academicJournals

Vol. 7(50), pp. 5669-5676, 18 December, 2013
DOI: 10.5897/AJMR12.1595
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Virulence of new South Carolinian heterorhabditid isolates (Rhabditida: Heterorhabditidae) to the beet armyworm, *Spodoptera exigua*

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Accepted 11 November, 2013

The virulence of new nine heterorhabditid isolates from South Carolina (*Heterorhabditis megidis* LEX, *H. zealandica* EDS and CHR, and *H. bacteriophora* WPS, SMP, PD, CFG, MF and CFM strains) against the beet armyworm was compared with two known heterorhabditid nematodes (*H. bacteriophora* Hb and HP88 strains) under laboratory conditions. The Petri-plate bioassay procedure was used to evaluate the susceptibility of the *Spodoptera exigua* larvae to the heterorhabditids at concentrations of 10, 25, 50, and 100 infective juveniles (IJs) per larva. Mortalities were counted for 4 days. At the final count, mortalities were 53.6-100, 72-100, 79.8-100, and 92.9-100% for all nematode species/strains at the concentrations of 10, 25, 50, and 100 IJs per larva, respectively. *H. megidis* LEX strain was superior and differed than others by having 100% mortality in all of the concentrations. It was second to cause early mortality. It had the highest mortality rate at 10 nematodes per larva and *H. bacteriophora* WPS, *H. zealandica* CHR, *H. bacteriophora* HP88 and *H. zealandica* EDS strains followed it with 92.9, 89.3, 85.7 and 82.1% mortality, respectively. LC_{50} for most of the nematodes was relatively low (10 IJs per larva). Virulence of *H. bacteriophora* WPS, HP88 and SMP and *H. zealandica* CHR strains were similar. The least virulent heterorhabditid was *H. bacteriophora* CFM strain with LC_{50} value of 14.8 IJs per larva. The LT_{50} value of *H. bacteriophora* WPS strain was the smallest and it was followed by *H. megidis* LEX, *H. zealandica* CHR and EDS, *H. bacteriophora* SMP, HP88, MF, PD, Hb, CFG and CFM strains, respectively.

Key words: Biological control, entomopathogenic nematodes, *Heterorhabditis, Spodoptera exigua.*

INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is an important polyphagus pest of cultivated crops worldwide primarily in the tropical and subtropical regions (Stewart et al., 1996; Ansari et al., 2007; Leiva et al., 2010). It has a wide host range, including vegetable, field, and flower crops. Among susceptible vegetable crops are asparagus, bean, beet, broccoli, cabbage, cauliflower, celery, chickpea, corn, cowpea, eggplant, lettuce, onion, pea, pepper, potato, radish, spinach, sweet potato, tomato, and turnip. Field crops damaged include alfalfa, corn, cotton, peanut, safflower, sorghum, soybean, sugar beet, and tobacco (Capinera, 2006). It cannot be adequately controlled by most commercial pesticides because of its wide range of resistance (Brewer and Trumble, 1989; Van Laecke and Degheele, 1991). Its decrease in pesticide susceptibility becomes more serious, especially when it develops into late instars (Kim et al., 1998). Also, because of environmental and regulatory concerns associated with chemical use (Luckman and Metcalf, 1982; National Research Council, 1989; Hamilton et al., 1997; Cohen, 2000; Römbke et al., 2008), there is much interest in biorational approaches

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(Kerns et al., 1998; Guerrero and Rosell, 2005).

Entomopathogenic nematodes (EPN) (Steinernematidae and Heterorhabditidae) are obligate parasites of insects (Poinar, 1990; Adams and Nguyen, 2002). They are mutualistically associated with bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively). Infective juveniles (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002). The nematodes molt and complete up to three generations within the host after which IJs exit the cadaver to search out new hosts (Kaya and Gaugler, 1993).

