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Full Length Research Paper

Comparison of efficacy of nine new heterorhabditid isolates (Rhabditida: Heterorhabditidae) in *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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The virulence of new nine heterorhabditid isolates from South Carolina (*Heterorhabditis megidis* LEX, *Heterorhabditis zealandica* EDS and CHR, and *Heterorhabditis bacteriophora* WPS, SMP, PD, CFG, MF and CFM strains) on the yellow mealworm 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 *Tenebrio molitor* larvae to the heterorhabditids at concentrations of 5, 10, 25, and 100 infective juveniles (IJs) per larva. Mortalities were counted for 4 days. At the final count, mortalities were 27.1-79.9, 48.7-85.2, 82-100, and 93.8-100% for all nematode strains at the concentrations of 5, 10, 25, and 100 IJs per larva, respectively. *H. megidis* LEX strain differed than others by having 100% mortality in both 25 and 100 nematode concentrations. It had also the highest mortality rate with 80% at 5 nematodes per larva and *H. bacteriophora* HP88, WPS and SMP strains followed it with 70.1, 70.1 and 64.6% mortality, respectively. LC_{50} value for the nematodes was relatively low ranging from 1.74 IJs per larva for *H. bacteriophora* WPS strain to 11.12 IJs per larva for *H. bacteriophora* Hb strain. The LT_{50} value ranged from 1.30 to 5.31 days. Our results suggest that *H. bacteriophora* WPS, SMP, and CFM, and *H. megidis* LEX strains may be considered first to be studied further as potential biocontrol agents of insects.

Key words: Biological control, entomopathogenic nematodes, *Heterorhabditis, Tenebrio molitor*, yellow mealworm.

INTRODUCTION

Entomopathogenic nematodes (EPNs) (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).

Despite the progress that has been made in the use of EPNs (Laznik et al., 2010a), knowledge about their natural host range and their efficacy on insect populations as biological control agents is still limited

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(Ansari et al., 2007). When new nematodes are isolated, studies are needed to determine their efficacy comparing them with other nematodes. Therefore, our goal was to compare the virulence of nine new heterorhabditid isolates from South Carolina with two known heterorhabditid nematodes (*Heterorhabditis bacteriophora* Hb and HP88 strains) in *T. molitor*, a susceptible host for in vivo mass production of entomopathogenic nematodes (Shapiro-Ilan et al., 2002; Shapiro-Ilan et al., 2008) under laboratory conditions.

Materials and Methods

Tenebrio molitor colony was sustained at 27 ± 2℃ and 50% RH on a wheat bran diet. Saturated polyacrylamide crystals were used as water source for adults and larvae by mixing them directly in the wheat bran. Fiberglass pans (35 × 25 × 20 cm, L × W × H) were used to rear adult beetles. The eggs glued to the bottom of the pan were recovered by replacing pans every 2 weeks. These pans with eggs were added fresh wheat bran for hatching larvae. Eggs and larvae were kept in the same pan until pupal stage approximately 3 months later. Wheat bran and water were added as needed. Pupae were separated from the diet by the use of a standard No. 6 sieve (3.35 mm openings) and allowed to complete development in 29 × 20 × 9 cm plastic boxes lined with tissue paper (Morales-Ramos et al., 2010).

H. bacteriophora Hb strain was obtained from Dr. David I. Shapiro-Ilan, 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).

EPNs 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 15 mm) with 15-20 ml of distilled water, was used to collect the infective juveniles (IJs). These IJs were stored at 7-8℃ 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 yellow mealworm larvae to heterorhabditids at concentrations of 5, 10, 25, and 100 IJs per larva in 1 ml of sterile distilled water (Woodring and Kaya, 1988; Shapiro-Ilan et al., 2008) Petri dishes (100 × 15 mm) were lined with two Whatman No.1 filter paper pieces (9 cm diameter). One hour 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 3rd instars of *T. molitor* 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℃ (Glazer et al., 1991). Controls consisted of 1 ml of sterile distilled water without nematodes. The bioassay was repeated two times.

T. molitor 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℃) and examin ed to confirm the presence of nematodes. The mortalities were converted to percentages and adjusted for control mortality, using Abbott's correction formula (Abbott, 1925). 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 5, 10, 25, and 100 nematode concentrations for each nematode strain were estimated by probit analysis (SPSS, 2003).

