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Effect of ethyl acetate on vitality and virulence of entomopathogenic nematode species

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Abstract:

Entomopathogenic nematodes (EPNs) are the most important agents used in classical and augmentative biological control. The aim of this research work is to study, the effect of different concentrations of ethyl acetate (EA) on survival and virulence of two Egyptian isolates of the EPNs, Heterorhabditis indica Poinar, Karunakar and David (Rhabditida: Heterorhabditidae) and Steinernema carpocapsae (Weiser) (Rhabditida: Steinernematidae) in laboratory assays. EA showed a nematicidal effect against both species at a concentration higher than 0.1%. Infective juveniles (IJs) of H. indica were much more sensitive to the lethal effect of EA than S. carpocapsae. The medium lethal concentration (LC50) of EA for H. indica treated for 24 hrs. was nearly four times less than that of S. carpocapsae. In contrary, EA at low concentrations increased EPNs virulence by enhancing the ability of IJs to penetrated and kill the host and by increasing the proportion of both actively moving IJs in H. Indica and jumper "sprinter" IJs in S. carpocapsae. It is suggested that the lethal effect of EA on Entomopathogenic nematode (EPN) could be related to the damage of the nematode sensory apparatus due to its neurotropic effects. The activation effect of lower concentration on EPNs could be due to their stimulation to nervous receptors in the amphidial channel and channel un-blockage which increase the sensorimotor reactivity of nematode. The overall results suggested that EA at very low concentration is a promising candidate for EPNs activation prior field application.

Introduction

The entomopathogenic nematodes (EPNs) that belong to the families; Steinernematidae and Heterorhabditidae, have been used for biological control of many agricultural insect pests as a safe alternative to chemical insecticides. The third stage infective juveniles (IJs) of these nematodes can penetrate and kill their hosts within 24-48

hours in the laboratory (Poinar, 1986). However, in the field, they expose to various environmental extremes such as low humidity, solar radiation, plant- and agro-chemicals, which may limit their survival and field efficacy both in the soil and on plant surface (Ishibashi and Takii, 1993). Accordingly, finding environmentally safe activators that increase nematode efficacy and do not adversely affect nematodes vitality could be of great importance. Of the explored activators, juice from kale and aloe plant can stimulate nematode activity (Ishibashi, 1987), the insecticide carbamate oxamyl stimulated the locomotor movement of the IJs (Gaugler and Campbell, 1991), the pyrimidine fungicide nuarimol showed a beneficial effect on nematode vitality and movement (Gordon *et al.,* 1996) and the chlorine-based bleach sodium hypochlorite has been mentioned to activated the nematodes (Dempsey and Griffin, 2003). However, most of the studied chemical activators either environmentally unsafe, human-toxic pesticides or proved to be ineffective in the field (Ishibashi and Takii, 1993). Abd Elrahman and Abd Elrahman (2005) showed that treatment of *Heterorhabditis indica* Poinar, Karunakar and David (Rhabditida: Heterorhabditidae) IJs with a very low concentration of ethyl acetate stimulate nematode activity without any adverse effect in nematode survival. In contrary, Monzer and Al-Elimi (2002) and Monzer and Abd Elrahman (2003) mentioned that ethyl acetate has toxic effect against *H. indica*. However, the above-mentioned research did not provide detailed data on the effect of various EA concentrations and treatment periods on nematode survival and virulence as well as its effect on other nematode species.

Ethyl acetate (EA) is a colourless liquid with a characteristic fruity smell and is naturally present in plants such as *Anthemis nobilis* (*Roman chamomile*), *Rubus* species, several fruits (apple, banana and nectarines), cereal crops, radishes, palm tissues and during fermentation of plant materials (Monzer and Abd Elrahman, 2003 and Khan *et al.,* 2017). It is considered as a relatively safe product because of low toxicity to humans, animals, and the environment, thus it exempted from the requirement for tolerance when used in accordance with good agricultural practices as inert ingredients in pesticides (OECD, 2002). It also evaluated by the joint WHO/FAO experts committee on food additive (JECFA) and approved by FDA for a direct food additive (IPCS, 2002). The above-mentioned properties make EA an important candidate as a practical entom entomopathogenic nematode (EPN) activator. Hence, the exact effects that may EA have on the EPNs - a thorough examination.