These nematodes are effective biocontrol agents of a variety of economically important insect pests (Klein, 1990; Shapiro-Ilan et al., 2002**;** Grewal et al., 2005) and they have been used in controlling insect pest for about 25 years, extending their usage from high value markets to large area crops, including forestry (Peters, 2010). A number of studies indicate that applications of EPN can result in high levels of control for a variety of noctuid pests including *S. exigua* (Feaster and Steinkraus, 1996; Medeiros et al., 2000; González-Ramírez et al., 2000; Kim et al., 2006; Kepenekçi and Evlice, 2009).

Despite the progress that has been made in the use of EPN (Laznik et al., 2010a), knowledge about their natural host range and their efficacy on insect populations as biological control agents is still limited (Ansari et al., 2007). Our overall goal was to determine the potential use of several heterordabditid nematodes for *S. exigua* suppression.

A crucial element to be successful in any biological control program with EPN is the pairing the most suitable nematode with the defined host, and relative pathogenicity among various nematodes is one of the important factors to consider in determining suitability (Georgis and Gaugler, 1991; Shapiro-Ilan et al., 2002; Shapiro et al., 2006). Therefore, this research aimed to compare the virulence of new nine heterorhabditid isolates from South Carolina against the beet armyworm with two known heterorhabditid nematodes (*Heterorhabditis bacteriophora* Hb and HP88 strains) under laboratory conditions.

MATERIALS AND METHODS

The larvae of *S. exigua* were reared on artificial pinto bean diet (Burton, 1969). *Heterorhabditis bacteriophora* Hb strain was obtained from Dr. David I. Shapiro-İlan, Integrated BioControl Systems, Inc. (Aurora, Indiana) and *H. bacteriophora* HP88 strain was provided by Dr. Khoung B. Ngyuen and Dr. Byron J. Adams of the University of Florida. The other nine heterorhabditids; *H. megidis* LEX, *H. zealandica* EDS and CHR, and *H. bacteriophora* WPS, SMP, PD, CFG, MF and CFM strains were obtained from soil on a survey in South Carolina, USA (Canhilal and Carner, 2006a).

EPN were produced on last-instar of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) following the standard rearing method described by Woodring and Kaya (1988). A modified White Trap (Canhilal and Carner, 2006b), consisting of a folded 11-cm filter paper (3 mm in depth after folding) in a Petri dish (100 x 15mm) with 15-20 ml of distilled water, was used to collect the infective juveniles (IJs). These IJs were stored at 7-8°C in tissue culture flasks for 15-20 days before being used for experiments (Kung et al., 1990). Before the assays, viability was confirmed by observing nematode activity (rapid wiggling) under a binocular microscope (Laznik et al., 2010b).

The Petri-plate bioassay procedure was used to evaluate the susceptibility of the beet armyworm larvae to heterorhabditids at concentrations of 10, 25, 50, and 100 IJs per larva in 1 ml of sterile distilled water, following the procedures described by Woodring & Kaya (1988). Petri dishes (100 x 15 mm) were lined with two Whatman No.1 filter paper pieces (9 cm diameter). 1 h before the beginning of the experiment, the IJs were applied and distributed evenly on the filter paper. For each treatment concentration, four groups of seven 4th instars of *S. exigua* were placed per dish containing IJs. The Petri dishes were placed in a double plastic bag and put in a dark incubator at 25 ± 1°C (Glazer et al., 1991). Controls consisted of 1 ml of sterile distilled water without nematodes. The bioassay was repeated two times.

S. exigua mortality was recorded every 24 h for 4 days (Epsky and Capinera, 1994). Dead insects were incubated on modified White Traps at room temperature (25 ± 1°C) and examined to confirm the presence of nematodes. The mortalities were converted to percentages and adjusted for control mortality, using Abbott's correction formula. The data were analyzed as a completely randomized factorial design and Least Significant Difference (LSD) mean separation procedure was used to detect differences among treatments. Lethal concentration (LC_{50}) values and median lethal time (LT_{50}) values at 10, 25, 50, and 100 nematode concentrations for each nematode strain were estimated by probit analysis (SPSS, 2003).

