EFFICACY OF SOME GEOGRAPHICAL ISOLATES OF ENTOMOPATHOGENIC NEMATODES AGAINST *LEPTINOTARSA DECEMLINEATA* (SAY) (COL.: CHRYSOMELIDAE)

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ABSTRACT: Entomopathogenic nematodes (EPNs) are used to control several agriculturally important insect pests of the different orders. In this study the ability of four geographical isolate of *Heterorhabditis bacteriophora* and three species of *Steinernema* include *S. bicornutum*, *S. carpocapsae* and *S. feltiae* were investigated for control of Colorado potato beetle in laboratory conditions at 25±2 ºC and photoperiod of 12:12 (L:D). The efficacy of EPNs was tested at five concentrations including 100, 200, 400, 500 and 1000 infective juvenile (IJs) per individual with three methods, filter paper assay, leaf assay and soil assay at four exposure times. In filter paper assay and leaf assay methods, *H. bacteriophora* IRA10 had the highest toxicity and *S. bicornutum IRA7* was the lowest one. There are no significant differences between strains at lowest concentration in all exposure times. In soil assay method, *H. bacteriophora* IRA12 had the highest mortality percentage and *S. bicornutum* IRA7 was the lowest one. Our study clearly shows that both species and geographical isolates of same species of etomopathogenic nematodes may have significantly different virulence against specific pest target. Nevertheless these results it is difficult to predict which species/isolate might be the most effective biological control agent for suppression of *L. decemlineata* in field conditions but at least we can expect that the most effective EPN among studied isolates might be *H. bacteriophora* IRA10.

KEY WORDS: Entomopathogenic nematods, geographical isolates, *Heterorhabditis, Steinernema, Leptinotarsa decemlineata.*

The Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata* (Say) is the most economically damaging pest to potatoes in most areas of the Iran. If potato field left uncontrolled, CPB can completely defoliate it. Although the potato is its favorite food, the beetle may also feed on tomato, eggplant, tobacco, pepper, ground cherry, petunia, and even cabbage crops. It also attacks a number of common weeds including jimson weed, henbane, horse nettle, belladonna, thistle, and mullein (Metcalf & Metcalf, 1993). The intensive use of insecticides against *L. decemlineata* has led to the appearance of pesticide resistance in Iran (Mohammadi et al., 2007) and other parts of world (Pap et al., 1997). Therefore with the aim of reducing chemical use and preventing pesticides resistance phenomenon, new strategies have developed in some regions.

Entomopathogenic nematodes (EPNs) are used to control several agriculturally important insect pests of the different orders. There are several species of EPNs used around the world against a variety of pests. Some of the important EPN species belonging to *Steinernema* and *Heterorhabditis* that they are obligate pathogens and are characterized by their association with symbiotic bacteria, carried in the digestive tract; *Xenorhabdus* in steinernematids and

Photorhabdus in heterorhabditids (Boemare et al., 1996). Infective juveniles invade the host through body openings or the cuticle and release symbiotic bacteria that induce septicemia and kill the host. The nematodes then develop and reproduce in the host. They are soil-inhabiting parasites and effective against a wide range of insects (Begley, et al., 1990 and Klein, et al., 1990). At first they were mostly known as the effective agents towards soil pests, but in recent years many investigations have demonstrated that they can also be effectively used against foliar pests (Arthurs et al., 2004). Developments in production of these biocontrol agents through liquid fermentation (Georgis, 1990), expansion of the number of invivo producers and the exemption from registration requirements in most countries (Gaugler, 1988) have favored their commercial development (Georgis, 1992). With the aim of determining virulence of different species and isolates, we investigated the ability of EPNs for control of Colorado potato beetle in laboratory conditions.

MATERIALS AND METHODS

Nematode sources

Four geographical isolate of *Heterorhabditis bacteriophora* and three species of *Steinernema* include *S. bicornutum*, *S. carpocapsae* and *S. feltiae* were prepared from Insect Pathology Lab. in Azarbaijan University of Tarbiat Moallem-IRAN.

Insect source

First instar larvae of *L. decemlineata* were collected from the potato shrub that left in wheat field vicinity to potato field (In that region potato altered with wheat) and they were reared on fresh potato leaves in Laboratory condition.