Accordingly, the objective of this study was to examine in the laboratory the effects of various EA concentrations and exposure periods on the survival and virulence of *H. indica* and and *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) that represent the two different genera of EPN with different foraging behaviour, as an attempt to find an effective EPN activator.

Materials and Methods

1.Nematode source:

Two Egyptian isolates of EPNs were tested in this study; *H. indica* (EGAZ2) and *S. carpocapsae* (EGAZ9). These two EPN species were identified using both morphometric analysis and molecular techniques (Azazy *et al.,* 2018). The nematodes were reared in late instar greater wax moths; *Galleria mellonella* L. (Lepidoptera Pyralidae), by the method of Dutky *et al.* (1964) and IJs were harvested with modified white traps (White, 1927). A suspension of 2000 IJ/ml was prepared and kept at 25 ± 1ºC for at least 24 h but for less than one week prior to testing. The concentration of IJs in the stock suspension was determined by counting aliquot samples and adjusting to the required concentrations by adding water.

2.Treatment of nematodes:

Seven concentrations of 0.0, 0.01, 0.1, 0.25, 0.5, 1.0 and 2.0% (v/v) of EA were prepared by adding 0.0, 0.01, 0.1, 0.25, 0.5, 1.0 or 2.0 ml of HPLC grade EA (Sigma Chemical Co., St. Louis, MO, USA) to 250 ml flasks each contained 100 ml from the stock nematode suspension and kept in the dark at 25 ± 1ºC.

3. Effect of ethyl acetate on nematode survival:

After 24 and 48 hrs. of nematode incubation in various concentrations of EA, the numbers of IJs in one ml of the suspension were counted under a stereomicroscope microscope and grouped into two categories: active and alive (sinusoidal undulation, "J"-shaped postured or inactive "S" posture; quiescent-looking till slight touch), or dead IJs (complete straight posture not responding to mechanical stimulation up to self-disintegrating). Sample extraction from each flask was repeated 3 times and survival percentages were calculated.

4.Effect of ethyl acetate on nematode virulence:

The ability of control and EA treated IJs to penetrate and cause host mortality was compared using both filter paper and sand row assays.

4.1. Filter paper assay:

Filter paper assays were conducted according to the standard filter paper method detailed by Monzer and Abd Elrahman (2003) with minor modifications as follows. Nematodes survived treatment with 0.0, 0.1 and 0.1 EA for 24 and 48 hrs. were concentrated and rinsed several time with distilled water and suspended in distilled water at a concentration of 100 IJs/ml. Eppendorf tubes (1.5 cm3) with several small holes made in their lids were lined with double layer filter paper (Whatman No. 1) and 200 µl (20 IJ) of nematode suspensions were transferred with a micropipette to each tube. Afterward, a single *G. mellonella* larva was placed directly inside each Eppendorf tube, which was then closed and kept at 25°C, in the dark. After 24 h, larvae were washed twice with distilled water to remove adhering IJ, dried and transferred to Petri dishes each lined with a piece of moistened filter paper. Over the following 5 days, the number of dead larvae was recorded before their dissection under stereomicroscope microscope to determine the number of nematodes in each. The penetration rate was determined by calculating the percentage of penetrated nematode in each cadaver relative to the total applied nematode (20 IJs).

4.2. Sand row assay:

The ability of EPNs to disperse and locate their host through the soil was tested using a sand assay as described by Abd Elrahman and Abd Elrahman (2005) and Azazy *et al.* (2014). A plastic tube (25 cm in length and 5 cm in diameters) was cut longitudinally into two symmetrical halves. The two ends of the half tube (25 cm in length and 2.5 cm in height) were sealed with wire mesh screens and were filled to height 2.0 cm with sterilized, wet sand (10% moisture with the particle size of 0.05-0.1 mm in diameter). Four full grown larvae of *G. mellonella* were used as bait providing host cues to attract the foraging IJs. Larvae were kept inside a wire screen cage (1mm pore size) filled with moist sand and placed at a trap zone located near one end of each container as shown in Figure (1). The prepared containers were incubated at 25±1ºC in the dark, for 24h to allow equilibration of any diffuses from the insects through the sand before applying the nematodes. Six thousand IJs in 3 ml distilled water were inoculated in sand of the inoculation zone opposing to the trap of each container. Containers were enclosed in plastic bags to minimize water evaporation and kept horizontally at 25 ± 1ºC in dark. After 24 hrs. of incubation, and in each container was divided into five equal sections (5 cm length for each) as illustrated in Figure (1) and every sand soil of each section (after excluding the first nematode inculcation section) was transferred to a separate petri dish, and the number of nematodes in each section was determined with a live-bait method modified from Fan and Hominick

