COMPARISON BETWEEN INOCULATIVE AND INUNDATIVE RELEASE FOR CONTROLLING SCARAB BEETLES IN STRAWBERRY USING ENTOMPATHOGENIC NEMATODES UNDER FIELD CONDITIONS

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ABSTRACT

White grubs, the root-feeding larvae of *Temnorhynchus baal* (Reiche) cause significant damage to strawberries in Egypt. Entomopathogenic nematodes (EPNs) belong to both Steinernema and Heterorhabdis genera were tested against this scarabaied beetle. Laboratory bioassay indicated that, Steinernema glaseri "NJ strain" (Steiner) caused a 100% mortality of the 1^{st} and the 2^{nd} instar larvae of T. *baal.* Whereas, 94% mortality of the 3^{rd} instar larvae and 96% mortality of the adult stages three days after nematode treatment were obtained. In contrast, Heterorhabdis bacteriophora "HP 88" (poinar) strains" caused 68, 62, 60, and 58% insect mortality when applied on the 1^{st} , 2^{nd} and 3^{rd} , larval instars and adult stages of T. baal respectively. The current data suggest that, S. glaseri was the most virulent nematode on the all stages of this scarab pest more than H. bacteriophora. Under field conditions, the inoculative release was effective (F value, 3.47, mean square "M.S.", 1.65; P<0.05 after three inoculate release) more than the inundative release (F value, 2.61, M.S., 1.26; P<0.05 after the first inundate release). Furthermore, the data of both the six and second applications for the inoculative and inundative releases respectively showed the same trend with S. glaseri. On the other side, there was a strong host population reduction according to the application strategy mentioned reporting a highly significant variation of both strategy : (F = 3.71; M.S. = 1.41; $P \le 0.05$ after the first three inoculative release) while, (F = 11.15; M.S. = 2.49; $P \le 0.05$ after the first inundate release) with H. bacteriophora. However there was no significant variation between the two methods of application with H. bacteriophora after inocultive and inundative release. In the present study, the nematodes S. glaseri was highly effective when applied on the soil surface at a concentration of 20000IJ/m^2 more than H. bacteriophora with the same concentration in both methods of application inundative and inoculative release. Inoculative release was effective more than the inundative release for controlling T. baal in strawberry fields in Egypt.

Key words: biological control, entomopathogenic nematodes, Heterorhabdis bacteriophora, inoculative and inundative release, Steinernema glaseri, Temnorhynchus baal.

1. INTRODUCTION

Most recent publications on entomopathogenic nematodes (EPNs) have focused on their potential use as biocontrol agents, but a little is known about the structure and dynamics of their natural populations. Accordingly, soil survey is conducted to assess the occurrence of EPN and to find out new isolates, across seasons, habitats, and geographic regions. Although results from many laboratory tests with EPN have been promising in controlling insect pests, field evaluation results have often been highly variable particularly against well-hidden insects of cryptic habitats such as soil insects "*e.g.* scarabaids" (Atwa; 2003). They are well protected from chemical insecticides with a high rate of survival. Thus, these insect hosts are capable of producing large populations and new generations that subsequently disperse or migrate or both to more susceptible plant hosts where more control measures are required. Therefore, field trials were conducted to validate laboratory findings. In addition, the efficacy of multiple inoculative releases of EPN against target insect pests at long-term was also monitored (Shamseldean and Atwa; 2004).

White grubs, the root-feeding larvae of scarab beetles, cause significant damage to many agricultural and horticultural plants. Their subterranean habit makes them the most difficult vegetables pests to control in Egypt (Shamseldean and Atwa, 2004). Despite the need for stronger implementation of integrated pest management (IPM) because of the importance of vegetables to humans and growing public concerns about safety, chemical pesticides are still the first choice of vegetables managers for the control of white grubs and other vegetables pests. However, white grub outbreaks are difficult to predict because of their localized and usually sporadic nature and the difficulty of sampling for white grubs in general and their eggs and first instars in particular. Therefore, the preventaive approach involves treating large turf areas that otherwise may have needed only partial or no treatment.