Filter paper assay

The efficacy of EPNs was tested at five concentrations including 100, 200, 400, 500 and 1000 infective juvenile (IJs) per individual into 5 ml of water per Petri dish. Ten last instar CPB larvae were put in each Petri dish (9 cm in diameter) lined with 2 layer of filter paper disks. The control was treated only with 5 ml of water. The Petri dishes were put in rearing chamber at 25±2 ºC and photoperiod of 12:12 (L:D). Each treatment was done in 5 replicates. The mortality rates were recorded after 48, 72, 96 and 120 h. of exposure.

Leaf assay

Leaf assay was done with same conditions except we used EPNs suspensions on potato leaf instead of filter paper. In each Petri dish (14 cm in diameter) 10 potato leaves were placed and sprayed with 10 ml of the EPNs suspensions. Twenty last instar CPB larvae were put in each Petri dish lined with potato leaves. The control was treated only with 10 ml of water. After two days, remnants of treated leaves were changed with fresh ones (and EPN-free). The number of dead larvae was determined from the second day with 24 h. intervals for 4 days. The mortality rates were recorded after 48, 72, 96 and 120 h. of exposure.

Soil assay

After completing larval development, CPB larvae enter to pre-pupa stage that migrate to the soil and form pupa. In order to study EPNs effects on CPB in this period, for each replicate, the plastic containers (8 cm in height and 3 cm in diameter) were used. The soil with sandy-loam texture was autoclaved, mixed thoroughly with 10 ml of each concentration of EPNs and poured in the plastic container. The control was treated only with 10 ml of water. Twenty CPB pre-pupa were transferred to each replicate and they were kept until mature insects were

appeared in control. The containers were put in rearing chamber at 25±2 ºC and photoperiod of 12:12 (L: D). Each treatment was done in 5 replicates.

Statistical analysis

Data were submitted to analysis of variance and the means were compared by the Tukey test, using SPSS 14.0 software program (SPSS, 2004). The data were transformed into √(x+0.5) before statistical analysis as necessary.

RESULTS

Filter paper assay

The effects of seven strains of entomopathogenic nematodes on CPB last instar larvae at different exposures time are shown in tables 1 to 4. There are no significant difference between concentrations at 48 h. exposures time except in *H. bacteriophora* IRA10, *H. bacteriophora* IRA12 and *S. carpocapsae* IRA18. There are significant differences between concentrations at 72, 96 and 120 h. exposures times. Mortality was increasing by concentration increase and there is positive correlation between concentration and mortality. The trend of toxicity of different strains in highest concentration in 48 h. expouser time was *H. bacteriophora* IRA10 > *S. carpocapsae* IRA18 = *H. bacteriophora* IRA12 > *S. feltiae* IRA22= *H. bacteriophora* IRA4= *H. bacteriophora* IRA3 > *S. bicornutum* IRA7. Also, The trend of toxicity of different strains in highest concentration in 120 h. expouser time was *H. bacteriophora* IRA10 > *S. carpocapsae* IRA18 = *H. bacteriophora* IRA12> *S. feltiae* IRA22= *S. carpocapsae* IRA18= *H. bacteriophora* IRA4> *S. bicornutum* IRA7. These results indicated that H. bacteriophora IRA10 had the highest toxicity and S. bicornutum IRA7 was the lowest one. There are no significant differences between strains at lowest concentration in all exposure times.