(1991). Briefly, *G. mellomella* larvae were transferred to each dish. Larvae in the trap section were also transferred to a separate dish. After 3 days incubation at 25±1ºC, larvae were removed, washed in tap water, dried on an absorbent paper and transferred to Petri dishes lined with a piece of moistened filter paper. After 2 days the number of adult nematodes in the body cavity was determined by dissecting the Galleria larvae under a stereomicroscope. An index was calculated to determine the average net distance [N (D)] travelled per individual IJ according to the following equation:

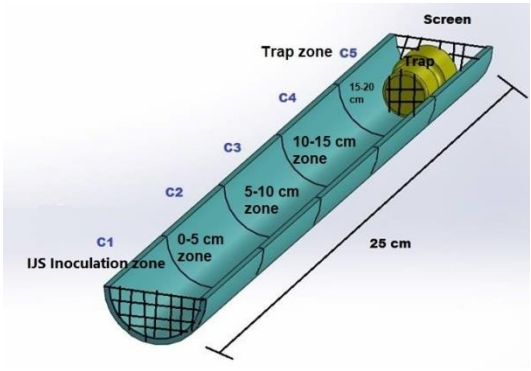
$$N(D) = (2.5a + 7.5b + 12.5c + 17.5T)/N$$

The values a, b, and c represent the number of nematodes recovered in a given section, T represents the number of nematodes recovered in larvae enclosed in trap, the constants (i.e., 2.5, 7.5, etc.) indicate the distance from the IJ inoculation zone to the midpoint of the section, and N is the total number of nematodes recovered outside the inoculation zone. This provides a weighted average of the distance travelled by nematodes within the column in 24 hrs., with greater values indicating increasing proximity to the hosts.

Nematode dispersal was quantified by:

a) Total number of IJ migrated outside the inoculation zone.
b) Percentage of IJs recovered in each section outside the inoculation zone relative to the total number of migrated IJs.
c) Total percentage of migrated IJs relative to total inoculated IJs (6000 IJs)
d) The average net distance N (D) travelled by each IJ outside the site of application.

Figure (1): Schematic of the sand assay (Modified from Azazy *et al.*, 2014)



5. Data analyses:

The entire assays were repeated using three generations of both *H. indica* and *S. carpocapsae* the results of the three experiments were combined for statistical analysis. Most of the results were expressed in percentage, although actual numbers were used for statistical tests. Probit analysis was done to calculate the Median Lethal Concentration (LC50) values and slope of EA, using LdP-Line® software (Bakr, 2007). Significantly different means were identified by analysis of variance (Tukey's honestly significant difference) at $P<0.05$ using CoStat® software (Costat, 2007). Results were recorded as the mean ± standard deviation (SD).

Results and Discussions

1. Effect of ethyl acetate on nematode survival:

Survival of IJs of *H. indica* and *S. carpocapsae* incubated in seven EA concentrations were recorded for 24 and 48 hrs. (Table, 1). There was no significant effect of EA at concentrations of 0.01 and 0.1% on the survival of IJs incubated for 24 and 48 hrs. of both nematode species (survival rate ranging from 100% to 95.1 ± 3.9%). However, significant differences were observed in the survival of IJs incubated in EA concentration higher than 0.1% ($p < 0.05$). Incubation of *H. indica* IJs in 0.25, 0.5, or 1.0% EA concentration for 24 hrs. sharply decreased nematode survival rate to 28.9 ± 2.5, 1.4 ± 2.5 or 1.2 ± 0.4, respectively. IJs of *S. carpocapsae* were more tolerant to the lethal effect of EA with a survival rate of 86.7 ± 1.5, 72.8 ± 3.7 or 38.9 ± 10, among IJs incubated in 0.25, 0.5, or 1.0% EA concentration, respectively, for 24 hrs. The difference in survival rate between *H. indica* and *S. carpocapsae* incubated in 0.25, 0.5, or

