</i>	63.75 Cb	9.82 \pm 4.83 Ab (3 - 20)	5.07 \pm 1.26 (3 - 8)	72.29Bb	8442.73 \pm 2338.65 Cb (5400 - 12200)
	<i>Agrotis ipsilon</i>	62.5 Cb	8.18 \pm 3.66 Ab (2 - 16)	4.86 \pm 1.18 (3 - 7)	71.29Bb	3954.51 \pm 696.40 Cc (3000 - 6000)
	<i>Spodoptera littoralis</i>	52.5 Ca	5.41 \pm 2.45 Ac (2 - 10)	4.73 \pm 1.14 (3 - 7)	71.36Bb	2560 \pm 403.95 Cd (2000 - 3200)
F value	For soils	97.3	2.047	0.97	6.49	101.97
	For pests	19.5	137.89	1.18	4.88	141.199
	Interaction between them	4.01	0.74	0.85	1.68	112.86
P	For soils	.0000 ***	14 n. s.	.38 n. s.	.004 **	.0000***
	For pests	.0000***	.0000***	.33 n. s.	.005 **	.0000***
	Interaction between them	.003**	.62 n. s.	.53 n. s.	.38 n. s.	.0000***

Number between brackets: (Range), Number \pm = Mean \pm SD, ** =Highly significant, ***=Very highly significant, n. s. =not significant, Numbers with different letter either small or capital have highly significant differences between them.