RESULTS

All nematodes tested were capable of killing the beet armyworm and reproducing in it. The dead larvae in the treatments showed typical symptoms of nematode infection. The mortality induced by nematodes increased, typically with increasing numbers of nematodes per larva and significant positive regressions were observed between mortality and dose rate for *H. megidis* LEX (r^2 = 0.500; F = 18.000; df = 1, 19; P = 0.001), *H. zealandica* EDS (r^2 = 0.635; F = 31.281; df = 1, 19; P = 0.001) and CHR (r^2 = 0.796; F = 70.198; df = 1, 19; P = 0.001), *H. bacteriophora* HP88 (r^2 = 0.578; F = 24.672; df = 1, 19; P = 0.001), Hb (r^2 = 0.675; F = 37.402; df = 1, 19; P = 0.001), WPS (r^2 = 0.551; F = 22.120; df = 1, 19; P = 0.001), SMP (r^2 = 0.588; F = 25.657; df = 1, 19; P = 0.001), PD (r^2 = 0.730; F = 48.785; df = 1, 19; P = 0.001), CFG (r^2 = 0.670; F = 36.628; df = 1, 19; P = 0.001), MF (r^2 = 0.796; F = 70.198; df = 1, 19; P = 0.001) and CFM strains (r^2 = 0.774; F = 58.164; df = 1, 19; P = 0.001). There was very low mortality in untreated controls (Figures 1 and 2).

Low mortality occurred during the first day after treatment except 100 nematode concentrations of some