1.0% EA concentration for 24 hrs. was significant (P<0.01). Almost all IJs from both species that survived incubation in 0.5 and 1.0% EA concentration were not actively moving (i.e., motionless with straight or quiescent-looking postured till slight touch) after 24 hrs. of incubation. At EA concentration of 0.25 for 48 hrs., 0.0 and 30.2 ± 3.2% survival rate were recorded for *H. indica* and *S. carpocapsae*, respectively. IJs of both nematode species did not survive 0.5, 1.0% or 2.0% EA for 48 hrs. or 2% EA for 24 hrs. (Table, 1). Generally, EA was more toxic to *H. indica* than *S. carpocapsae* IJs as reflected by its calculated LC50 (Table, 1). The LC50 of EA for *H. indica* was 0.16 %, which is significantly lower than that for *S. carpocapsae* (0.78%).

Table (1): Mean survival rate (% ± SD) of *Heterorhabditis indica* and Steinernema *carpocapsae* treated with different concentrations of ethyl acetate for 24 and 48 hrs.

EA Concentration (%)	*H. indica*		*S. carpocapsae*	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
0 (control)	97.0 ± 4.2 a	95.2 ± 3.9 a	99.6 ± 0.4 a	99.2 ± 0.4 a
0.01	97.9 ± 1.5 a	95.1 ± 3.5 a	100 a	99.4 ± 0.3 a
0.1	95.3 ± 2.6 a	96.5 ± 0.4 a	99.9 ± 0.2 a	98.9 ± 0.7 a
0.25	28.9 ± 2.5 b	0.0 b	86.7 ± 1.5 b	30.2 ± 3.2 b
0.5	1.4 ± 2.5 c	0.0 b	72.8 ± 3.7 c	0.0 c
1.0	1.2 ± 0.4 c	0.0 b	38.9 ± 10 d	0.0 c
2.0	0.0 c	0.0 b	0.0 e	0.0 c
LC50	0.16e		0.78f	
Slope	2.37 ± 0.21		2.5 ± 0.2	

Means within the same column followed by the same letters do not differ significantly (p >0.05), SD = standard deviation.

2.Effect of EA on nematode virulence:

