Migration of infective juveniles of entomopathogenic nematode, *Heterorhabditis indica* in soil after their application through Low Pressure Drip Irrigation System

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Abstract: *Application of infective juveniles (IJs) of entomopathogenic nematode,* Heterorhabditis indica *in the field through Fully Automatic High Pressure Drip Irrigation System may affect migration behaviour of nematodes in the soil due to exposure to hydrodynamic stresses during their flow through laterals and emitters.*

This experiment was conducted at CPCT, ICAR-IARI, New Delhi where three heads (1.0, 1.5 and 2.0 m) and two types of pipes having diameters of 16 mm and 20 mm were evaluated. The migration of IJs was observed at 2, 3 and 4 inch distance at four time intervals viz. 24, 48, 72 and 96 h post release. Factorial CRD using OP Stat software was used for the statistical analysis of the data on vertical and horizontal migration studies. After 96 h, horizontally maximum numbers of IJs were concentrated at 3 inch distance compared to 2 and 4 inch distance; while vertically maximum concentration IJs was recorded at 4 inch distance compared to 2 and 3 inch distance. Also, movement of IJs was positively correlated with time. The rate of downward migration was faster compared to lateral movement, suggesting that IJs showed positive geotropism. Head up to 2.0 m of drip irrigation system or use of pipes having diameters of 16 or 20 mm had no effect on the migration behaviour of IJs of H. indica *post application in the field.*

Keywords: *EPN, Migration, Drip irrigation,* Heterorhabditis, *Pressure.*

INTRODUCTION

Entomopathogenic nematodes (EPNs) being non-toxic to humans and relatively specific to their target pests, have been widely used as biological insecticides in pest management programs. EPNs can be applied with standard pesticide equipments (hand pumps, spray cannons etc.) as well as through spinning discs, micro-injectors, subsurface syringes, different irrigation systems and other application types such as cadaver application (Wang et al. , 2009; Raja et al. , 2015).

Heterorhabditis indica confer high virulence against soil insect pests and have been used widely against many economically important insect pests of crops (Grewal *et al.,* 2005; Mohan *et al.,* 2016). As EPNs are tolerant to shear stress, they can survive under high pressure (Fife *et al.*, 2003). Some studies showed that some EPN species can resist up to 14 bar (Wright *et al.,* 2005).

Widespread application of *H. indica* under Indian scenario requires information on post-application survival and behaviour under field conditions. In the current agricultural scenario, there is a shift towards precision farming and resource conservation. Use of drip irrigation technology, wherein precise and slow application of water in the form of discrete or continuous drops

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through mechanical devices called emitters in the root zone of the plants, provides more efficient utilization of water. By the use of drip irrigation technology, water and agrochemicals (e.g., fertilizers and pesticides) are applied directly to the root zones of the plants at a desired rate best suited to meet the needs of the plants being irrigated. The objective of this study was to evaluate the migration behaviour of infective juveniles of *H. indica* after their passing through Low Pressure Drip Irrigation System in the field to facilitate *H. indica* to establish itself as a potent bio-control agent under Indian conditions.

MATERIALS AND METHODS

The experiment was conducted at CPCT, ICAR-IARI, New Delhi. Infective juveniles (IJs) of *H. indica* were multiplied in the laboratory on *Galleria mellonella* larvae *in vivo*, following the standard procedures (Kaya and Stock, 1997). Freshly emerged infective juveniles from the wax moth larval cadavers were collected in sterilized distilled water using White traps (White, 1927) and were used for subsequent studies within 2-4 weeks.

A low pressure drip irrigation system consisted of a 100 litre plastic tank with 30 metres (100 feet) of drip line connected to the bottom of the tank. The tank was placed at 2.5 feet above the ground so that gravity provides sufficient water pressure to ensure even watering for the entire crop. Clean water was poured into the tank through a filter/strainer. The water in the bucket filled the main drip line and distributed to watering points. The drip line was engineered to dispense water through openings spaced at 30cm. A filter after the control valve was installed to prevent blockages. Before injecting IJs, the drip lines were flushed. The irrigation water was checked for the presence of nematodes before the application. There were three heads (1.0, 1.5 and 2.0 m) and two types of pipes having diameter 16 mm and 20 mm were used.Treatments were assigned in a completely randomized design and were replicated three times.