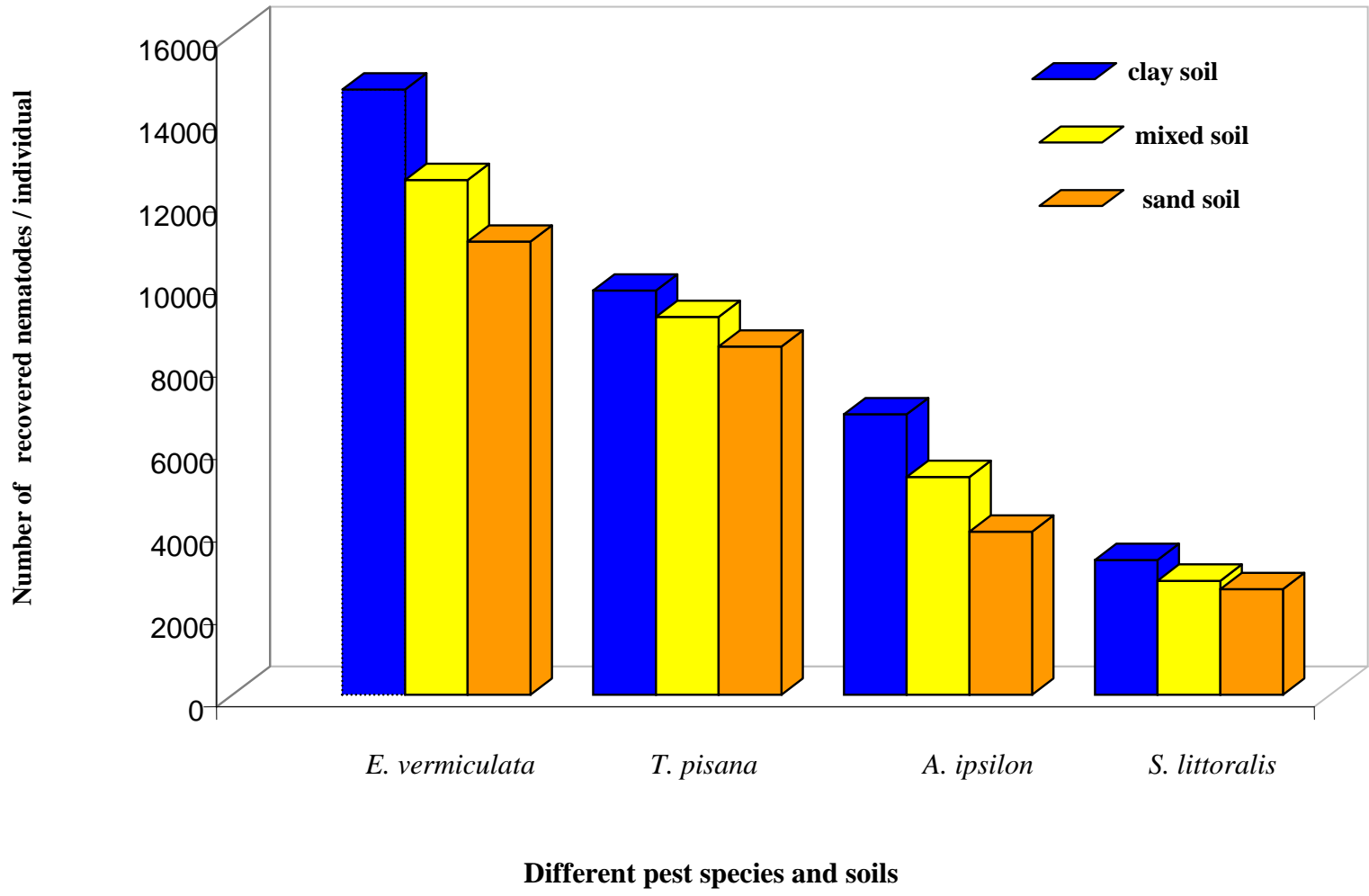


Fig.(1): Number of nematodes recovered from different pest species in three types of soil.

existed between *S. littoralis* and snails. The highest number of recovered nematodes (14679.09 ± 2290.65) was reported for the snail *E. vermiculata* infected in the clay soil followed by *T. pisana* and *A. ipsilon* (Fig.1). Reversely, the lowest number (2560 ± 403.95) was associated with *S. littoralis* in the sandy soil. *E. vermiculata* had the highest number in all types of soils. This may be attributed to its larger size and because it is the natural host of the nematode *Ph. tawfiki* Azzam .

Statistical analysis showed very high significant differences between the three types of soils and between *E. vermiculata* and all other pest species in all types of soils. Highly significant differences existed between *T. pisana* and both species of insect larvae and significant differences between *S. littoralis* and *A. ipsilon* in all soil types. Concerning the period from infection to death, no significant differences existed between the types of soils but very high significant differences existed between different pest species (Table,1). No significant differences existed between different pest species or soils in the period from death to recovered nematodes (Table, 1).

The aforementioned results indicate more suitability of the clay soil to the ability of *Ph. tawfiki* to infect *Vermiculata*, *T. pisana*, *A. ipsilon* and *S. littoralis* than the sand or mixed soil . Azzam (2004a&b) reported more suitability of the clay soil to persistence and activity of *Ph. tawfiki* than the sand and mixed soil when infected *E. vermiculata* . Reversely, Molyneux and Bedding (1984) mentioned that *Steinernema glaseri* showed more infectivity in sand than in mud, while *Heterorhabditis* sp. became more effective in the last (mud) soil.

The present investigation showed clearly that the snail parasitic nematodes, *P. tawfiki*, produced high enough mortality rates to play an important role in controlling the abovementioned pest species especially those infesting plants in clay soil.

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تأثير نيماتودا القواقع *Phasmarhabditis tawfiki* Azzam علي بعض أنواع القواقع والحشرات في انواع مختلفة من التربة تحت ظروف الصوبة الزجاجية

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ملخص

تم دراسة قدرة النيماتودا *Phasmarhabditis tawfiki* Azzam علي مكافحة القواقع *Eobania vermiculata* (Müller) و القواقع *Theba pisana* (Müller) و علي مكافحة يرقات حشرة الدودة القارضة *Agrotis ipsilon* (Hüfnagel) و يرقات دوده ورق القطن *Spodoptera littoralis* (Boisd.) تحت ظروف الصوب باستخدام ثلاثة انواع من التربة (تربة طميية, خليط رمل مع طمي , تربة رملية) وحوالي 200 طور معدي لكل قوقع او يرقة عرضت لمدة اربعة اسابيع للعدوي.
كانت نسبة الموت التي دونت في التربة الطميية للقواقع *T.pisana*, *E. vermiculata* و يرقات الحشرات *S. littoralis*, *A. ipsilon* 88.94 , 83.75 , 93.75 و 71.25% , علي التوالي . و في التربة الخليط من الرمل والطمي سجلت نسبة الموت 67.5 , 72.5 , 70 و 60% , علي التوالي بينما دونت في التربة الرملية 53.75 , 63.75 , 62.5 و 52.5% علي التوالي .
كما تم تسجيل أكبر عدد من الطور المعدي للنيماتودا (14579.09) للقواقع الواحد من *E. vermiculata* المعدي في التربة الطميية علي العكس من ذلك سجل اقل عدد (2560) طور معدي لليرقة الواحدة من دودة ورق القطن *S. littoralis* في التربة الرملية في حين تم تسجيل 9807.17 طور معدي من قوقع *T.pisana* و 6803.28 طور معدي من اليرقة الواحدة من الدودة القارضة *A. ipsilon* في التربة الطميية .
كانت نسبة الافراد التي انتجت النيماتودا في التربة الطميية 89.94 , 58.04 , 82.79 و 79.01% لكل من قوقع *pisana* , *T. E. vermiculata* و يرقات *S. littoralis* , *A. ipsilon* علي التوالي