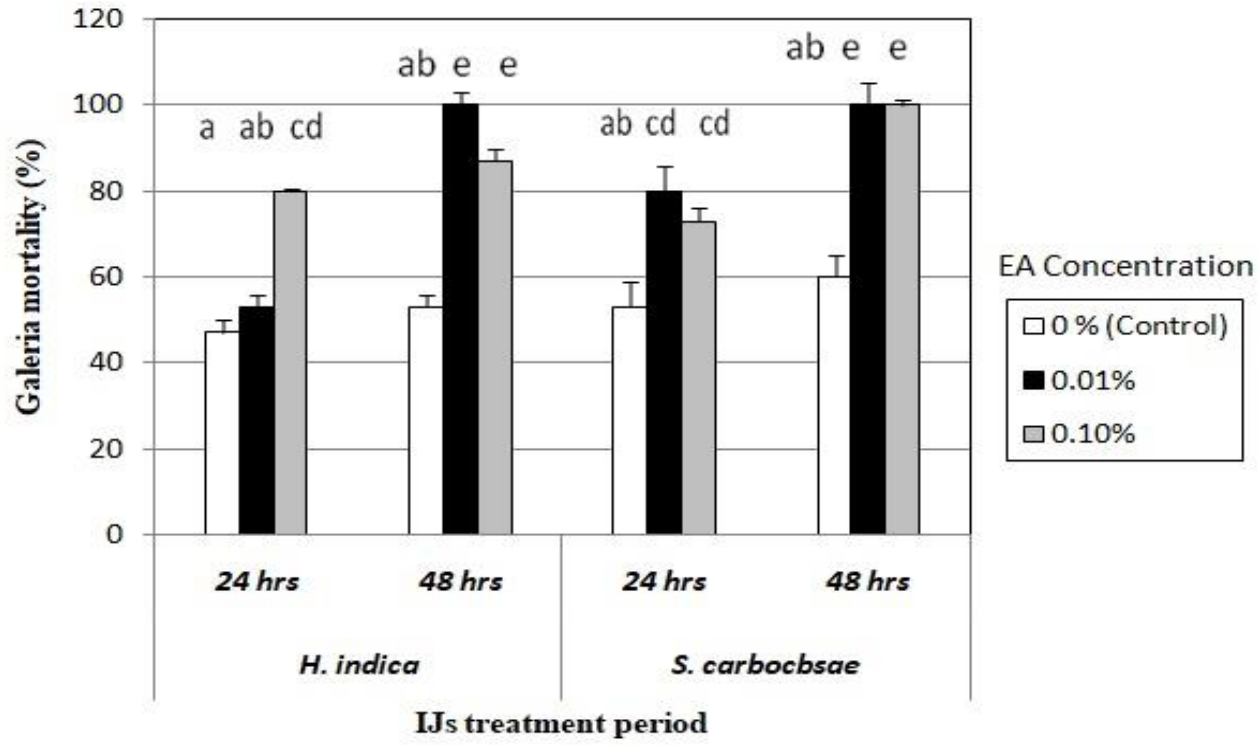
Results of nematode penetration rate in filter paper assays (Table, 2) indicated that treatment of IJs with EA generally increased nematode penetration rate. However, EA at a concentration equal to 0.01% for 48 hrs. increased nematode penetration rate significantly than untreated IJs for both nematode species. At such concentration of EA, the penetration rate of IJs reached 58.3 ± 12.6% and 61.7 ± 11.3% among *H. indica* and *S. carpocapsae*, respectively compared with 16.7 ± 7.6% and 30 ± 5.0 for untreated IJs, respectively. Figure (2) illustrated the effect of IJs treatments with EA on their ability to kill *G. mellonella* larvae in filter paper assays. Treatment of *H. indica* IJs with EA for 24 hrs. increased their ability to kill *G. mellonella* larvae than untreated control IJs, reached 80 ± 1.0 % among larvae exposed to IJs that previously treated with EA concentration of 0.1% for 24 hrs. The difference in mortality percentage among larvae exposed to EA-treated and control IJs was significant, P>0.01 at EA concentration of 0.1% and was not significant at EA concentration of 0.01%, p>0.01), However, IJs of *H. indica* that previously treated with 0.01 or 0.1% EA for 48 hrs. caused significantly higher mortality percentage among *G. mellonella* larvae compared with untreated IJs (100 and 87 ± 3.0% vs. 53 ± 3.0 %, respectively). On the other hand, treatment of *S. carpocapsae* IJs with either 0.01 or 0.1% concentration of EA significantly increase their ability to kill *G. mellonella* (P<0.01) than untreated control IJs.

Table (2): Filter paper assay: the average percentage (Mean ± SD) of nematodes counted in infected cadavers of *Galleria mellonella* larvae following exposure IJs of *Heterorhabditis indica* and Steinernema *carpocapsae* treated with 0.0, 0.01and 0.1% ethyl acetate concentration for 24 and 48 hrs.

Nematode species	EA concentrations	Exposure time	
		24 hrs.	48 hrs.
H. indica	0 (control)	11.7 ± 7.6 a	16.7 ± 7.6 acd
	0.01	13.3 ± 7.6 ab	58.3 ± 12.6 e
	0.1	26.7 ± 2.9 cd	28.3 ± 2.9 cd
S. carpocapsae	0 (control)	38.3 ± 5.8 c	30 ± 5.0 bcd
	0.01	56.7 ± 7.6 e	61.7 ± 11.3 e
	0.1	33.3 ± 2.9 c	36.7 ± 5.8 e

Means sharing the same letter are not significantly different (P>0.01), SD = standard deviation

Figure (2): Filter paper assay: the percentage mortality (Mean ± SD) of *Galleria mellonella* larvae following exposure to IJs of *Heterorhabditis indica* or *Steinernema carpocapsae* that previously treated with 0.0, 0.01and 0.1% ethyl acetate for 24 and 48 hrs. (Columns sharing the same over headed letter(s) do not differ significantly (p>0.05).