In relation to the use of EPNs in the field against insect pests, very economic insect pest was chosen. This insect live in cryptic habitat such as below the soil in habitats close to the natural habitats of EPNs where humidity is relatively high and the nematodes will reach their host easily. In these habitats, the nematodes are also well protected from direct sunlight (ultra violet) which harm the nematodes and lower their efficacy against target insect pests. White grubs of the scarabaied beetle, Temnorhynchus baal (Reich) is a serious insect pest on the roots of strawberry plants in Egypt (Atwa 2003). Strawberries are one of important crops exported to Europe every year and the use of chemical insecticides should be limited. The slow inoculative release at a rate of 6 intervals of EPNs vs inundative release at rate of 2 intervals against this scarab leads to achieve good results to control this pest. Some field studies were conducted to use these EPNs against white grubs (Koppenhöfer et al., 1999).

The main objectives of the present study was to compare two methods of biological control (inoculative vs inundative releases) of two EPNs for controlling the white grubs in strawberry fields under drip irrigation system.

2. MATERIALS AND METHODS 2.1. Nematodes preparation for laboratory and field trials

Nematode species, *Steinernema glaseri* (NJ), and *Heterorhabditis bacteriophora* (Hp88) were cultured on the last instar larvae of *Galleria mellonella* (L) according to the method by Dutky *et al.* (1964). The infective juveniles (IJs) were harvested from nematode traps as described by White (1927) at $25 \pm 2^{\circ}$ C. A stock suspension of the IJs (for laboratory experiment) in sterilized distilled water was stored at 10°C until needed. All nematodes were used within 2 weeks of harvest and a new infection cycle and a stock of IJs was made every 2 weeks. The larval (1st, 2nd, and 3rd) and adult stages of *Temnorhynchus baal* were used for laboratory evaluation and field efficacy tests. While, *S. glaseri* and *H. bacteriophora* were cultured on the last instar larvae of the greater wax moth "G. mellonella". The emerging (IJs) were harvested from nematode traps as described by White (1927) at $25 \pm 2^{\circ}$ C and stored in sterilized water with sponge at room temperature ($20 \pm 2^{\circ}$ C) for 2 - 3 months before field application. This method of storage is a technique under study by the author. It is a part of a large tria for storing nematodes with different materials or substrates and it is under study.

2.2. The target host and experimental sites

The larval stages and sometimes the adults of the white grubs of the scarabaied beetle, "T. baal" are destructive stages for strawberry plants in Egypt. This soil insect is ideal to be controlled by EPN because they live in cryptic habitats; *i.e.* the scarabaied larvae and pupa live in the soil at the root area of strawberry plants (Atwa, 2003). Two groups (three of each) infested field plots by T. baal were treated with S. glaseri and H. bacteriophora and one infested field was left without treatment. Each treated field divided into two subplots was treated with an inoculative method (6 treatments), other three infected plots were treated with an inundative release (2 treatments). The experiment site is located at El-Rodah village, El-Kantarah West, Ismailia. The area of plots was 0.25 feddan (1050 m²) during two seasons of plantation 2005/2006 and 2006/2007.

2.3. Laboratory bioassay

In the laboratory experiment, the different stages of *T. baal* were tested using units of plastic containers (7 cm high, 6.5 cm in diameter) covered with plastic lid. Each contained 50 gm of autoclaved sandy soil were prepared for the experiment . Five ml of sterile distilled water were added to all tested units, including the nematodes suspension (500 IJS / replicate). Fifty instars from each of the 1^{st} , 2^{nd} and 3^{rd} and adult stages were used for the bioassay experiment. Each given stage, was placed in each container. The mortality percentage was determined after 72 – 96 hours.