RESULTS

All nematodes tested were capable of killing the yellow mealworm 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. There was low mortality in untreated controls (Table 1 and 2).

Low mortality (<50%) occurred during the first day after treatment except 100 nematode concentrations of *H. zealandica* CHR and *H. megidis* LEX strains and *H. megidis* LEX strain was the best performer with the highest mortality at the first day count (Table 1). In general, mortality rates increased from day 2 to day 4 (Tables 1 and 2). On the second day; mortalities at 25 and 100 nematode rates reached usually over 70%. Hundred nematode concentrations of *H. bacteriophora* WPS strain and *H. megidis* LEX strain, and 25 and 100 nematode concentrations of *H. zealandica* CHR killed about 90% of larvae in the treatments (Table 1).

On the 3rd day count; only 100 nematode concentration of *H. zealandica* CHR caused 100% mortality. All nematode strains except *H. bacteriophora* Hb strain produced over 90% mortality at 100 nematode concentration. However they were not significantly different including *H. bacteriophora* Hb strain (Table 2). At 25 nematode concentration, all nematodes except *H. bacteriophora* Hb, SMP and MF strains gave more than 90% mortality but they were not significantly different except *H. bacteriophora* Hb strain. At 10 nematode rate; nematode strains produced usually more than 50% mortality. *H. megidis* LEX, *H. bacteriophora* SMP, and *H. zealandica* CHR strains performed better at the same group statistically with 81.01, 72.32, 71.73% mortality, respectively (Table 2). At 5 nematodes rate, *H. megidis* LEX, *H. bacteriophora* WPS and HP88 strains were not significantly different with 79.9, 56.3, and 58.3 mortality, respectively. *H. zealandica* CHR, *H. bacteriophora* SMP and CFM strains created the other group with 50.0, 47.9, and 45.8% mortality, respectively (Table 2).

At the final count, mortalities were 27.1-79.9, 48.7-85.2, 82-100, and 93.8-100% for all nematode strains at the concentrations of 5, 10, 25, and 100 IJs per larva, respectively. All larvae died in the treatments of *H. megidis* LEX, *H. zealandica* CHR and *H. bacteriophora* MF strains at 100 nematode rate. Mortalities were 97.9% for *H. zealandica* EDS, *H. bacteriophora* CFM and PD strains, 97.5% for *H. bacteriophora* WPS strain, 96.4% for *H. bacteriophora* SMP strain, 95.8% for *H. bacteriophora* HP88 and Hb strains, and 93.8% for

Table 1. Mean percent mortality of 3rd instars of the yellow mealworm after 1 and 2 d by heterorhabditid species/strains in a Petri-plate bioassay at 5, 10, 25, and 100 IJs per larva.