Results of virulence of *H. indica* against *G. mellonella* larvae in the sand assay were tabulated in Table (3). More than half of the dispersed IJs reached the trap zone of the container (64.9 ± 3.91% and 52.2 ±7.8% for control and treated IJs, respectively). No significant differences (p>0.05) were observed in the percentage of IJ recovered from 0-5, 10-15 cm zone, or trap zone between EA treated and control IJs. The total number of dispersed IJs was significantly (P<0.05) higher in EA-treated IJs relative to control (74.0 ± 3.0 versus 59 ± 5.8% IJs, respectively).

Table (3): Effect of treatment with 0.0 and 0.01% EA for 48 hrs. on the percentage of *Heterorhabditis indica* IJs dispersed after 24 hrs. of inoculation in the sand assay.

Distance (Cm)	%dispersed IJs relative to total dispersed IJs	
	0.0 (Control)	**0.01 %**
0-5	13.6 ± 5.44a	11.3 ± 6.9a
5-10	12.4 ± 0.01a	30.2 ± 5.5b
10-15	9.0 ± 7.0a	6.3 ± 2.8a
15-20 (Trap)	64.9 ± 3.91c	52.2 ±7.8c
	Total number of dispersed IJs	
0-20	59 ± 5.8 a	74.0 ± 3.0b
	Total dispersed IJs relative to inoculated IJs	
0-20	0.98 ± 0.04a	1.2 ± 0.05b

Means sharing the same letter within each section of the table are not significantly different (P>0.01), SD = standard deviation

On the other hand, most of *S. carpocapsae* IJs were recovered from the first two zones of the column for both control and EA-treated IJs of *S. Carpocapsae* (Table, 4). Table (4) also showed that percentage of IJs recovered from the trap zone was significantly higher in EA-treated *S. carpocapsae* IJs than control (41.6 ± 5.9% vs. 21.2 ± 7.0%, respectively), while, No significant difference was observed in total no of dispersed IJs between control and EA treated *S. carpocapsae*. Only 0.62 ± 0.03% of the inoculated IJ dispersed outside the inoculation zone for *S. carpocapsae* nematode, while the significantly higher proportion of *H. indica* IJ ($p<0.01$) were found outside the inoculation zone after 24 hrs. of inoculation (0.98 ± 0.04% of total inoculated IJs).

Table (4): Effect of treatment with 0.0 and 0.01% ethyl acetate for 48 hrs. on the percentage of Steinernema *carpocapsae* IJs dispersed after 24 hrs. of inoculation in the sand assay.

Distance (Cm)	%dispersed IJs relative to total dispersed IJs	
	0.0 (Control)	**0.01 %**
0-5	38.1 ± 8.6a	19.8 ± 1.7b
5-10	36.3 ± 13.6a	34.7 ± 10.4a
10-15	4.4 ± 1.5c	3.9 ± 1.7c
15-20 (Trap)	21.2 ± 7.0b	41.6 ± 5.9a
Total number of dispersed IJs		
0-20	37.7 ± 2.1a	33.7 ± 4.9a
Total dispersed IJs relative to inoculated IJs		
0-20	0.62 ± 0.03e	0.56 ± 0.08e

Means sharing the same letter within each section of the table are not significantly different (P>0.01), SD = standard deviation

The calculated N(D) (Table, 5) indicated that there was no significant difference in the average net distance travelled by EA-treated and untreated IJs of *H. indica*, while it was significantly higher in EA-treated IJs of *S. carpocapsae* than control ($P<0.05$). On the other hand, N (D) was significantly higher in *H. indica* than *S. Carpocapsae* IJs for both control and EA-treated IJs.

Table (5): Effect of treatment with 0.01% ethyl acetate for 48 hrs. on the average net distance N (D) travelled by dispersed IJs of *Heterorhabditis indica* and Steinernema *carpocapsae* in the sand assay.

Nematode species	ND (cm)	
	0.0 (Control)	**0.01 %**
H. indica	13.7 ± 0.8a	12.5 ± 0.6a
S. carpocapsae	7.90 ± 0.78b	11. 0 ± 0.30c

Means sharing the same letter are not significantly different (P>0.01), SD = standard deviation.