2.4. Field application and evaluation

Drip irrigation system was used for nematodes applied to the soil surface within the barriers at 4:00 PM before dusk (sunset was around 5:00 – 5:30 PM during the application period which was a wintertime) (Atwa, 2003). The EPN were used through the fertilizing system via the net of drip irrigation. Nematode concentrations were adjusted to reach 2000 IJs per each nozzle in the drip irrigation system for the inoculative release, while, nematode concentrations were 6000 IJs per each nozzle for the inundative release. To irrigated one feddan, 20,000 nozzles were used to irrigate one feddan (ca 4,200 m²). Meanwhile, 40,000 plants were grown in this feddan in rows, 1 m. wide, and 50 m. long. Nematode viability was checked just before application by pouring samples of the nematode solution into a glass beaker. These samples were examined for nematode movement and curled nematodes with a 10X hand lens immediately before application to verify that the nematodes were alive before the application. Also, nematodes were collected from dripping nozzles and examined to make sure that they had not been affected by either the drip pressure or any irrigation contaminations in the system. Pathogenecity of the nematodes S. glaseri and H. bacteriophora was determined by dripping 5 random nematode samples from each plot during the application. The suspension was examined according to Atwa (2003) by placing it into a 15cm Petri dish lined with filter paper. For the inoculative release 6 nematodes treatments, (one every three weeks) were started after three weeks of plantations. While for the inundative release (2 nematodes treatetment), the first treatment was after 9 weeks and the second was after 18 weeks. To evaluate the performance and efficacy of EPN in the six treated field plots, ten replicates 1 m^2 in each plot were tested for nematode persistence. The nematode solution was introduced through the drip irrigation system into the field plots, after one week from the introduction of the nematodes, the top soil was removed from a 1 m² where dead and alive insects found in the soil were counted. These tests were accomplished at the six treated field plots and the control field directly before the treatments and after one week from the treatment to resolve the population reduction of the white grubs by using Henderson and Tilton formula (1955) and a means separated at the $p \le 0.05$ level by Fisher's test (SAS institute, 1988).

3. RESULTS AND DISCUSSION 3.1. Laboratory bioassay

Data in Table (1) epitomize that the laboratory experiment conducted to bioassay nematode species Steinernema glaseri (NJ) and Heterorhabditis bacteriophora (HP88) to be used in the biological control of the scarabaied beetle (Temnorhynchus baal). The experiments were done using two different nematode species on different stages of whitegrubs and adults of the scarabaied beetle, *T. baal*. The data from Table (1) indicate that, Steinernema glaseri caused a 100% mortality of the 1^{st} and the 2^{nd} instar larvae of T. baal. Whereas, 94% mortality of the 3rd instar larvae and 96% mortality of the adult stages three days after nematode treatment were obtained. In contrast, H. bacteriophora has caused 68, 62, 60,

and 58% insect mortality when applied on 1^{st} , 2^{nd} , 3^{rd} , larvae and adult stages respectively (Table 1). Although the current data reveal far above the ground medium infection in the exposed scarbs to the H. bacteriophora. However, the data recorded by Atwa, (2003) have shown that S. glaseri was the most virulent nematode on the all stages of this scarab pest. This paper preaches the use of both active cruiser species (e.g., S. glaseri, and H. bacteriophora) both of them had the ability of cursing and host finding in the soil. Also because the symbiotic bacterium kills insects so quickly, there is no intimate host-parasite relationship as is characteristic for other insect-parasitic nematodes. Consequently, EPNs are lethal to an extraordinarily broad range of insect pests in the laboratory. Field host range is considerably more restricted, with some species being quite narrow in specificity. Steinernematid host and heterorhabditid nematodes are exclusively soil Yield research organisms. progresses and improved insect-nematode matches are made by Gaugler et al. (1992).

3.2. Field application and evaluation

In order to obtain the best field efficacy of EPNs against particular pest, one must use the most infective nematode and optimize the application regime (Martin, 1997 and Grewal, 2002). In the present study we used S. glaseri and H. bacteriophora with nontraditional application methods (inoculative and inundative release) via drip irrigation system to control scarab beetles in strawberry fields in Egypt. Further studies, under natural conditions, are needed to optimize application efficiency and evaluate the commercial utilization of these biological control agents. On the other hand efficacy showed that **EPNs** in the genera Steinernema and *Heterorhabditis* can be effective biological control agents against a wide variety of soil insect pests in various cropping systems, such as black vine weevil (Otiorhynchus sulcatus F.) in cranberry bogs and strawberry fields, citrus root weevils (Diaprepes abbrevatus L. and Pachnaeus litus Germar) in citrus groves, or the alfalfa fields (Fife, et al., 2003). The inoculative or inundative releases of nematode-based biological control agents would be effective when; 1) the pest is present throughout most of the year or at the time of application, 2) the pest has a high economic threshold, and 3) soil conditions are favorable to nematode survival. All these criteria can be met in crops irrigated with drip irrigation system, in which the scarabs have larvae present in the soil for most of the year and the crops are irrigated during dry conditions unfavorable to nematodes, these conditions are close to the conditions of

unter ent stages of Tennornynchus baar under laboratory conditions.								
Nematode isolates and/or strains	% insect mortality							
		A dulta						
and/or strains	1 st instar	2 nd instar	3 rd instar	Mean	Adults			
S. glaseri (NJ)	100.0	100.0	94.0	98.0 ^a	96.0 ^a			
H. bacteriophora (EKB20)	68.0	62.0	60.0	60.0 ^b	58.0 ^b			

 Table (1): Effect of Steinernema glaseri and Heterorhabditis bacteriophora on different stages of Temnorhynchus baal under laboratory conditions.