Nematodes[b]	Nematodes per sunn pest[a]							
	5	10	25	100	5	10	25	100
	1st day reading				2nd day reading			
H.b.HP88	7.44^{abcde}	8.93^{abcdef}	26.79^{defghi}	23.51b^{cdefghi}	46.43^{ghijklm}	46.43^{ghijklm}	78.27st	83.33st
H.b.Hb	0^{a}	0^{a}	3.57^{abc}	3.57^{abc}	10.72^{abc}	23.51^{abcdefg}	39.29^{efghijk}	65.18^{lmnoprs}
H.b.CFG	5.36^{abcd}	3.57^{abc}	21.43^{abcdefg}	25.3^{cdefghi}	10.72^{abc}	23.51^{abcdefg}	51.19^{jklmno}	68.15^{mnoprs}
H.b.PD	1.79^{ab}	0^{a}	3.57^{abc}	10.72^{abcdefg}	23.22^{abcdefg}	39.88^{efghijk}	76.19^{prst}	86.61st
H.b.CFM	1.79^{ab}	0^{a}	3.57^{abc}	10.72^{abcdefg}	25.00 ^{bcdefgh}	25.30^{bcdefgh}	63.10^{klmnopr}	76.19^{prst}
H.b.SMP	8.93^{abcdef}	14.29^{abcdefg}	21.43^{abcdefgh}	28.87^{efghi}	35.72^{defghij}	42.26^{efghijkl}	53.87^{jklmnop}	71.43^{oprs}
H.z.EDS	1.79^{ab}	1.79^{ab}	25^{cdefghi}	36.01^{hij}	21.43^{abcdefg}	19.35^{abcde}	87.20st	82.14st
H.b.WPS	1.79^{ab}	0^{a}	21.43^{abcdefgh}	42.86^{ijk}	42.86^{efghijkl}	39.58^{efghijk}	83.93st	96.13^{t}
H.b.MF	0^{a}	5.36^{abcd}	10.72^{abcdefg}	11.01^{abcdefg}	12.50^{abcd}	26.19^{bcdefghi}	56.55^{jklmnopr}	69.34^{noprs}
H.z.CHR	9.53^{abcdef}	8.93^{abcdef}	30.36^{fghi}	50^{jk}	50.00^{ijklmn}	49.11^{hijklmn}	96.43^{t}	98.21^{t}
H.m.LEX	17.86^{abcdefgh}	14.29^{abcdefg}	32.14g^{hij}	57.14^{k}	57.14^{cdefghij}	69.94^{ghijklmn}	84.82^{ghijklm}	89.88^{ghijklm}
Untreated	1.79^{ab}	5.36^{abcd}	0^{a}	1.79^{ab}	0^{a}	10.72^{abc}	3.57^{ab}	7.15^{ab}

[a]Means within the same columns followed by the same letters are not significantly different, [b]H.b.HP88: *Heterorhabditis bacteriophora* HP88, H.b.Hb: *H. bacteriophora* H.b, H.b.CFG: *H. bacteriophora* CFG, H.b.PD: *H. bacteriophora* PD, H.b.CFM: *H. bacteriophora* CFM, H.b.SMP: *H. bacteriophora* SMP, H.z.EDS: *H. zealandica* EDS, H.b.WPS: *H. bacteriophora* WPS, H.b.MF: *H. bacteriophora* MF, H.z.CHR: *H. zealandica* CHR, H.m.LEX: *H. megidis* LEX.

H. bacteriophora CFG strain (Table 2). However, they all are not significantly different.

At 25 nematode concentration; while only *H. megidis* LEX strain was producing 100% mortality, the others caused mortality over 90% except *H. bacteriophora* Hb strain. All treatments were at the same group statistically. *H. megidis* LEX, *H. zealandica* CHR, and *H. bacteriophora* SMP and HP88 strains were better with more than 80% mortality at 10 IJs per larva (Table 2). *H. megidis* LEX was superior with 80% mortality at 5 nematode per larva rate and it was followed by *H. bacteriophora* HP88, WPS and SMP with 70.1, 70.1 and 64.6% mortality, respectively at the same statistical group (Table 2).

The LC_{50} and LC_{90} data are summarized in Table 3. LC_{90} ranged from 12.30 to 54.12 IJs per larva. LC_{50} value for the nematodes was relatively low (<9 IJs per larva) except *H. bacteriophora* Hb strain. The lowest LC_{50} value was 1.74 IJs per larva for *H. bacteriophora* WPS strain followed by *H. bacteriophora* HP88 and SMP, *H. megidis* LEX, *H. bacteriophora* CFM, *H. zealandica* CHR, *H. bacteriophora* CFG and MF, *H. zealandica* EDS, *H. bacteriophora* PD and Hb strains with the LC_{50} of 2.66, 3.10, 4.23, 4.62, 4.73, 5.67, 6.44, 8.37, 8.40, 11.12 IJs per larva.

The LT_{50} and LT_{90} data are given in Table 4. The LT_{50} values ranged from 2.56 to 5.31 days for *H. megidis* LEX and *H. bacteriophora* Hb strains at 5 IJs per larva, from 2.04 to 3.41 days for *H. megidis* LEX and *H. zealandica* EDS strains at 10 IJs per larva, from 1.30 to 2.58 days for *H. zealandica* CHR and *H. bacteriophora* Hb strains at 25 IJs per larva, and from 1.09 to 1.95 days for *H. zealandica* CHR and *H. megidis* LEX, and *H. bacteriophora* Hb strains at 100 IJs per larva, respectively.