Our laboratory studies were conducted by directly immersing the nematodes in series concentrations of EA for 24 and 48 hrs. Results showed that survival of IJs was generally unaffected by EA concentration up to 0.1%, then, significantly decreased with EA concentration for both nematode species and exposure periods. Furthermore, no IJs survived treatment with 0.5 and 1.0% EA for 48 hrs. or 2% EA for 24 hrs., indicating that EA has an obvious nematicidal effect. IJs of *H. indica*, on the other hand, proved to be much more sensitive to the lethal effect of EA, as the calculated EA LC50 for *H. indica* was nearly four times less than that of *S. carpocapsae*. This finding indicates species-specific differences in the response among nematodes. Campbell and Gaugler (1992) mentioned that heterorhabditids tends to be less tolerant of environmental stress than steinernematids. Popiel and Vasquez (1991) observed that the survival rate of *H. bacteriophora*, exposed to 22% glycerol for 24 hours, was three-fold lower than that of *S. carpocapsae* exposed to the same glycerol concentration. Also, Negrisoli *et al.* (2008) reported that the insecticide thiamethoxam and the fungicide cyproconazole were toxic to *H. bacteriophora*, but did not cause any toxic effect on *S. carpocapsae*. Our results, in general, are consistent with the observations of the effect of EA on nematodes reported by previous studies (Monzer and AL-Elimi, 2002 and Monzer and Abd Elrahman, 2003). As treatment with EA concentrations of 0.01% and 0.1% for 24 and 48 hrs. did not show any significant effect on IJs from both *H. indica* and *S. Carpocapsae* survival relative to control, they selected for studying their effect on nematode virulence.

Virulence of EPNs is the ability of IJs to search, recognize, penetrate and kill insect hosts (Glazer, 1992). It was evaluated in this study using both filter paper and sand assays. The filter paper assay put the IJs in close proximity to the host, thereby assuring their contact with the host and measured their ability to penetrate and kill the host. In the sand assay, there is no host contact, and host finding by IJ is required, thus measured the nematode's ability to detect, disperse, reach and penetrate the target host (Campbell and Gaugler, 1992 and Ricci *et al.*, 1996). Results of filter paper assay indicated that EA-treated nematode were significantly more efficient in penetrating *G. mellonella* larvae than control, especially in IJs of *H. indica* and *S. carpocapsae* treated with 0.01% EA for 48 hrs. Mating is essential for further reproduction inside host in *S. carpocapsae*, thus penetration with a high number of individuals increases the probability of mating and further reproduction, and consequently, increases nematode efficiency (Ricci *et al.*, 1996). However, a single juvenile of *H. indica* can potentially reproduce and few IJs will be sufficient to establish the second generation, thus penetration with high number may not imply that *H. indica* nematode has lower efficiency in killing the host. Accordingly, percentage mortality of *G. mellonella* larvae following exposure to EA treated IJs of both nematode species for 24 and 48 hrs. were calculated. Again, results of this study indicated that treatment of IJs with EA at 0.01% concentration for 48 significantly increases their efficiency in killing their contact host for both nematode species, and suggest that EA treatment enhanced the nematode's host- penetration and killing abilities.

Due to the above-discussed results of filter paper assay, IJs treated with 0.0% (control) and 0.01% EA for 48 hrs. from both species were selected to further explore their ability to detect, disperse, and penetrate the target host in the sand assay. Results of virulence in sand assay revealed that very small proportion (less than 1%) of inoculated untreated IJs from both species dispersed laterally throughout the sand toward the host within 24 hrs. Corroborating our findings, Lacey *et al.* (2001) and Manimaran et al. (2012) reported that only around 0.1% of both S. *carpocapsae* and *H. indica* IJs dispersed 8 cm after 24 hrs. of inoculation in sand column assay in the presence of a host. The sand assay also indicated that a total number of dispersed IJs related to *H. indica* was significantly higher than that of S. *carpocapsae*. In addition, the highest proportion of dispersed *S. carpocapsae* IJs was recovered from the first (0-5) zone adjacent to inoculation zone, while the highest proportion of dispersed *H. indica* IJs was recovered from the trap zone at the far end of the sand row. This reflects the difference in foraging behaviour between the two nematode species in the soil. According to foraging behaviour, EPN has been classified into cruisers (active searchers) and ambushers (sit and wait foragers) (Bal *et al.*, 2015). The dispersed IJs of *H. indica*, which is cruiser forager species, moved actively toward *G. mellonella* cue thus higher proportions of them reached the trap zone after 24 hrs. of inoculation. On the other hand, S. *carpocapsae* is ambush forager and do not disperse very well in sand as most of IJs prefers to wait for the host, although a small number of IJs disperse slowly by waving (Campbell and Kaya, 2000 and Lacey *et al.*, 2001). However, the results of this study showed that a significant proportion of dispersed *S. carpocapsae* IJs reached the trap zone after 24 hrs. of inoculation despite their ambush foraging nature. This could be attributed to jumping or "sprinter" behaviour. Bal *et al.* (2014) and Labaude and Griffin (2018) mentioned that even so *S. carpocapsae* is ambush foraging, it possesses a small group of sprinters that able to fast disperse on the soil toward the host cue by jumping movement.

