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EVALUATION OF THE ENTOMOPATHOGENIC NEMATODES, *STEINERNEMA* SPP AND *HETERORHABDITIS BACTERIOPHORA*, IN THE CONTROL OF MOLE CRICKET,*GRYLLOTALPA AFRICANA*

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ABSTRACT: *This study aimed to evaluate the efficacy of a new approach in the control of the mole cricket, Gryllotalpa africana using Steinernema spp (Siwa strain) ; Steinernema riobrave ; Heterorhabditis bacteriophora (B_{20}) and , (HP_{88}) under laboratory conditions. Entomopathogenic nematodes were added at four concentrations (1000, 2000, 3000 and 4000 infective juveniles/ pot). Results revealed that, the use of Steinernema spp (Siwa strain) at 3000 IJs/ pot resulted 70%, 80% and 80% mortality after 6 days of the 1st, 2nd and 3rd nymphal instars, respectively, while Steinernema riobrave at the same concentrations caused 80% mortality of the 1st , 2nd and 3rd nymphal instars after 6 days. Concentrations of 4000 IJs of S. riobrave recorded 80%, 90% and 90% mortality of the 1st, 2nd and 3rd nymphal instars after 6 days respectively. The concentrations 4000 IJs of Heterorhabditis bacteriophora (B_{20}) recorded 100%, 90% and 90% mortality of the 1st , 2nd and 3rd nymphal instar after 6 days respectively, while Heterorhabditis bacteriophora (HP_{88}) at the same concentrations caused 100% , 80% and 80% mortality against 1st, 2nd and 3rd nymphal instars after 6 days ,respectively.*

Key words: *Gryllotalpa africana , Entomopathogenic nematodes, Steinernema, Heterorhabditis.*

INTRODUCTION

The *Gryllotalpa africana* has an economic importance as insect pest to many plants in seedling stage causing great damage (El-Malki, 1990 and Mohamed, 1993) *Steinernemtids* and *Heterorhabditids* are entomopathogenic nematodes that have been recovered from many regions through out the world. Entomopathogenic nematodes associated with bacteria, are commercially available to control soil pests (Georgis and Manweiler, 1994 ; Kella, 2004 and Shamseldean *et al.*,1996). This entomopathogenic nematode is excellent biological control candidate. They selectively infect many insects and a few other pests but do not adversely affect mammals or plants

(Akhurst and Smith, 2002). The goal of this study is using *Steinernema* and *Heterorhabditis* as a biological control agent against *Gryllotalpa africana*.

MATERIALS AND METHODS

1- Entomopathogenic nematodes:

The nematode population used in this research originated from applied center for entomonematodes, Faculty of Agriculture, Cairo University, where Greater wax moth *Galleria mellonella* was used as the host insect to in vivo culture *Steinernema* spp (Siwa strain); *Steinernema riobrave*; *Heterorhabditis bacteriophora* (B_{20}) and *Heterorhabditis bacteriophora* (HP_{88}) (Kaya and Stock, 1997). Infective juveniles were harvested from white traps (White, 1927). Live and active infective juveniles were used within the first week of emergence from their host cadavers.

2- Rearing techniques of *Gryllotalpa africana*:

Adults of *G. africana* were collected from Sears-Ellayan locality, Minufiya governorate, by the aid of light traps and used as a stock culture of *G. africana* according to Wineriter *et al.*, (1991). Field collected adults were paired, and each pair was placed in a plastic jar of 1 kg capacity, filled with 450 gm dry sifted sand, and moistened with 125 ml. of water. *G. africana* food was consisted of an equal mixture of ground maize seeds and ready chicken food containing 15-20% animal protein. After one week the female was presumed to be mated and the male was removed. Containers were held at 25-28 °C in an incubator. Eggs were removed from the egg chamber at the bottom of the container. Petri dish 10 cm was filled with 34 g of dry sifted sand and 5ml of water. The dishes were stored on a shelf out of direct light. Nymphs were removed as they hatch.

3- Application of entomopathogenic nematodes on the G. africana:-

540 plastic pots (13 cm in diameter and 7 cm height), each was filled with 250g sterilized sand and received 70 ml distilled water. These pots were divided to three groups.