* Values followed by the same letter within rows or columns are not significantly different (LSD test, P < 0.05).

Table (2): Population reduction of Temnorhynchus ball estimated before and after
nematode applications of Steinernema glaseri and Heterorhabdities
bacteriophora in strawberry field plots in both inoculative and inundative
releases.

	releases.							
Treated plots with different nematodes application		Mean No. before application	<u>After the 3^{rd} app</u>	blication for (#)	after the 6 th application for (#)			
			and 1 st applica		and 2 nd application for (**)			
			Mean numbers*	% population reduction	Mean numbers*	% population reduction		
	Control	2.95	4.2 ^d		6.7 ^d			
S. glaseri	1 #	2.65	0.3 ^a	92.1	0.2 ^a	96.7		
	2 #	2.75	0.25 ^a	93.6	0.15 ^a	97.6		
	3 #	2.6	0.15 ^a	96.0	0.25 ^a	95.8		
	Average	2.67	0.23 ^a	94.0	0.2 ^a	96.7		
	Mean square (M. S.)		1.6	55	1.03			
	F value		3.4	7	1.95			
	1 **	3.05	0.7 ^b	83.9	0.45 ^b	93.5		
	2 **	3.0	0.75 ^b	82.4	0.6 ^b	91.2		
	3 **	2.6	0.65 ^b	82.4	0.5 ^b	91.5		
	Average	2.88	0.7 ^b	82.9	0.52 ^b	92.1		
	M. S.		1.2		1.53			
	F value		2.6	51	4.65			
H. bacteriophora	1 #	3.1	1.05 °	76.21	1.2 °	83.0		
	2 #	2.75	1.05 °	73.2	1.05 °	83.2		
	3 #	2.85	1.1 ^c	72.9	1.0 ^c	84.6		
	Average	2.9	1.07 ^c	74.1	1.15 °	82.5		
	M. S.		1.4	1	1.75			
	F value		3.7	'1	4.84			
	1 **	3.1	$0.7^{\rm b}$	84.1	0.8 ^b	88.6		
	2 **	3.1	0.85 ^b	80.7	0.75 ^b	89.4		
	3 **	3.2	0.75 ^b	83.5	0.8 ^b	89.0		
	Average	3.13	0.77 ^b	82.7	0.78^{b}	89.0		
	M. S.		2.4		1.61			
	F value		11.	15	4.22			
* Values followed by the same letter within rows or columns are not significantly different								

* Values followed by the same letter within rows or columns are not significantly different (LSD test, P<0.05).

Field plots treated with inoculative release.

** Field plots treated with inundative release.

strawberry field in Egypt (Atwa, 2003 and Shamseldean and Atwa, 2004).

The field experiment showed the same trends as the laboratory bioassay experiment illustrated. Analysis of all data together (Using Henderson and Tilton. 1955 formula) showed that there is a significant effect between the inoculative release and inundative release of S. glaseri in the three treated fields (Table 2). Data in Table (2) explain the difference and significantly effect of both nematode application strategy and untreated Whereas: there control. is а significant differentiation of S. glaseri inoculative and inundative release. The inoculative release was more effective (F value, 3.47; mean square "M.S.", 1.65; $P \leq 0.05$ after three inoculate release of nematodes) than the inundative release (F =2.61; M.S. = 1.26; P<0.05 after the first of nematodes inundate release). Statistical analysis in Table (2) explain the significant difference the two application strategy, where the data after six applications and second application for the inoculate and inundate release, respectively showed the same trend with S. glaseri. Data analysis with inoculative and inundative release of *H. bacteriophora* for comparing the differentiation of host mortality required to different application strategy mentioned a highly significant variation of both strategy (F = 3.71; M.S. = 1.41; P<0.05 after three inoculate release of nematodes) On the other side, significant difference was detected after the first of nematodes release, (F = 11.15;M.S.= 2.49; P < 0.05 after the first of nematodes inundate release). While, there is no significant variation between the two methods of application with H. bacteriophora after inocultive and inundative release (Table 2).