The main result of this study is that treatment of *H. indica* IJ with 0.01% EA for 48 hrs. increased slightly but significant number of dispersing IJs than untreated control although it did not affect the average net distance N(D) travelled by dispersed IJs. On the other hand, treatment of *S. carpocapsae* with EA under the same concentrations did not affect a number of dispersing IJs but significantly increased the percentage of IJs that detect and reach *G. mellonella* larvae enclosed in the trap zone than untreated control. The activation effect of EA on the ability of *S. carpocapsae* IJs to disperse was reflected by the average net distance N(D) they travelled which was significantly longer in EA treated IJs than control. It could be concluded that treatment with EA increased proportion of actively moving IJ relative to total *H. indica* IJs that was injected in the inoculation zone, while increase proportion of "sprinters" in *S. carpocapsae* relative to the total dispersing IJs, but not total injected in the inoculation zone.

It is not yet understood the exact mechanism by which EA act on EPNs. Monzer and Abd Elrahman (2003) related the lethal effect of EA on EPNs to the damage of the sensory apparatus of the IJs due to its neurotropic effects. EA is not likely to penetrate inside nematode body as it adsorbs by the glycoprotein surface of the IJ duple sheath coat (Djian *et al.*, 1991 and Glazer, 2002). In addition, natural openings of the IJs, such as the mouth and anus, are closed while living outside hosts (Endo and Nickle, 1994). The only vital organ in contact with the external environment and that could expose to EA would have been the nervous receptors in the amphidial channel opening near the head of IJ to detect aqueous chemo-attractants and repellents (Ashton *et al.*,1999). Compounds such as carbamates and organophosphates act through their neurotropic effects on the

nervous system throught amphidial channel (Hara and Kaya, 1982). However, neurotropic effects of EA do not explain the detected activation effect of EA low concentrayions on IJ active movement in *H. indica* and jumbling movement in case of S. *carpocapsae*. Bowen and Balster (1997) reported increase sensorimotor reactivity among mice inhaled low concentration of EA. Accordingly, the increase in nematode virulence by low concentrations of EA could be due to its stimulation to nervous receptors in the amphidial channel, which increases sensorimotor reactivity and response of nematode to external stimuli such as host cue, higher concentrations damage of the sensory apparatus as postulated by Monzer and Abd Elrahman (2003). Alternatively, we hypothesized that an increase in nematode virulence due to EA treatment could be related to amphidial channel un-blockage. IJs naturally expose inside decomposed *G. mellonella* or during the extraction process, to microscopic fat micro-droplets and other organic debris, various micro-organisms and/or fungal adhesion that could infiltrate inside and block the amphidial channel in a significant number of IJs. Nematodes with blocked amphidial channel will be unable to detect chemical host cue that triggering their actively moving response in *H. indica* or sprint in case of *S. carpocapsae*. EA as an effective organic solvent; capable of dissolving many polar and non-polar organic compound, may act through dissolving many amphidial channel blocker. Removal of such blocker by EA could enhance the response of IJs to host cue and increase their virulence.

Based on the present study we conclude that EA at low concentration (0.01-0.1%) increases EPNs virulence, while at higher concentrations acts as a nematicide. Pending further research on the effect of EA on other nematode species and its exact mode of action on EPNs, EA appears as a promising candidate for nematode activation prior field application. However, the biological significance of the above discussed results in the field is yet to be determined since laboratory bioassays are generally thought to provide better results than field tests (Ishibashi and Takii, 1993).

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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