Leaf assay

The effects of studied entomopathogenic nematodes on CPB in larva - leaf treatment at different exposures time are shown in tables 5 to 8. There are no significant difference between concentrations at 48 h. exposures time except in *H. bacteriophora IRA10* and *S. feltiae IRA22* that mortality was increasing by concentration increase. Also there are no significant difference between nematode isolates at 48 h. exposures time, except in concentrations 4 and 5, that highest mortality is related to *H. bacteriophora IRA10* and *S. feltiae IRA22* isolates. There are significant differences between concentrations at 72, 96 and 120 h. exposures times. Mortality was increasing by concentration increase and there is positive correlation between concentration and mortality. The trend of toxicity of different isolates in highest concentration in 48 h. espouser time was *H. bacteriophora IRA10* > *S. feltiae IRA22*> *S. carpocapsae IRA18*= *H. bacteriophora IRA12* = *H. bacteriophora IRA4*= *H. bacteriophora IRA3*= *S. bicornutum IRA7*. Also, The trend of toxicity of different isolates in highest concentration in 120 h. exposure time was *H. bacteriophora IRA10* > *H. bacteriophora IRA3*= *S. feltiae IRA22*= *H. bacteriophora IRA12*> *S. carpocapsae IRA18*= *H. bacteriophora IRA4*= *S. bicornutum IRA7*. These results indicated that *H. bacteriophora IRA10* had the highest mortality percentage and *S. bicornutum IRA7* was the lowest one. There are no significant differences between isolates at lowest concentration in all exposure times.

Soil assay

The effects of seven isolates of entomopathogenic nematodes on CPB in pre-pupa – soil treatment at different exposures time are shown in tables 9 to 12. There are no significant difference between concentrations at 48 h. exposures time except in *H. bacteriophora* IRA3 *and H. bacteriophora* IRA12. There are significant differences between concentrations at 72, 96 and 120 h. exposures times. Mortality was increasing by concentration increase and there is positive correlation between concentration and mortality. The trend of toxicity of different isolates in highest concentration in 48 h. expouser time was *H. bacteriophora* IRA10 > *H. bacteriophora* IRA3 > *H. bacteriophora* IRA12 > *S. feltiae IRA22 = S. carpocapsae* IRA18 > *H. bacteriophora* IRA4 > *S. bicornutum* IRA7. Also, The trend of toxicity of different isolates in highest concentration in 120 h. expouser time was *H. bacteriophora* IRA10 > *H. bacteriophora* IRA12 = *H. bacteriophora* IRA3 = *S. feltiae* IRA22> *S. carpocapsae* IRA18> *H. bacteriophora* IRA4 > *S. bicornutum* IRA7. These results indicated that *H. bacteriophora* IRA12 had the highest mortality percentage and *S. bicornutum* IRA7 was the lowest one.

DISCUSSION

Laboratory screening of entomopathogenic nematodes for various beneficial traits has been used to identify superior candidates for insect suppression and has reduced the number of strains or species that need to be tested in the field (Mannion & Jansson, 1992; Patterson Stark & Lacey, 1999; Shapiro & McCoy, 2000; Shapiro et al., 2003). Our study clearly shows that both species and geographical isolates of same species of etomopathogenic nematodes may have significantly different virulence against specific pest target.

Different levels of susceptibility of CPB to different isolates of one species have been previously reported too (Wright et al., 1987). Understanding the underlying mechanisms caused these differences is necessary in using EPNs as biological agents because with such information we can select and maintain desired traits in organisms. Four *H. bacteriophora* isolates that used in this study belonged to different geographical regions with more or less different conditions. *Heterorhabditis bacteriophora* IRA10 was isolated from orchard but others from alfalfa field or grasslands (Eivazian et al., 2009). These differences can affect isolate in two ways. First under specific abiotic and biotic factors that govern isolate niche natural selection affects population so that some specific morphological and behavioral novelty may be appears in population. At least such morphological differences among isolates of a species were reported for *S. rarum* (Nguyen, 2006).

The virulence of *H. bacteriophora* IRA10 against CPB larvae and pupa was remarkable and had significant difference with other isolates and species. *Heterorhabditis bacteriophora* IRA12, *H. bacteriophora* IRA3 and *S. feltiae* caused similar mortality in both larvae and pre-pupa. *Heterorhabditis bacteriophora* IRA4 and *S. bicornutum* IRA7 caused minimum mortality and therefore don't recommended against CPB. *Steinernema feltiae* is cold adapted species; therefore the low virulance could be attributing the relatively high temperature (25±2). In the case of *S. bicornutum* our preliminary study showed that this isolate is cold adapted and low temperature needed (20±2) for successful invivo production (Unpublished data). *Steinernema carpocapsae* IRA18 caused moderate mortality.