Injecting IJs into the drip line

A 100 ml dilution of one lakh *H. Indica* IJs was prepared. The number of nematodes in each 100 ml treatment was estimated by counting three aliquots of 1ml each. The IJs were injected at the point where the drip line left the filter. The injection port was then sealed to avoid nematode leakage. The irrigation was stopped during the nematode injection process and the lines were opened after application. Each line constituted one replicate. There were 10 openings per lateral. To evaluate migration of IJs of *H. indica* applied via drip irrigation system in the field, nematodes release points on the soil by emitters of each of the six laterals (total 60 emitters) were marked. 30 emitters were marked for horizontal migration studies and rest thirty for vertical migration studies. The migration of IJs was observed at 2, 3and 4 inch distance at four time intervals viz. 24, 48, 72 and 96 h post release. Three replicates of each treatment were maintained. After 24, 48, 72 and 96 hours, about 20g of soil was taken in plastic tubes at a distance of 2, 3 and 4 inch from the release point of 30 emitters randomly on all the four sides for horizontal migration studies. Soil samples were also collected at a depth of 2, 3 and 4 inch from the release point of 30 emitters for vertical migration studies. Samples were collected destructively from seven release points at a time for each type of migration study. This soil was brought to the laboratory and tested for presence of larvae of *H. indica* by baiting it with late instar larvae of *G. mellonella* and infectivity of *H. indica* IJs was quantified. Each sample set up was accompanied with control, in which soil from the same field was taken where no EPNs were released. Additionally, a check of zero pressure (approximately 1500 IJs in an aqueous suspension of 10 ml were released in the same field on pre-determined spots marked 1m apart along the drip lines) was also taken. This arrangement was replicated thrice. Mortality of larvae was observed every 24 h up to 96 h, by which the percentage mortality was calculated.

Statistical analysis

Factorial CRD using OP Stat software was used for the statistical analysis of the data on vertical and horizontal migration studies.

5.1.2. *Vertical and Horizontal migration of* **Heterorhabditis indica** *in the soil post application via low pressure drip irrigation system*

The data obtained on the mortality of *Galleria mellonella* larvae as an indicator of migration of IJs of *Heterorhabditis indica* applied in the field via low pressure drip irrigation system after 24, 48, 72 and 96 h is presented in tables 1 to 4.

Table 1 presents data recorded after 24 h on the mortality of larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs as a parameter for horizontal and vertical foraging. Pooled mean (A) revealed no movement by IJs up to 4 inch, as indicated by no mortality recorded in larvae kept embedded in soil taken from horizontal as well as vertical distance of 4 inch from point of release. Movement of IJs up to 7.5 cm was observed after 24 h, but it was not significant since only 10.96% mortality at 3 inch horizontal distance and 27.59% mortality at vertical distance was recorded. The IJs could not move beyond 3 inch after 24 h. Maximum number of IJs appeared to have migrated up to 2 inch causing 48.09% morality horizontally and 45.71% mortality vertically. Thus, mortality obtained was inversely proportional to distance. The interactions of all the three factors with each other and among themselves were non-significant.

Table 2 presents data recorded after 48 h on mortality of larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs. Although migration of IJs in terms of *Galleria* larval mortality was recorded 10 cm vertically and 7.5 cm horizontally, yet the mortality decreased with increasing distance from the point of release of IJs. Pooled mean (A) for horizontal migration revealed that maximum number of IJs apparently migrated up to 2 inch resulting in maximum mortality (23.98%) which is significantly more compared to horizontal distance of 3 inch (15.48%). Thus, the data on larval mortality and horizontal distance were inversely proportional. Pooled mean (A) for vertical migration showed that the highest number of IJs migrated up to 2 inch causing maximum mortality (45.87%) which is significantly more compared to vertical distance of 3 inch (23.33%) and 4 inch (10.18%). The interactions of all the three factors with each other and among themselves were non-significant.