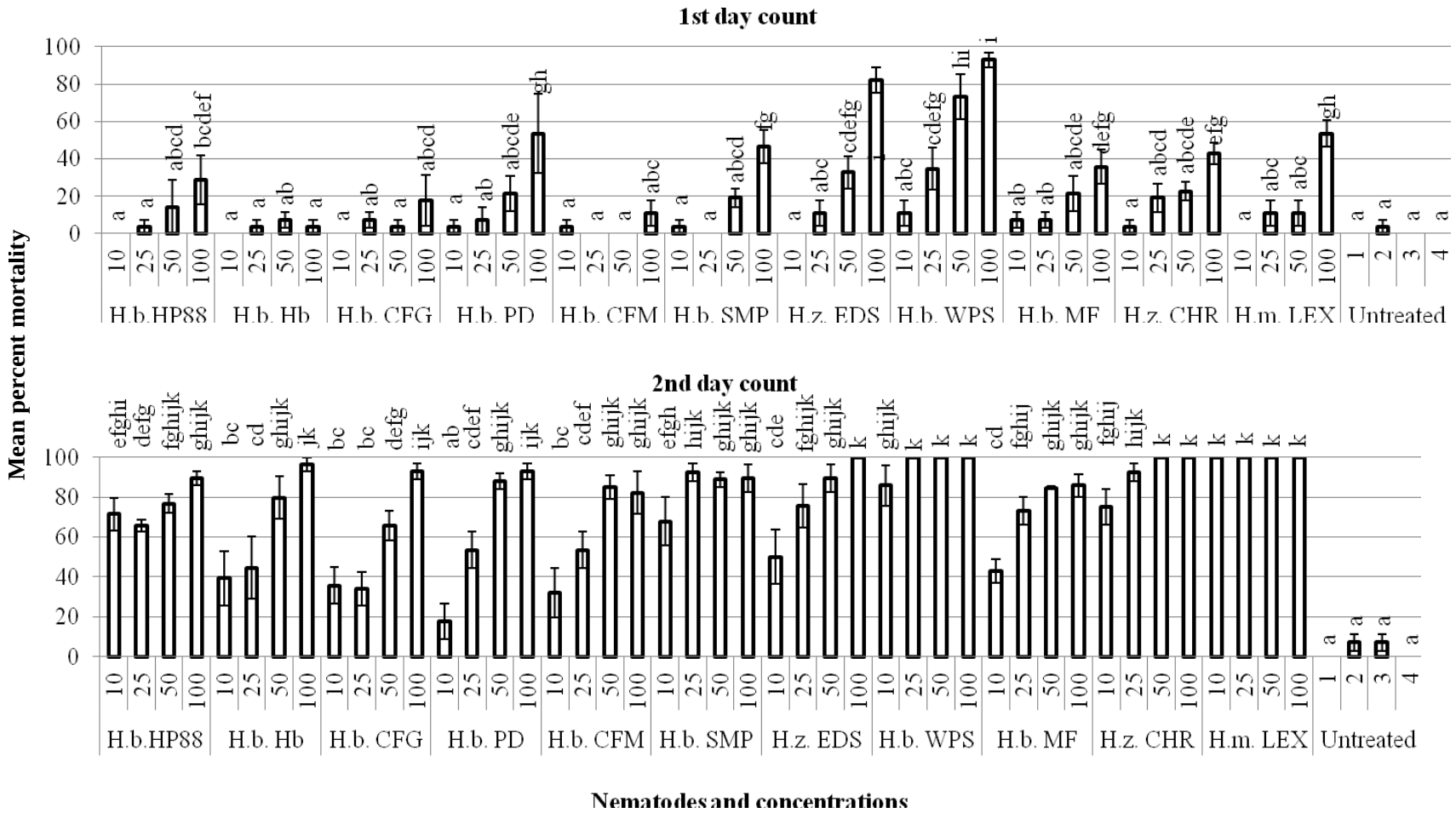

Figure 1. Mean percent mortality of 4th instars of the beet armyworm after 1 and 2 d by heterorhabditid species/strain in a Petri-plate bioassay at 10, 25, 50 and 100 IJs per larva.

nematodes and 50 nematode concentration of *H. bacteriophora* WPS strain. *H. bacteriophora* WPS and *H. zealandica EDS* strains were the best performer at the first day count (Figure 1). In general, mortality rates increased from day 2 to day 4 (Figures 1 and 2).

On the second day; mortalities at 50 and 100 nematode rates reached usually over 80%. All rates of *H. megidis* LEX strain, 50 and 100 nematode rates of *H. zealandica* CHR strain, 25, 50 and 100 nematode rates of *H. bacteriophora* WPS strain, and 100 nematode rate of *H. zealandica* EDS strain killed all larvae in the treatments (Figure 1). At 25 nematode rate; *H. megidis* LEX and *H. bacteriophora* WPS strains produced 100% mortality which were not significantly different than *H. zealandica* CHR and EDS and *H. bacteriophora* SMP strains with 92.3,