The one possible reason for high virulence of *H. bacteriophora* IRA10 compared to other studied species against *L. decemlineata* may result from extra IJs sheet. In *Heterorhabditis* spp. the second-stage cuticle plays an important role in resist against encapsulation (Dowds et al., 2002). Studies on *T. oleracea* suggest that *Heterorhabditis* spp. avoid non-self recognition by slipping off the second juvenile stage cuticle (J2-cuticle) just before or after entering haemocoel (Peters et al., 1997).

In most dissected cadaver, we see dead nematodes in different number and apparently this was due to encapsulation or other insect defense, however they caused mortality and then they were able to inoculate haemocoel before dead. In *Leptinotarsa decemlineata* larvae a maximum of 21 encapsulated *S. carpocapsae* were found but at least one nematode escaped encapsulation when more than nine nematodes had invade (Thurston et al., 1994).

Nevertheless these results it is difficult to predict which species/isolate might be the most effective biological control agent for suppression of *L. decemlineata* in field conditions but at least we can expect that the most effective EPN control (among studied isolates) might be *H. bacteriophora* IRA10 in warm condition.

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Table 1. The effects of seven isolates of entomopathogenic nematodes on CPB last instar larvae at filter paper assay at 48 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 a A	0 c A	0 b A	0 c A
H. bacteriophora IRA4	0 a A	0 a A	0 bc A	6 ± 2.91 b A	7 ± 3.74 bc A
H. bacteriophora IRA3	0 a A	4 ± 2.44 a A	0 bc A	0 b A	5 ± 2.23 bc A
H. bacteriophora IRA12	0 a B	0 a B	5 ± 2.23 abc AB	8 ± 2.54 ab AB	15 ± 4.47 b A
H. bacteriophora IRA10	0 a C	5 ± 2.23 a C	9 ± 2.91 ab BC	19 ± 4 a AB	29 ± 4.3 a A
S. bicornutum IRA7	0 a A	0 a A	0 bc A	0 b A	0 bc A
S. carpocapsae IRA18	0 a B	0 a B	11 ± 2.44 a A	10 ± 2.73 ab A	9 ± 1.87 bc AB
S. feltiae IRA22	0 a A	0 a A	4 ± 1.58 abc A	5± 2.73 b A	8 ± 2.54 bc A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 2. The effects of seven isolates of entomopathogenic nematodes on CPB last instar larvae at filter paper assay at 72 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 c A	0 d A	0 d A	0 e A
H. bacteriophora IRA4	0 a B	0 c B	0 cd B	13 ± 1.22 bc A	15 ± 2.23 cd A
H. bacteriophora IRA3	0 a B	8 ± 1.22 a AB	5 ± 1.87 cd AB	10 ± 1.58 bcd A	9 ± 1 de A
H. bacteriophora IRA12	0 a C	5 ± 1.58 abc BC	12 ± 3.39 abc BC	16 ± 1.87 b B	32 ± 4.63 b A
H. bacteriophora IRA10	0 a C	6 ± 1.87 ab C	23 ± 2.54 a B	29 ± 6 a B	51 ± 4.3 a A
S. bicornutum IRA7	0 a C	0 bc C	5 ± 1.58 cd AB	0 cd AB	8 ± 2.54 de A
S. carpocapsae IRA18	4 ± 1.87a B	4 ± 1.87 abc B	17 ± 2.54 ab A	16 ± 2.91 b A	24 ± 3.31 bc A
S. feltiae IRA22	0 a B	0 bc B	8 ± 3.39.22 bcd B	6 ± 2.44 bcd B	19 ± 1.87 bc A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 3. The effects of seven isolates of entomopathogenic nematodes on CPB last instar larvae at filter paper assay at 96 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 b A	0 d A	0 e A	0 e A
H. bacteriophora IRA4	0 a B	5 ± 1.58 ab B	6 ± 1.87 cd B	17 ± 1 .22 bc A	18 ± 2 cd A
H. bacteriophora IRA3	0 a B	9 ± 1.87 a A	9 ± 1 bc A	10 ± 1.58 cd A	9 ± 1 de A
H. bacteriophora IRA12	4± 1.87 a C	5 ± 1.58 ab C	18 ± 2.54 ab B	19 ± 1.87 b B	36 ± 3.67 b A
H. bacteriophora IRA10	5 ± 2.23 a C	7 ± 2 ab C	27 ± 2.54 a B	38 ± 2.54 a B	60 ± 4.18 a A