The first group 180 pots each pots contained one individual of the first instar nymph of *G. africana* /pot and inoculated with nematode as follow:-

1-Ten pots inoculated with 1000 IJs of *Steinernema* spp (Siwa strain).
2-Ten pots inoculated with 2000 IJs of *Steinernema* spp (Siwa strain).
3-Ten pots inoculated with 3000 IJs of *Steinernema* spp (Siwa strain).
4-Ten pots inoculated with 4000 IJs of *Steinernema* spp (Siwa strain).
5-Ten pots inoculated with 1000 IJs of *Steinernema riobrave*.
6-Ten pots inoculated with 2000 IJs of *Steinernema riobrave*.
7-Ten pots inoculated with 3000 IJs of *Steinernema riobrave*.
8-Ten pots inoculated with 4000 IJs of *Steinernema riobrave*.

9-Ten pots no inoculum of entomopathogenic nematode as check.
10-Ten pots inoculated with 1000 IJs of *H. bacteriophora* (HP_{88}).
11-Ten pots inoculated with 2000 IJs of *H. bacteriophora* (HP_{88}).
12-Ten pots inoculated with 3000 IJs of *H. bacteriophora* (HP_{88}).
13-Ten pots inoculated with 4000 IJs of *H. bacteriophora* (HP_{88}).
14-Ten pots inoculated with 1000 IJs of *H. bacteriophora* (B_{20}).
15-Ten pots inoculated with 2000 IJs of *H. bacteriophora* (B_{20}).
16-Ten pots inoculated with 3000 IJs of *H. bacteriophora* (B_{20}).
17-Ten pots inoculated with 4000 IJs of *H. bacteriophora* (B_{20}).
18-Ten pots with no inoculum of entomopathogenic nematode.

The second group 180 pots each pot contained one individual of second instar nymph of G. africana/ pot and the same above mentioned treatments.

The third group 180 pots each pot contained one individual of third instar nymph of *G. africana*/ pot and the same above mentioned treatments.

Nematode inoculum was added as a liquid suspension to the food (10 g/ pot). The mortality percentages of *G. africana* were counted after 2 , 4 and 6 days from the beginning of the experiment.

RESULTS AND DISCUSSION

Results showed that all treatments were satisfactory affect the different nymphal stages of *G. africana*. The obtained results in Table (1) reveal that the use of Steinernema sp. (Siwa strain) of 3000 infective Juveniles/ pot achieving 70 , 80, and 80 % mortality of the 1st , 2nd and 3rd nymphal instars, after 6 days respectively, while *Steinernema riobrave* caused 80% mortality of the 1st , 2nd and 3rd nymphal instars after 6 days at the same concentration. Concentrations of 4000 IJs of *S. riobrave* recorded 80, 90 and 90 % mortality of the 1st, 2nd and 3rd nymphal Instars ,after 6 days , respectively..

Data in Table (2) reveal that, the application of *H. bacteriophora* (B20) at the rate of 4000 IJs recorded 100, 90 and 90% mortality of the 1st, 2nd and 3rd nymphal instars, after 6 days , respectively.

Results also showed that *H. bacteriophora* (Hp88) in 4000 IJs recorded 100% mortality at the 1st nymphal instars , after 6 days. While concentrations of 3000 IJs /pot recorded 60% mortality against the 1st, 2nd and 3rd nymphal instars after 6 days, respectively.

Table (1): Efficacy of four concentrations of two strains of *Steinernema* spp on the mortality of different nymphal stages of *G. africana*.

Nematodes and Concentrations (IJs)		% mortality at 2 , 4 ,6 days after treatment								
		1st Instar nymph			2nd Instar nymph			3rd Instar nymph		
		2 day	4 day	6 day	2 day	4 day	6 day	2 day	4 day	6 day
Steinernema spp (Siwa strain)	1000	0	20	20	0	10	20	10	20	20
	2000	20	40	50	20	40	60	20	30	60
	3000	30	40	70	30	50	80	30	40	80
	4000	30	60	70	30	50	80	40	60	80
Steinernema riobrave	1000	20	30	30	10	20	20	20	20	30
	2000	20	30	50	30	40	60	30	50	60
	3000	30	50	80	40	80	80	40	50	80
	4000	50	70	80	50	80	90	40	70	90
Check		0	0	0	0	0	0	0	0	0

Table (2): Efficacy of four concentrations of two strains of *Heterorhabditis* sp on the mortality of different nymphal stages of *G. africana*.