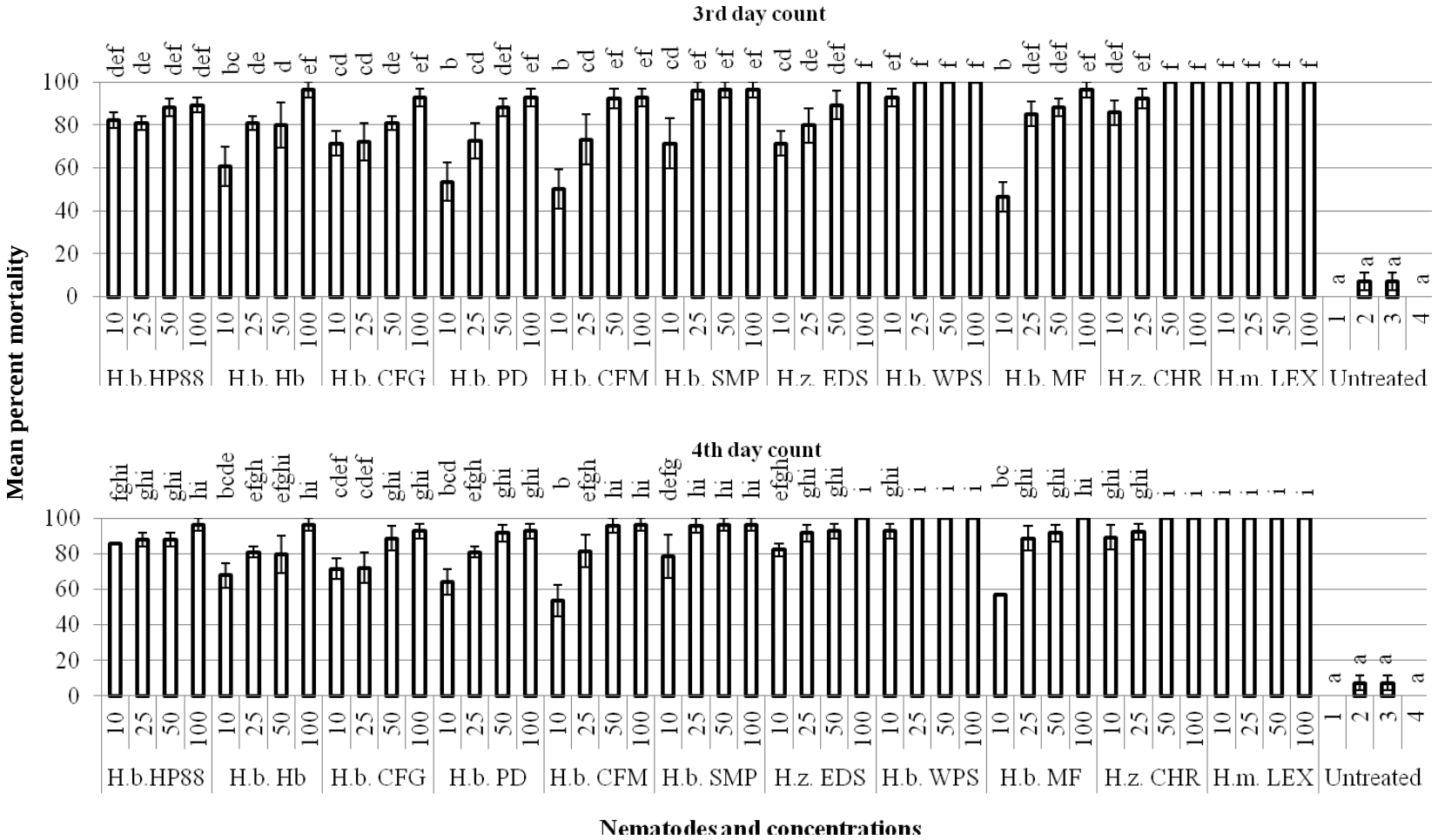

Figure 2. Mean percent mortality of 4th instars of the beet armyworm after 3 and 4 d by heterorhabditid species/strain in a Petri-plate bioassay at 10, 25, 50 and 100 IJs per larva.

75.6 and 92.3% mortality, respectively. They were followed by *H. bacteriophora* MF and HP88 strains with 73.2 and 65.5% mortality, respectively (Figure 1). There was similar trend at 10 nematode rate with 25. The lowest rate of *H. megidis* LEX strain which gave 100% mortality was not significantly different than *H. bacteriophora* WPS with 85.7% mortality. They were followed by *H. zealandica* CHR, *H. bacteriophora* HP88 and SMP, *H. zealandica* EDS strains with 75, 71.4, 67.9 and 50% mortalities, respectively. Others produced less than 50% mortality (Figure 1).

On the 3rd day count; only same strains killed all larvae in the second day caused 100% mortality

Table 1. LC_{50} and LC_{90} values of heterorhabditid nematodes for beet armyworm larvae.