DISCUSSION

In determining an entomopathogenic nematode as a 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

Table 2. Mean percent mortality of 3rd instars of the yellow mealworm after 3 and 4 d by heterorhabditid species/strains in a Petri-plate bioassay at 5, 10, 25, and 100 IJs per larva.

Nematodes[b]	Nematodes per sunn pest[a]							
	5	10	25	100	5	10	25	100
	3rd day reading				4th day reading			
H.b.HP88	58.33 fghi	66.96 ghijkl	92.86 lmnop	92.56 lmnop	70.84 efghi	80.77 ghijkl	94.64 kl	95.71 kl
H.b.Hb	6.25 a	43.15 def	65.72 ghijk	87.14 klmnop	27.08 ab	60.66 defg	82.03 ghijkl	95.83 kl
H.b.CFG	20.84 abc	53.87 efgh	91.07 kmnop	93.33 mnop	33.33 bc	72.98 efghij	94.35 kl	93.75 kl
H.b.PD	22.92 abc	58.33 fghi	93.93 nop	97.92 op	35.42 bc	58.75 cdef	93.93 kl	97.92 kl
H.b.CFM	45.83 defg	45.83 defg	91.07 lmnop	93.33 mnop	54.17 cdef	65.89 defghi	96.13 kl	97.92 kl
H.b.SMP	47.92 defg	72.32 hijklm	86.79 klmnop	94.64 op	64.58 defgh	85.18 hijkl	90.36 ijkl	96.43 kl
H.z.EDS	29.17 bcd	36.91 cde	90.36 lmnop	98.21 op	41.67 bcd	48.69 bcde	96.13 kl	97.92 kl
H.b.WPS	56.25 efghi	59.52 fghij	94.64 op	97.92 op	70.83 efghi	68.81 efghi	96.43 kl	97.50 kl
H.b.MF	20.83 abc	65.48 ghijk	80 klmnop	96.43 op	41.67 bcd	78.39 fghijk	94.34 kl	100 l
H.z.CHR	50 efg	71.73 hijklm	98.21 op	100 p	56.25 cdef	81.96 ghijkl	98.21 kl	100 l
H.m.LEX	79.92 ijklmno	81.01 ijklmno	95.71 klmnop	95.71 klmnop	79.92 ghijkl	81.01 ghijkl	100 l	100 l
Untreated	14.29 ab	10.72 ab	7.14 a	10.72 ab	14.29 a	14.29 a	10.72 a	25 ab

[a]Means within the same columns followed by the same letters are not significantly different, [b]H.b.HP88: *Heterorhabditis bacteriophora* HP88, H.b.Hb: *H. bacteriophora* H.b, H.b.CFG: *H. bacteriophora* CFG, H.b.PD: *H. bacteriophora* PD, H.b.CFM: *H. bacteriophora* CFM, H.b.SMP: *H. bacteriophora* SMP, H.z.EDS: *H. zealandica* EDS, H.b.WPS: *H. bacteriophora* WPS, H.b.MF: *H. bacteriophora* MF, H.z.CHR: *H. zealandica* CHR, H.m.LEX: *H. megidis Lex.*

Table 3. LC_{50} and LC_{90} values of heterorhabditid nematodes for yellow mealworm larvae.

Nematodes	No. larvae	*LC_{50}	*LC_{90}	χ^2	*P*
H. bacteriophora CFG	56	5.67	54.12	74.00	0.001
H. bacteriophora CFM	56	4.62	31.52	6497.70	0.001
H. zealandica CHR	56	4.73	12.30	63.81	0.001
H. zealandica EDS	56	8.37	34.13	6967.40	0.001
H. bacteriophora Hb	56	11.12	40.64	333.70	0.001
H. bacteriophora HP88	56	2.66	29.58	2542.30	0.001
H. megidis LEX	56	4.23	37.64	166.10	0.001
H. bacteriophora MF	56	6.44	16.78	19.29	0.001
H. bacteriophora PD	56	8.40	34.22	6563.30	0.001
H. bacteriophora SMP	56	3.10	43.81	88.47	0.001
H. bacteriophora WPS	56	1.74	30.48	3065.60	0.001

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

Table 4. LT_{50} and LT_{90} values of heterorhabditid nematodes at 5, 10, 25, and 100 infective juveniles per larva for yellow mealworm larvae.