Nematodes and concentrations (IJs)		% mortality at 2 , 4 ,6 days after treatment								
		1st Instar nymph			2nd Instar nymph			3rd Instar nymph		
		2 day	4 day	6 day	2 day	4 day	6 day	2 day	4 day	6 day
Heterorhabditis bacteriophora (B_{20})	1000	20	30	40	10	30	40	20	30	30
	2000	50	50	70	40	50	60	40	50	60
	3000	60	70	100	50	70	90	50	80	80
	4000	60	80	100	50	80	90	50	80	90
Heterorhabditis bacteriophora (Hp_{88})	1000	0	0	20	0	20	20	0	20	20
	2000	20	20	40	10	20	40	10	20	30
	3000	40	40	60	20	40	60	20	40	60
	4000	40	50	100	40	60	80	30	50	80
Check		0	0	0	0	0	0	0	0	0

The obtained results illustrated in Fig. (1) showed that percentages of mortality of the different insect stages of *G. africana* were increased by increasing the concentrations of nematodes, *Steinernema* (Siwa strain), *S. riobrave*, *H. bacteriophora* (B_{20}) and *H. bacteriophora* (HP_{88}). The highest mortality percentages (100%) of 1st nymphal instar , after 6 days, was achieved by using 4000 IJs/pot of *H. bacteriophora* (B_{20}) or (HP_{88}).

Entomopathogenic nematodes are obligate parasites of soil-living insects. Nearly 80 species have been described from two genera .They are used successfully as biological control agents in several cropping systems to reduce populations of soil insects . Their intended effect on soil food webs is to reduce the density of herbivorous insects, thereby protecting crop plants. The symbiotic bacteria associated with steinernematids, *Xenorhabdus* spp, produce metabolites that are toxic to insects.

These results are agree with those reported previously by (Kella, 2004) who controlled the African mole cricket under laboratory and field conditions by entomopathogenic nematodes . According to Woodring and Kaya (1988) certain metabolites produced by entomopathogenic nematode or their associated symbiotic bacteria *Xenorhabdus* spp were toxic to *Gryllotalpa africana* . The bacteria rapidly multiply and kill the host within 48 hours by septicemia.

Recently, Shamseldean, *et al.*, (2008) studied the effect of Egyptian entomopathogenic nematode isolates on some economic insect pests, they conducted experiments to evaluate the infectivity of *Heterorhabditis bacteriophora* and *H.indica* as biocontrol candidates against some economic agricultural insect pests; *Spodoptera littoralis, Agrotis ipsilon, Sesamia cretica, Phthorimaea operculella and Spodoptera exigua*,. The tested nematode species grew faster in *S.littoralis* and *Ph.operculella* larvae than in the other host larvae. The survival of *H.bacteriophora* and *H.indica* in distilled water was affected by the two combined factors, exposure time and temperatures. All individuals of *H. bacteriophora* and *H. indica* were viable after exposure for 48 hours to temperatures ranged from 5-30°C. At lower (0°C and -5°C) and higher temperatures (35 and 40°C), the survival decreased especially at higher temperatures. Effect of temperature and exposure time on the infectivity of the nematode *H. indica* (EASD77 isolate) and *H. bacteriophora* (EASD98 isolate) was higher against *S. littoralis*.

Finally it could be recommended that the use of *Steinernema* and *Heterorhabditis* for controlling *G. Africana* ,especially on organic cultivars gave satisfied control results if used in the field with high numbers , this will be help in eliminating the use of chemical pesticides .