Data in Figure (1) represent the corrected percentage mortality of the scarab insect pest (Henderson and Tilton, 1955). The data indicate that, the inoculative release of tested EPN species S. glaseri, during the 2005/2006 and 2006/2007 seasons showed a high virulence against the population of *T. baal* after the second application. As illustrated in Figure (1) generally, there is a significant variation between the treated field plots with the inoculaive and inundative releases. The inundative release was highly significant after the first release of S. glaseri in 2006/2007 seasons. On the other hand, the population reduction required to the inoculatve release was highly significant after the second treatment (Figure 1). Data in Figure (2) represent the corrected percentage mortality of the scarab insect pest (Henderson and Tilton, 1955) and remain that, the tested EPN H. bacteriophora, during the 2005/2006 and 2006/2007 seasons showed a moderate virulence against *T. baal* infestation as inoculative release after the second application. As shown in Figure (2), there are a highly significant variation between the field plots treated with the inoculative release and inundative releases in general. The inundative release was highly significant after the first release of *H. bacteriophora* in both seasons of application. On the other wise, the population reduction required to the inoculative release was highly significant after the second treatment (Figure 2).

Conservation strategies are poorly developed and largely limited to avoid applications onto sites where the nematodes are ill-adapted; for example, where immediate mortality is likely (*e.g.*, exposed foliage) or where they are completely ineffective (*e.g.*, aquatic habitats) (Lewis *et al.*, 1997). Minimizing deleterious effects of the aboveground environment with a post-application rinse the infective juveniles into the soil that is also a useful approach to increasing persistence and efficacy in the inoculative and inundative release *via* drip irrigation systems in strawberries fields.

The use of inoculative release for the three and six field applications gave almost the same degree of percentage reduction of larval population with slight superiority of S. glaseri, but the insect population after the three application was still high enough to cause economic damages to strawberry plants. Consequently, the application continued tells the six application of inoculative release was better than the two inundative release (Figure 3), during the insect activity in the early Spring. On the strength of the data in Figure (3) either of the inoculative or inundative releases of S. glaseri was significantly effective and caused population reduction compared with those of Н. bacteriophora. This high level of reduction in pest population was obtained due to the use of EPNs (crop / insect / nematodes, complex) application in cryptic habitat such as the soil (Atwa, 2003). Once applied to the soil surface, the nematodes locate their insect host by detecting movement and following CO₂ emissions (Atwa, 2003 and Shamseldean and Atwa, 2004). They enter the pest larva via natural openings such as the mouth, anus or breathing spiracles, or hack their way directly through weak spots in the hosts outer covering. Once inside the insect larva, they release bacteria that multiply up on the insect tissue, quickly killing it. The nematode then feeds off the bacteria, and starts producing many thousands of infective juvenile offspring. When the host insect cadaver finally disintegrates, these infective juveniles then move into the soil to locate a new host, and start the process over again. The short persistence of EPNs in sandy soil is attributable to

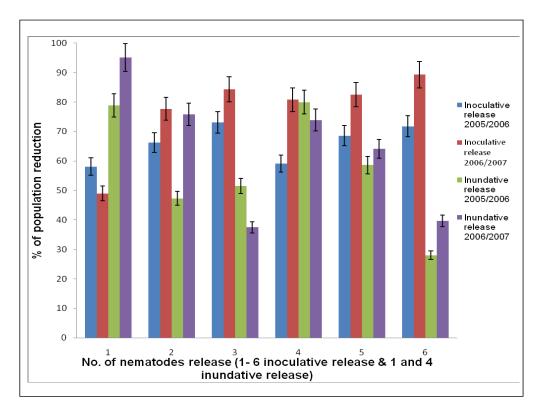


Figure (1): Comparison of *Steinernema glaseri* inoculative and inundative release during 2005/2006 and 2006/2007 seasons in strawberry fields.