Nem[a]	No. larvae	5 infective juveniles				10 infective juveniles				25 infective juveniles				100 infective juveniles			
		*LT_{50}	LT_{90}	χ^2	P	*LT_{50}	*LT_{90}	χ^2	P	*LT_{50}	*LT_{90}	χ^2	P	*LT_{50}	*LT_{90}	χ^2	P
CFG	56	4.14	6.48	2.18	0.585	2.81	4.54	2.59	0.460	1.95	3.28	4.66	0.198	1.66	2.88	10.63	0.014
CFM	56	3.86	6.72	15.29	0.002	3.04	4.71	6.19	0.103	1.83	3.01	6.91	0.075	1.67	2.64	18.43	0.001
CHR	56	3.66	7.16	39.89	0.001	2.30	3.83	11.72	0.008	1.30	2.18	423.38	0.001	1.09	1.74	2.01	0.570
EDS	56	4.33	7.07	12.24	0.007	3.41	5.42	8.49	0.037	1.55	2.74	27.03	0.001	1.35	2.37	42.55	0.001
Hb	56	5.31	8.04	4.34	0.227	3.16	4.89	9.99	0.019	2.58	4.04	7.288	0.063	1.95	2.90	13.07	0.004
HP88	56	2.69	4.60	12.18	0.007	2.37	4.04	8.02	0.046	1.61	2.89	21.70	0.000	1.56	2.73	24.65	0.001
LEX	56	2.56	4.93	19.04	0.001	2.04	3.72	25.58	0.001	1.40	2.36	8.54	0.036	1.09	2.04	18.95	0.001
MF	56	3.84	5.63	3.08	0.380	2.63	4.12	4.91	0.178	2.07	3.33	6.47	0.091	1.71	2.52	1.19	0.756
PD	56	3.94	6.27	7.19	0.066	2.88	4.80	22.99	0.001	1.86	2.89	51.69	0.001	1.59	2.40	262.36	0.001
SMP	56	2.93	4.88	5.24	0.155	2.24	3.78	3.86	0.277	1.98	3.42	8.67	0.034	1.55	2.75	17.25	0.001
WPS	56	2.74	4.43	11.73	0.008	2.76	4.41	19.28	0.001	1.58	2.68	33.31	0.001	1.18	2.11	358.50	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 d counts and expressed in days.

al., 2011). The virulence of nematodes to insects varies significantly. (Mbata and Shapiro-Ilan, 2005; Mederios et al., 2000). 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 (27.1-100%) in the current study. Although the yellow mealworm larva was susceptible to each nematode species and strain tested, there were differences among these nematodes in their ability to kill the insect. *H. bacteriophora* WPS, HP88, SMP, *H. megidis* LEX, *H. bacteriophora* CFM, *H. zealandica* CHR strains were more efficacious than others against *T. molitor* larva as it was reflected in the LC_{50}, LT_{50} and percent mortality data. The mortalities were higher, LC_{50} values were lower and LT_{50} values were shorter for these nematodes. These differences may be due to difference of the origins of the strains (Mannion and Jansson, 1992).

No statistical difference obtained among nematode strains at 25 and 100 nematode concentrations. Therefore 5 and 10 nematode per larva were distinctive rates to differentiate the nematodes' biological efficacy on the yellow mealworm. All the heterorhabditid strains tested showed virulence to 3rd instars of the yellow mealworm, producing a significantly higher mortality (27.1-100%) at all concentrations than the untreated control at the final count. Apparently, they are effective bio-control agents of insects. However, environmental factors such as soil structure, temperature, humidity and host density under greenhouse and field conditions have huge impact on the efficacy of EPNs (Koppenhöfer, 2000; Georgis et al., 2006). Therefore future studies need to be directed to the greenhouse and field conditions with these heterorhabditid isolates on various insect pests.

Our results suggest that *H. bacteriophora* WPS, SMP, and CFM, *H. megidis* LEX, and *H. zealandica* CHR strains should be considered first to be studied further as potential biocontrol agents of insects. The others may also be valuable material to be studied.

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