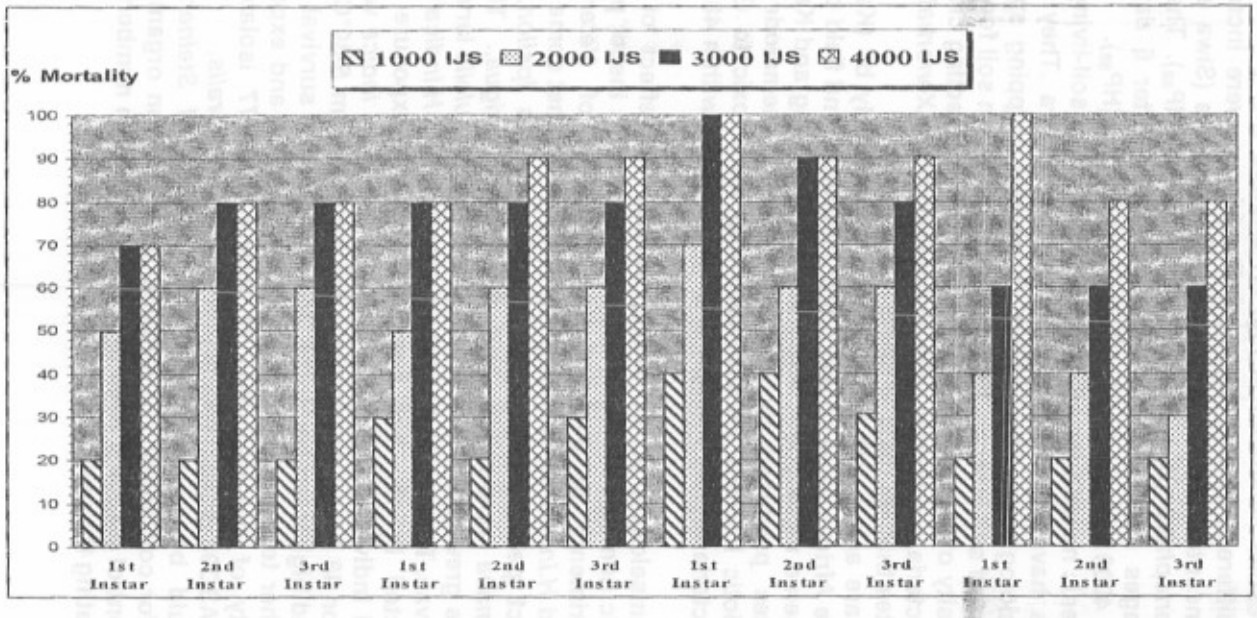

Fig (1) : Mortality percentages of *Gryllotalpa africana* nymphal stages as affected by four nematode concentrations of *Steinernema* spp and *Heterorhabditis* spp ,after 6 days of application

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تقييم النيماتودا *Steinernema* spp. ، *Heterorhabditis bacteriophora.* في مكافحة الحفار الأفريقي *Gryllotalpa africana*

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الملخص العربي

يعتبر الحفار الأفريقي من أكثر أنواع الحفار انتشارا في مصر وهو من أهم آفات البادرات ومحاصيل الخضر حيث إنه يسبب أضرار للنبات وخاصة في طور البادرة. ونظراً لخطورة استخدام المبيدات لمكافحة الحفار فقد تم اختبار أربع تركيزات : ١٠٠٠-٢٠٠٠-٣٠٠٠-٤٠٠٠ طور معدي لكل حشرة من ***Steinernema*** **spp (Siwa strain),** ***S. riobrave*** ***Heterorhabditis. bacteriophora*** **(B20) ,** ***H. bacteriophora*** **(HP88)** وهذه النيماتودا هي من النيماتودا الممرضة للحشرات التابعة لمجموعة النيماتودا الحرة والتي لها مستقبل في برنامج المكافحة الحيوية للآفات الحشرية حيث أنها ذات قدرة عالية في البحث عن الآفة واختراقها .

تم استخدام هذه النيماتودا لمكافحة أطوار الحفار في طور الحورية (عمر أول وثاني وثالث) وقد أظهرت النتائج أن استخدام نيماتودا ***Steinernema*** **spp (Siwa strain)** بتركيز ٣٠٠٠ طور معدي لكل حشرة أعطي نسب موت ٧٠ ،٨٠، ٨٠٪ على الحوريات عمر (أول و ثاني وثالث) على الترتيب بعد ٦ أيام، بينما نيماتودا ***S. riobrave*** عند نفس التركيز أعطت نسبة موت ٨٠٪ على الحوريات عمر(أول وثاني وثالث) أما التركيز ٤٠٠٠ طور معدي من نيماتودا ***S. riobrave*** على الحوريات عمر(أول وثاني وثالث) أعطي نسب موت ٥٠ ، ٥٠ ، ٤٠٪ على الترتيب بعد ٢ يوم بينما ارتفعت إلى ٨٠، ٩٠، ٩٠٪ بعد ٦ أيام. بينما في حالة نيماتودا ***H. bacteriophora*** **(B20)** بتركيز ٣٠٠٠ طور معدي لكل حشرة كانت نسبة

الموت في الحوريات عمر (أول وثاني وثالث) ٦٠، ٥٠،٥٠ على الترتيب بعد ٢ يوم بينما وصلت نسبة الموت بعد ٦ أيام إلى ١٠٠، ٨٠،٩٠٪ على الترتيب. نيماتودا *H. bacteriophora* (HP88) بتركيز ٣٠٠٠طور معدي لكل حشرة أعطت موت ٦٠٪ للحوريات بعد ٦ أيام بينما أعطت ١٠٠، ٨٠، ٨٠ ٪ على الترتيب عند التركيز ٤٠٠٠ طور معدي لكل حورية. وفى النهاية أوضحت الدراسة إمكانية استخدام النيماتودا الممرضة في مكافحة الحفارات وذلك بإضافة الطور المعدي من النيماتودا على الطعم المقدم للحفار حيث وصلت نسبة الموت لحوريات الحفار إلى أكثر من ٥٠٪ بعد يومان من التطبيق.