Nematode	Number larvae	*LC_{50}	*LC_{90}	χ^2	df	*P*
H. megidis LEX	56	3.5	5.9	0.02	3	0.999
H. bacteriophora WPS	56	5.5	9.4	0.00	3	1.000
H. bacteriophora HP88	56	5.5	48.3	92.81	3	0.001
H. bacteriophora SMP	56	6.1	39.9	445.41	3	0.001
H. zealandica CHR	56	7.2	17.1	43.27	3	0.001
H. zealandica EDS	56	8.5	30.4	72.15	3	0.001
H. bacteriophora PD	56	12.3	59.1	143.51	3	0.001
H. bacteriophora CFG	56	12.4	63.6	59.36	3	0.001
H. bacteriophora MF	56	13.2	34.1	37.15	3	0.001
H. bacteriophora Hb	56	13.3	58.1	51.60	3	0.001
H. bacteriophora CFM	56	14.8	46.9	232.68	3	0.001

*LC_{50} and LC_{90} values were calculated over 4 rates applied and expressed in number of nematodes per larva.

(Figure 2). All nematode strains except *H. bacteriophora* HP88 produced over 90% mortality at 100 nematode rate. However they were not significantly different including *H. bacteriophora* HP88 strain.

At 50 nematode concentrations, all nematodes except *H. bacteriophora* Hb strain gave more than 80% mortality. Even it was mostly over 90% and they were not signify-cantly different except *H. bacteriophora* CFG and Hb strains. At 25 nematode rate; all nematodes strains pro-duced more than 70% mortality. It was usually over 80%. *H. megidis* LEX, *H. zealandica* CHR, *H. bacteriophora* MF, WPS and SMP strains were at the same group statistically with 100, 92.3, 85.1, 100 and 95.8% mortality, respectively (Figure 2).

At 10 nematodes rate; *H. megidis* LEX, *H. zealandica* CHR, *H. bacteriophora* WPS and HP88 strains were not significantly different with 100, 85.7, 92.9 and 82.1% mortality, respectively. *H. zealandica* EDS, *H. bacteriophora* SMP and CFG strains made other group with 71.4% mortality. Another group was *H. bacteriophora* Hb, PD, CFM and MF strains with 60.7, 53.6, 50 and 46.4% mortality (Figure 2).

At the final count, mortalities were 53.6-100, 72-100, 79.8-100, and 92.9-100% for all nematode strains at the concentrations of 10, 25, 50, and 100 IJs per larva, res-pectively. All larvae died in the treatments of *H. bacteriophora* WPS and MF, *H. zealandica* EDS and CHR, and *H. megidis* LEX strains at 100 nematode rate. Mortalities were 96.4% for *H. bacteriophora* SMP, CFM, Hb, and HP88 strains and 92.9% for *H. bacteriophora* PD and CFG strains. However, they all are not significantly different (Figure 2).

At 50 nematode concentrations, while *H. megidis* LEX, *H. zealandica* CHR, and *H. bacteriophora* WPS strains were producing 100% mortality, the others caused mortality mostly over 90%. All treatments were at the same group statistically. *H. megidis* LEX and *H. bacteriophora* WPS strains again were superior with 100% mortality at 25 IJs per larva and it was followed by *H. bacteriophora* SMP, *H. zealandica* CHR and EDS, *H. bacteriophora* HP88 an MF strains (Figure 2).

H. megidis LEX had the highest mortality rate with 100% at 10 nematode per larva. *H. bacteriophora* WPS, *H. zealandica* CHR, *H. bacteriophora* HP88 and *H. zealandica* EDS strains followed it with 92.9, 89.3, 85.7 and 82.1% mortality, respectively at same statistical group (Figure 2).

LC_{50} for most of the nematodes was relatively low (<10 IJs per larva). The LC_{50} and LC_{90} data are summarized in Table 1. The lowest LC_{50} value was obtained by *H. megidis* LEX strain (3.5 IJs per larva). Virulence of *H. bacteriophora* WPS, HP88 and SMP and *H. zealandica* CHR strains were similar (LC_{50}s ranging from 5.5 IJs per larva in *H. bacteriophora* WPS strain to 7.2 IJs per larva in *H. zealandica* CHR strain). The least virulent heterorhabditid was *H. bacteriophora* CFM strain with LC_{50} value of 14.8 IJs per beet armyworm larva.