S. bicornutum IRA7	0 a C	0 b C	5 ± 1.58 cd AB	4 ± 1 de AB	9 ± 1.87 de A
S. carpocapsae IRA18	5 ± 2.23 a C	5 ± 1.58 ab C	20 ± 1.58 a B	19 ± 1.87 b B	33 ± 2.54 b A
S. feltiae IRA22	0 a C	0 b C	10 ± 3.53 bc B	9± 1.87 de B	25 ± 2.23 bc A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 4. The effects of seven isolates of entomopathogenic nematodes on CPB last instar larvae at filter paper assay at 120 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 c A	0 c A	0 f A	0 e A
H. bacteriophora IRA4	0 a B	5 ± 2.23 abc B	6 ± 1.87 c B	18 ± 1 .22 bcd A	19 ± 1 cd A
H. bacteriophora IRA3	0 a B	9 ± 1.78 a A	10 ± 1.58 bc A	11 ± 1.87 cde A	10 ± 0.00 de A
H. bacteriophora IRA12	4± 1.87a C	5 ± 1.58 abc C	19 ± 1.87 b B	19 ± 1.87 bc B	38 ± 3.74 b A
H. bacteriophora IRA10	5 ± 2.23 a C	8 ± 2.54 ab C	30 ± 3.53 a B	39 ± 2.91 a B	60 ± 4.18 a A
S. bicornutum IRA7	0 a C	0 bc C	5 ± 1.58 c AB	4 ± 1 ef AB	9 ± 1.87 de A
S. carpocapsae IRA18	5 ± 2.23 a C	5 ± 1.58 abc C	20 ± 1.58 ab B	22 ± 2.54 b B	33 ± 2.54 b A
S. feltiae IRA22	0 a C	0 bc C	10 ± 3.53 bc B	10± 1.58 de B	27 ± 2 bc A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 5. The effects of seven isolates of entomopathogenic nematodes on CPB in larva - leaf treatment at 48 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 b A	0 a A	0 b A	0 c A
H. bacteriophora IRA4	0 a A	0 b A	0 a A	0 b A	0 c A
H. bacteriophora IRA3	0 a A	0 ab A	0 a A	0 b A	0 c A
H. bacteriophora IRA12	0 a A	0 a A	0 a A	0 b A	0 c A
H. bacteriophora IRA10	0 a C	0 ab C	7 ± 3 a BC	19 ± 5.33 a B	42 ± 3.74 a A
S. bicornutum IRA7	0 a A	0 b A	0 a A	0 b A	0 c A
S. carpocapsae IRA18	0 a A	0 b A	0 a A	0 b A	0 c A
S. feltiae IRA22	5 ± 3.16 a B	5 ± 3.87 b B	0 a B	6 ± 2.91 b B	22 ± 3.39 b A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 6. The effects of seven isolates of entomopathogenic nematodes on CPB in larva - leaf treatment at 72 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 a A	0 b A	0 d A	0 d A
H. bacteriophora IRA4	0 a A	0 a A	8 ± 2.54 b A	5 ± 1.58 cd A	0 d A
H. bacteriophora IRA3	0 a B	5 ± 2.23 a B	9 ± 3.31 b B	6 ± 3.67 cd B	27 ± 2.54 bc A
H. bacteriophora IRA12	0 a C	0 a C	0 b C	23 ± 3.39 b B	35 ± 3.53 b A
H. bacteriophora IRA10	0 a D	9 ± 4 a D	25 ± 3.53 a C	46 ± 3.31 a B	70 ± 2.73 a A
S. bicornutum IRA7	0 a B	0 a B	0 b B	0 d B	7 ± 2.54 d A
S. carpocapsae IRA18	0 a BC	0 a BC	0 b C	14 ± 1.87 bc A	12 ± 5.14 cd AB
S. feltiae IRA22	6 ± 2.91 a B	9 ± 3.31 a B	9 ± 2.91 b B	26± 3.67 b B	35 ± 6.12 b A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 7. The effects of seven isolates of entomopathogenic nematodes on CPB in larva - leaf treatment at 96 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 b A	0 d A	0 d A	0 d A
H. bacteriophora IRA4	0 a B	0 b B	12 ± 1.22 bc A	6 ± 1.87 cd AB	7 ± 2 d AB
H. bacteriophora IRA3	0 a B	12 ± 3.39 ab B	15 ± 3.53 b B	12 ± 3.39 cd B	38 ± 3.39 b A
H. bacteriophora IRA12	0 a B	6 ± 4 b B	0 cd B	29 ± 5.09 b A	41 ± 4 b A
H. bacteriophora IRA10	0 a E	19 ± 1.87 a D	34 ± 1.87 a C	53 ± 1.22 a B	79 ± 2.91 a A
S. bicornutum IRA7	0 a B	0 b B	0 cd B	5 ± 3.16 cd B	15 ± 3.53 cd A
S. carpocapsae IRA18	4 ± 1.87 a C	4 ± 2.44 b C	5 ± 2.23 cd C	18 ± 2 bc B	28 ± 2 bc A
S. feltiae IRA22	7 ± 3.74 a B	9 ± 3.31 ab B	12 ± 2.54 bc B	29± 2.91 b A	37 ± 6.04 b A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 8. The effects of seven isolates of entomopathogenic nematodes on CPB larva - leaf treatment at 120 h. exposure time.