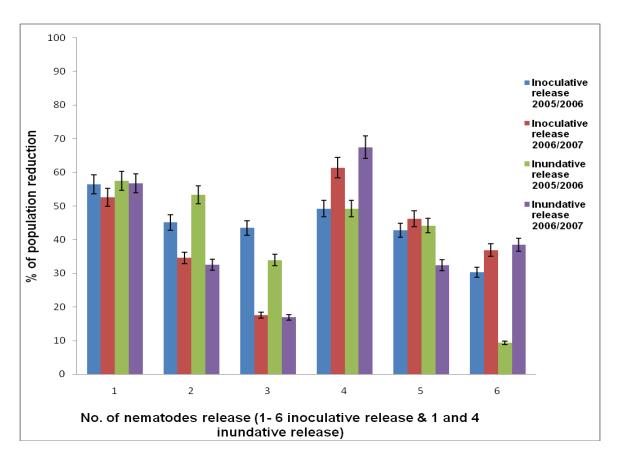


Figure (2): Comparison of *Heterorhabdities bacteriophora* inoculative and inundative release during 2005/2006 and 2006/2007 seasons in strawberry fields.

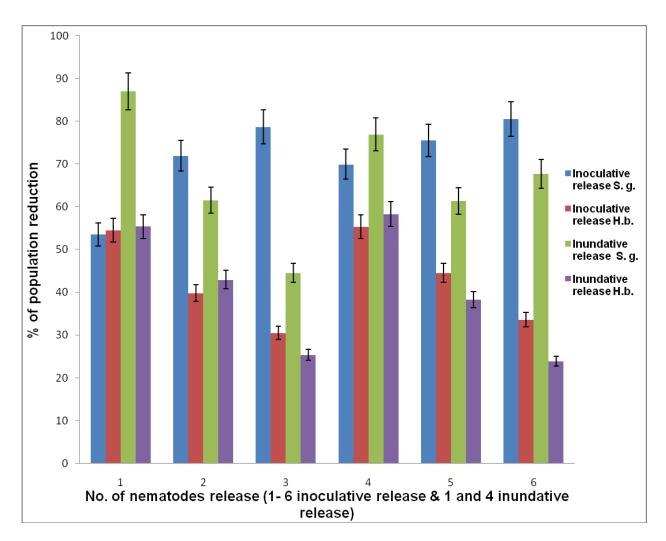


Figure (3): Comparative of mean average of population reduction by inoculative and inundative release of *Steinernema glaseri* and *Heterorhabdities bacteriophora* in strawberry fields during 2005/2006 and 2006/2007 seasons.

the quick loss of humidity from the sandy soils, but the drip irrigation used here keep the moisture for the nematodes activity. The effect of soil / pest (scarabs and weevils) / nematodes" systems is probably facilitating the long persistence of EPNs (Jansson *et al.*, 1991). The irrigation system (drip irrigation) wash the nematodes quickly from the root area (rhizosphere), in the same time, the daily irrigation causes a high levels of nematode recovery throughout the application period.

The nematodes work extremely well with the presence of large numbers of whitegrubs in the soil (high larval population), because, the more dense of the grubs population, the greater the chance of EPNs finding their host, and the more chance of a second wave of parasitism (where thousands of infective juveniles produced in the cadavers of whitegrubs killed by the initial application move back into the soil (Koppenhöfer, *et. al.*, 2000), looking for new hosts). Meanwhile, if the comparing efficacy of EPNs (Bedding and Nickson, 1999) with another control method such

as the chemical pesticides, it can be safely stated that, the larger the larvae of whitegrubs the harder to kill by chemical larvicides because the bigger grubs are hard to kill, and they are difficult to be killed using high concentration of chemical insecticides in the soils, while, EPNs work extremely well with large grub larvae (Atwa, 2003 and Shamseldean and Atwa, 2004). The EPNs find and kill the bigger larvae, with a more chance of a second wave of parasitism, where thousands of infective juveniles produced in the cadavers of grubs, the emerged infective juveniles move back into the soil, looking for new insect hosts to infect.

Atwa (2003) and Shamseldean and Atwa, (2004), mentioned that the long-term population level survival of EPNs in the soil is even more difficult to address. The records of long-term survival of applied nematodes indicate that recycling through hosts must have occurred. Records of epizootics suggest that under certain conditions dense populations of EPNs occur, presumably in response to host abundance.

However, the presence of dense populations of acceptable hosts doesnot seem to be the sole requirement for EPNs epizootics. For example, outbreaks of scarab grubs in turf in the northeastern U.S.A. do not always give rise to dense nematode populations (Akhutst et al. 1992). Studies of EPNs population dynamics reveal only that they generally lack seasonality. To establish more complete guidelines for conservation of natural populations it is needed first to understand the requirements and structure of natural populations. Conservation practices for EPNs can potentially decrease costs of control and increase the efficacy and predictability of control for both inundative and inoculative release. Long-term predictability of entomopathogenic nematode influence on host populations lags far behind short-term predictability (Gaugler et al., 1992., Booth, et al., 2002 and Gaugler et al., 2002).