The LT_{50} and LT_{90} data are given in Table 2. The LT_{50} values ranged from 1.5 to 3.4 days for *H. megidis* LEX and *H. bacteriophora* CFM strains at 10 IJS per larva, from 1.1 to 2.6 days for *H. bacteriophora* WPS and *H. bacteriophora* CFG and CFM strains at 25 IJs per larva, from 0.8 to 2.1 days for *H. bacteriophora* WPS and CFG strains at 50 IJs per larva, and from 0.6 to 1.7 days for *H. bacteriophora* WPS and CFM strains at 100 IJs per larva, respectively. The LT_{50} value of *H. bacteriophora* WPS strain was the smallest and it was followed by *H. megidis* LEX, *H. zealandica* CHR and EDS, *H. bacteriophora* SMP, HP88, MF, PD, Hb, CFG and CFM strains, respectively when the average of LT_{50} values at four concentrations were evaluated.

DISCUSSION

In determining an entomopathogenic nematode as a

Table 2. LT_{50} and LT_{90} values of heterorhabditid nematodes at 10, 25, 50, and 100 infective juveniles per larva for beet armyworm.

Nem[a]	Number of larvae	10 infective juveniles				25 infective juveniles			
		*LT_{50}	*LT_{90}	χ^2	P	LT_{50}	LT_{90}	χ^2	P
HP88	56	1.5	1.8	1.16	0.763	1.3	1.6	0.09	0.994
Hb	56	1.7	2.9	50.89	0.001	1.1	1.5	0.08	0.994
CFG	56	2.0	3.3	37.10	0.001	1.6	2.8	56.44	0.001
PD	56	2.2	3.6	41.08	0.001	2.1	3.5	26.37	0.001
CFM	56	2.3	4.0	35.40	0.001	1.7	2.6	284.97	0.001
SMP	56	2.5	4.0	19.42	0.001	1.8	3.2	23.87	0.001
EDS	56	2.8	4.4	18.96	0.001	2.6	4.3	12.82	0.005
WPS	56	2.9	4.7	17.45	0.001	2.4	3.8	20.32	0.001
MF	56	3.2	5.6	14.50	0.002	2.0	3.3	28.48	0.001
CHR	56	3.2	5.0	4.62	0.202	2.3	3.9	17.31	0.001
LEX	56	3.4	5.6	11.63	0.009	2.6	4.1	18.33	0.001
		50 infective juveniles				**100 infective juveniles**			
WPS	56	0.8	1.6	0.11	0.991	0.6	1.3	0.36	0.949
CHR	56	1.2	1.2	0.01	1.000	1.1	0.9	0.05	0.997
LEX	56	1.3	1.5	0.09	0.994	0.9	1.4	0.05	0.997
EDS	56	1.4	3.2	40.82	0.001	0.8	2.8	0.17	0.982
SMP	56	1.5	2.6	69.37	0.001	1.3	2.4	51.74	0.001
PD	56	1.6	2.8	44.86	0.001	1.2	1.1	50.29	0.001
MF	56	1.7	3.5	32.34	0.001	1.3	2.8	1.90	0.593
HP88	56	1.8	3.6	33.67	0.001	1.5	2.5	26.40	0.001
CFM	56	1.8	3.0	78.17	0.001	1.7	2.1	34.52	0.001
Hb	56	2.0	2.9	49.73	0.001	1.6	2.7	288.01	0.001
CFG	56	2.1	2.7	26.37	0.001	1.6	2.8	59.90	0.001