Teatment	Concentrate				
	1	2	3	4	5
control	0 a A	0 c A	0 d A	0 d A	0 e A
H. bacteriophora IRA4	0 a C	0 c C	14 ± 1 bc AB	8 ± 2 cd BC	15 ± 1.58 de A
H. bacteriophora IRA3	4 ± 1.87 a C	18 ± 1.22 ab B	20 ± 1.58 b B	24 ± 3.67 bc B	43 ± 1.22 b A
H. bacteriophora IRA12	0 a B	7 ± 3.74 c B	7 ± 3 cd B	32 ± 4.06 b A	44 ± 3.67b A
H. bacteriophora IRA10	7 ± 2.54 a E	24 ± 1.87 a D	36 ± 1.87 a C	58 ± 1.22 a B	83 ± 3.39 a A
S. bicornutum IRA7	0 a B	7 ± 1.22 c B	8 ± 1.22 cd AB	6 ± 2.91 cd B	17 ± 3.39 d A
S. carpocapsae IRA18	6 ± 1.87 a C	7 ± 3 c BC	7 ± 2.54 cd BC	18 ± 1 cd B	28 ± 2 cd A
S. feltiae IRA22	9 ± 4 a C	9 ± 3.31 bc C	13 ± 2.54 bc BC	29± 2.91 b AB	37 ± 6.04 bc A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 9. The effects of seven isolates of entomopathogenic nematodes on CPB in pre-pupa – soil treatment at 48 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 b A	0 b A	0 c A	0 b A	0 c A
H. bacteriophora IRA4	0 b A	0 b A	0 c A	0 b A	0 c A
H. bacteriophora IRA3	4 ± 1.87 ab B	5 ± 2.73 ab B	8 ± 2.54 bc AB	19 ± 4 a A	18 ± 2.54 ab A
H. bacteriophora IRA10	11± 2.44 a AB	9 ± 2.91 a B	26 ± 5.78 a A	22 ± 4.33 a AB	24 ± 3.31 a AB
H. bacteriophora IRA12	5 ± 2.73 ab A	0 ab A	7 ± 3.74 bc A	19 ± 4.47 a A	15 ± 1.93 abc A
S. bicornutum IRA7	0 b A	0 b A	0 b A	0 b A	0 c A
S. carpocapsae IRA18	0 b A	0 b A	0 bc A	5 ± 2.23 b A	8 ± 2.54 bcd A
S. feltiae IRA22	0 b A	0 b A	13 ± 1.22 b A	11± 2.44 ab A	9 ± 2.91 bcd A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 10. The effects of seven isolates of entomopathogenic nematodes on CPB in pre-pupa – soil treatment at 72 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 b A	0 b A	0 c A	0 c A	0 e A
H. bacteriophora IRA4	0 b A	0 b A	0 c AB	0 c AB	8 ± 2.5 de A
H. bacteriophora IRA3	8 ± 3.39 b BC	6 ± 2.44 b C	19 ± 1.87 b BC	29 ± 6 b AB	38 ± 2.54 b A
H. bacteriophora IRA10	17± 2.54 a B	23 ± 2.54 a B	51 ± 4.84 a A	55 ± 6.89 a A	71 ± 5.78 a A
H. bacteriophora IRA12	6 ± 2.44 b C	0 b C	15 ± 2.23 b BC	29 ± 6 b AB	32 ± 4.63 bc A
S. bicornutum IRA7	0 b A	0 b A	0 c A	4 ± 1.87 c A	0 e A
S. carpocapsae IRA18	0 b B	4 ± 1.87 b B	0 c B	6 ± 2.44 c B	19 ± 1.87 cd A
S. feltiae IRA22	4 ± 1.87 b B	0 b B	17 ± 1.22 b A	17± 2.54 bc A	23 ± 2.54 c A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 11. The effects of seven isolates of entomopathogenic nematodes on CPB in pre-pupa – soil treatment at 96 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 c A	0 c A	0 c A	0 d A	0 e A
H. bacteriophora IRA4	0 c B	0 c B	4 ± 1.8 c AB	5 ± 2 .23 d AB	9 ± 1.87 e A
H. bacteriophora IRA3	10 ± 3.53 b D	9 ± 1.87 b D	25 ± 2.23 b C	38 ± 2.54 b B	54 ± 2.91 b A
H. bacteriophora IRA10	20± 1.58 a D	27 ± 2.54 a D	62 ± 2.54 a C	78 ± 2.54 a B	90 ± 3.16 a A
H. bacteriophora IRA12	9 ± 1.87 bc C	6 ± 1.87 bc C	18 ± 2 b B	42 ± 1.22 b A	43 ± 2 c A
S. bicornutum IRA7	0 c AB	0 c B	0 c B	5 ± 2.23 d AB	6 ± 1.87 e A
S. carpocapsae IRA18	0 c B	5 ± 2.23 bc B	6 ± 1.87 c B	7 ± 2 d B	25 ± 2.23 d A
S. feltiae IRA22	5 ± 2.23 bc C	5 ± 1.58 bc C	18 ± 1.22 b B	20± 1.58 c AB	27 ± 2.54 d A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).