Finally the present study demonstrates that, the nematodes S. glaseri used here are highly effective when applied on the soil surface at a concentration of 20000IJ/m^2 more than *H*. bacteriophora with the same concentration in both methods of application (inundative and inoculative releases). This nematode species is most likely adapted to the climatic conditions in these regions and will be suitable for application in the strawberry fields for controlling scarab beetle (Atwa, 2003 and Shamseldean and Atwa, 2004). This notion is further emphasized in a previous study (Glazer et al., 1999) where nematode efficacy was compared at soil conditions for controlling soil insects. The possibility of longterm control, although important for certain pests in various cropping systems, is of little practical importance for strawberry beetle control when immediate curative applications are necessary.

4.REFERENCES

- Akhutst R. J., Bedding R. A., Bull R. M., and Smith K. R. J. (1992). An epizootic of *Heterorhabdities* spp. (Heterorhabditidae: Nematoda) in sugar cane scarabaeids (Coleoptera). Fund. Appl. Nematol. 15, 71-73.
- Atwa A. A. (2003). Identification, Mass Eulture, and Utilization of Entomopathogenic Nematodes Against Insect Pests. Ph. D. Thesis, Faculty of Agriculture, University of Cairo, Giza, Egypt. 172 pp.
- Bedding R. A. and Nickson D. (1999). Biological control of Black beetle larvae. Australian Turfgrass Management, 12: 34-37.
- Booth S. R., Tanigoshi L. K. and Shanks JR. C. H. (2002). Evaluation of entomopathogenic nematodes to many root weevil larvae in

Washington State cranberry, strawberry, and red raspberry. Environ. Entomol. 31 (5): 859 – 902.

- Dutky S. R., Thompson J. V. and Cantwell G. E. (1964). A technique for the mass propagation of the DD-136 nematode. J. Insect pathol. 6, 417-422.
- Fife J.P., Derksen R.C., Ozkan H. E., and Grewal P. S. (2003). Effect of pressure differentials on viability and infectivity of entomopathogenic nematodes. Biological Control, 27: 65-72.
- Gaugler R., Brown I., Shapiro-Ilan D. and Atwa A. A. (2002). Automated technology for *in vivo* mass production of entomopathogenic nematodes. *Biological control*, 24, 199-206.
- Gaugler R., Campbell J. F., Selvan S., and Lewis E. E. (1992). Large – scale, inoculative release of the entomopathogen: *Steinernema glaseri*, assessment 50 years later. Biol. Control. 2, 181-187.
- Glazer I., Salame L., Goldenberg S. and Blumberg D. (1999). Susceptibility of sap beetles (Coleoptera: Nitidulidae) to entomopathogenic nematodes. Biocontrol Sci. Technol. 9: 259 - 266.
- Grewal P. S. (2002). Formulation and application technology. In: R. Gaugler (ed), Entomopathogenic Nematology. CABI Publishing, Wallingford, UK. p. 189-_204.
- Henderson C. F. and Tilton W. (1955). Tests with Acaricides against the Brown Wheat Mite. *J.* Econ. Entomol., 48(2): 157-161.
- Jansson R. K., Lecrone Scott H. and Gaugler R. (1991). Comparison of single and multiple releases of *Heterorhabiditis bacteriophora* Poinar (Nematoda: Heterorhabditidae) for control of *Cylas formicarius* (Fabricius) (Coleoptera: Apionidae). Biological control 1: 320-328.
- Koppenhöfer A. M., Brown I. M., Gaugler R., Grewal P. S., Kaya H. K. and Klein M. G. (2000). Synergism of entomopathogenic nematodes and Imidacloprid against white grubs: greenhouses and field evaluation. *Biological Control*, 19: 245 – 251.
- Koppenhöfer A. M., Choo H. Y., Lee, Kaya H. K., and Gelernter W. D. (1999). Increased field and greenhouse efficacy against scarab grubs with combination of an entomopathogenic nematodes and Bacillus thuringiesis. Biological Control, 14, 37 – 44.
- Lewis E. E., Campbell J. F. and Gaugler R. (1997). The effect of aging on the foraging behavior of *Steinernema carpocapsae* (Rhabdita: Steinernematidae). Nematologica 43: 355-362