[a]Nem= Nematodes; WPS= *H. bacteriophora* WPS; CHR= *H. zealandica* CHR; LEX= *H. megidis* LEX; EDS= *H. zealandica* EDS; SMP= *H. bacteriophora* SMP; PD= *H. bacteriophora* PD; MF= *H. bacteriophora* MF; HP88= *H. bacteriophora* HP88; CFM= *H. bacteriophora* CFM; Hb= *H. bacteriophora* Hb; CFG= *H. bacteriophora* CFG. *LT_{50} and LT_{90} values were calculated over 4 days counts and expressed in days.

biological control agent, it is important to look at several attributes of the agent such as attraction, penetration, movement, host defense mechanisms, and biotic and abiotic environmental factors. Although many factors are responsible for the level of infectivity, some basic data may be gathered through lab studies (Mannion and Jansson, 1992; Shapiro-Ilan et al., 2002; Laznik et al., 2011).

The virulence of nematodes to noctuids and other lepidopters varies significantly (Mbata and Shapiro-Ilan, 2005; Mederios et al., 2000). Mederios et al. (2000) reported that *H. bacteriophora* Az33 strain caused 23% mortality to the sixth instar of another noctuid, *Pseudaletia unipuncta*, the armyworm at 200 IJs per larva, whereas, the mortality of *H. bacteriophora* strains in our research was much higher even at lower rates. This may be because of different host and difference of strain which was collected from different locality.

Some heterorhabditid nematodes may possess additional positive attributes compared with others as demonstrated with ranging percent mortalities on large scales (53.6-100%) in the current study. Although the beet army worm larva was susceptible to each nematode species and strain tested, there were differences among these nematodes in their ability to kill the insect. *H. megidis* LEX, *H. bacteriophora* WPS, HP88 and SMP, *H. zealandica* CHR and EDS strains were more efficacious than others against *S. exigua* larva as it was reflected in the LC_{50}, LT_{50} and percent mortality data. Mortalities were higher, LC_{50} values were lower and LT_{50} values were shorter for these nematodes. Kim et al. (2006) reported that LC_{50} value of *H. bacteriophora* Hamyang strain was 5.5 IJs per 4th instar of the beet armyworm. This value is the same as LC_{50}s of *H. bacteriophora* WPS and HP88 strains (5.5 IJs per larva) and close to *H. bacteriophora* SMP strain (6.1 IJs per larva) in our study. On the other hand, it was quite different than LC_{50}s of other *H. bacteriophora* strains which ranged from 12.3 to 14.8 IJs per larva. These differences may be due to difference of the origins of the strains (Mannion and Jansson, 1992).

No statistical difference was obtained among nematode strains at 50 and 100 nematode concentrations. Therefore

10 and 25 nematode per larva were distinctive rates to differentiate the nematodes' biological efficacy on the beet armyworm. *H. megidis* LEX strain was superior and differed than others by having 100% mortality in all of concentrations and it was second to cause early mortality.

All the heterorhabditid strains tested showed high virulence to 4th instars of the beet armyworm, producing a significantly higher mortality (53.6-100%) at all concentrations than the untreated control at the final count. Apparently, they are effective bio-control agents of *S. exigua* and our results corroborate the finding of the study of Kim et al. (2006). However, environmental factors such as soil structure, temperature, humidity and host density under greenhouse and field conditions have huge impact on the efficacy of EPN (Koppenhöfer, 2000; Georgis et al, 2006). Therefore future studies need be directed to the greenhouse and field conditions with these heterorhabditid isolates.

Our results suggest that *H. megidis* LEX strain, *H. bacteriophora* WPS, SMP and HP88 strains, and *H. zealandica* EDS and CHR strains should be consider first to be studied further as potential biocontrol agents of the beet armyworm and other similar lepidopters. The others, *H. bacteriophora* PD, CFG, MF, Hb and CFM strains may also be valuable material to be studied.

ACKNOWLEDGEMENT

I thank Dr. David Boyd of Bob Jones University, South Carolina, USA for reviewing of the manuscript and Dr. Halil Kutuk of Adana Plant Protection Research Institute for helping in the statistical analysis.

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