Table 12. The effects of seven isolates of entomopathogenic nematodes on CPB in pre-pupa – soil treatment at 120 h. exposure time.

Treatment	Concentrate				
	1	2	3	4	5
control	0 bc A	0 c A	0 d A	0 d A	0 d A
H. bacteriophora IRA4	0 c B	0 c B	4 ± 1.87 d AB	5 ± 2 .23 d AB	9 ± 1.87 cd A
H. bacteriophora IRA3	10 ± 3.53 b B	10 ± 1.58 b B	27 ± 2 b AB	39 ± 2.91 b A	42 ± 2.79 b A
H. bacteriophora IRA10	20± 1.58 a D	30 ± 3.53 a D	62 ± 2.54 a C	80 ± 2.23 a B	93 ± 2 a A
H. bacteriophora IRA12	10 ± 1.5 b C	6 ± 1.87 bc C	19 ± 1 bcB	44 ± 2.23 b A	44 ± 1.87 b A
S. bicornutum IRA7	0 bc A	0 c A	0 d A	5 ± 2.23 d A	6 ± 1.87 d A
S. carpocapsae IRA18	5 ± 1.58 bc B	5 ± 2.23 bc B	6 ± 1.87 d B	8 ± 2 d B	27 ± 2 bc A
S. feltiae IRA22	5 ± 1.23 bc C	9 ± 2.91 bc BC	18 ± 2.54 c B	20± 1.54 c AB	30 ± 3.53 b A

There are no significant difference between means with similar words (large words in each row and small words in each column) (Tokey test α=0.01).