- Martin W. R. (1997). Using entomopathogenic nematodes to control insects during stand establishment. Hortscience 32: 196-200.
- SAS Institute. (1988). "SAS User's Guide: Statistics." SAS Inst., Cary, Nc.
- Shamseldean M. M. and Atwa A. A. (2004). Laboratory and field tests of entomopathogenic nematodes against the scarab beetle *Temnorhynchus baal* (Reiche) a

novel insect pest of strawberry in Egypt. Proceeding of the 1st Arab Conference for Applied Biological Pest Control, Cairo, Egypt, 5-7 April 2004. Egyptian J. Biol. Pest. Cont., 14 (1): 127-133.

White C. F. (1927). A method for obtaining infective larvae from culture. Science 66: 302-303.

المقارنة بين الإطلاق المحدود والإطلاق الكثيف لمكافحة الخنافس الجعلية في الفراولة بإستخدام النيماتودا الممرضة للحشرات تحت الظروف الحقلية.

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ملخص

تسبب يرقات الخنفساء الجعلية تيمنور هينكس بال (Temnorhynchus baal) والتي تتغذي على جذور نباتات الفراولة أضراراً إقتصادية في مصر. ولقد تم إختبار النيماتودا الممرضة للحشرات والتابعة لجنسي شتينرنيما و هيتيرورابديتيس ضد هذه الخنفساء الجعلية. أظهرت النتائج المعملية أن النوع شتينر نيما جلاسيري (Steinernema glaseri Steiner) أعطى نسبة موت100 % للعمر اليرقى الأول والثاني للخنفساء الجعلية تيمنور هينكس بُال بينما كانت نسّبة الموت للعمر اليرقى الثالث 94% والحشرة الكاملة 96% بعد التعرض للأطوار النيماتودية المعديه لمدة 3 أيام. وفي المقابل أظهر النوع هيتيرور ابديدس باكتيريوفور ا (Heterorhabdis bacteriophora Poinar) نسبة موت 68 ، 62 ، 60 ، 58 % للأطوار الحشرية البرقية الأول والثاني والثالث والحشرة الكاملة على التوالي للخنفساء الجعلية تيمنور هينكس بال وعموماً فإن النوع شتينرنيما جلاسيري كان أكثر فعالية من النوع هيتيرور ابديدس باكتيريوفور الكل أطوار هذه الخنفساء الجعلية. وعلى مستوي التطبيق الحقلي كان الإطلاق المحدود (اللقاحي) للنوع شتينرنيما جلاسيري بعد ثلاث إطلاقات أكثر فاعلية من الإطلاق الكثيف (الإغراق) الأول ، علاوة على ذلك كانت النتائج الحقاية لنفس النوع بعد ست إطلاقات محدودة. ويسير الإطلاق الكثيف الثاني في نفس الاتجاه. على الجانب الآخر هناك إختلاف معنوي في الخفض في تعداد العائل الحشري وفقاً لإختلاف طريقة التطبيق ٱلحقلي سواء الإطلاق المحدود بعد الإطلاقة الثالثة أو الإطلاق الكثيف بعد الإطلاقة الأولى في حالة الإستخدام الحقلي للنوع هيتيرور ابديدس باكتيريوفورا ، بينما لم يكن هناك أي إختلاف معنوي لكلا نوعي التطبيق الحقَّلي بعد الإطلاقة السادسة في حالة الإطلاق المحدود أو الإطلاقة الثانية في حالة الإطلاق الكثيف. ولقد أشارت النتائج في هذه الدر اسة أن النوع النيماتودي شتينرنيمًا جلسيري كان أكثر فاعلية عندمًا أستخدم مع ماء الري بالتنقيط علي سطح التربة بتركيز 2000 طور معدى لكل متر مربع أكثر من النوع هيتيرور ابديدس باكتير يوفور اعندما أستخدم بنفس الطريقة ونفس التركيز في كلا طريقتي التطبيق الحقلي (الإطلاق المحدود أو الإطلاق الكثيف) ، وعموماً كانت طريقة الإطلاق المحدود أكثر فاعلية من طريقة الإطلاق الكثيف تحت الظروف الحقلية لمكافحة الجعلية تيمنور هينكس بال في حقول الفراولة بمصر

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (60) العدد الثاني (أبريل 2009):197-205.