Table 3 presents data recorded after 72 h on mortality of larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs. The data clearly reveals that IJs could migrate up to 10 cm vertically as well as horizontally after 72 h. The data on pooled mean (A) for horizontal migration indicates that irrespective of pipe diameter and pressure, number of IJs was more concentrated at a horizontal distance of 3 inch compared to 2 and 4 inches from the point of release and this is evident by significantly maximum larval mortality at a distance of 3 inches (19.05%) from the point of release. Pooled mean (A) for vertical migration depicted that maximum number of IJs migrated up to 4 inch vertically causing maximum mortality (48.57%) as compared to 2 and 3 inch. The interactions of all the three factors with each other and among themselves were non-significant.

Table 4 includes data recorded after 96 h on mortality of larvae at various horizontal and vertical distances from the point of release of IJs as a parameter for horizontal and vertical foraging. IJs were found distributed up to 4 inch horizontally as well as vertically after 96 h, but number of IJs was more concentrated at a horizontal distance of 3 inch compared to 2 and 4 inches from the point of release and is revealed by pooled mean (A) for horizontal migration. Significantly maximum mortality at a horizontal distance of 3 inches from the point of release was recorded compared to 2 and 4 inch distances. Pooled mean (A) for vertical migration revealed that maximum number of IJs could migrate up to 4 inch vertically causing maximum mortality (48.57%) as compared to 2 and 3 inch. The interactions of all the three factors with each other and among themselves were non-significant.

RESULTS AND DISCUSSION

The data obtained on the mortality of *Galleria mellonella* larvae as an indicator of migration of IJs of *Heterorhabditis indica* applied in the field via fully automated high pressure drip irrigation system after 24, 48, 72 and 96 h are presented in tables 1 to 4.

Table 1 presents data recorded after 24 h on the mortality of *Galleria* larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs as a parameter for horizontal and vertical foraging. Pooled mean (A) revealed no movement of IJs up to the point of sampling, i.e., 4 inch from the release point, as is evident by no mortality recorded in larvae kept embedded in soil taken from horizontal as well as vertical distance of 4 inch from point of release. Movement of IJs up to 3 inch after 24 h was indicated, but fewer IJs moved away from the point of release that resulted in only 10.78% mortality horizontally and 21.43% mortality vertically. The IJs could not move beyond 3 inch after 24 h. Maximum number of IJs seems to have migrated up to 2 inch causing 48.09% mortality horizontally as well as vertically. Thus, mortality obtained was inversely proportional to distance. The interactions of all the three factors with each other and among themselves were non-significant. Table 2 presents data recorded after 48 h on mortality of larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs as a parameter for horizontal and vertical foraging. Nil mortality of *Galleri*a larvae in soil samples collected 10 cm horizontally and vertically from the point of release of IJs indicates that the IJs do not migrate up to 10 cm. However, after 48 h, vertical movement up to 4 inch and 3 inch migration horizontally was indicated; although the insect larval movement decreased with increasing distance from the point of release of IJs. The IJs could not move beyond 3 inch horizontally after 48h. Pooled mean (A) for horizontal migration revealed that maximum number of IJs seems to have migrated up to 2 inch causing maximum mortality (20%) which is significantly more compared to horizontal distance of 3 inch (12.38%). Thus, mortality obtained was inversely proportional to horizontal distance. Pooled mean (A) for vertical migration revealed that maximum number of IJs migrated between 2 to 3 inch causing maximum mortality (45.71%) which is significantly more compared to vertical distance of 2 inch (24.29%) and 4 inch (10.48%). The interactions of all the three factors with each other and among themselves were non-significant. Table 3 presents data recorded after 72 h on mortality of *Galleria* larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs. Migration of IJs 4 inch horizontally as well as vertically is recorded after 72 h of release. Pooled mean (A) for horizontal migration revealed that irrespective of pipe diameter and pressure, number of IJs was more concentrated at a horizontal distance of 3 inch compared to 2 and 4 inches from the point of release and is evident by significantly maximum mortality at a distance of 3 inches (20%) from the point of release. Pooled mean (A) for vertical migration depicted that maximum number of IJs migrated up to 4 inch vertically causing highest mortality (48.57%) as compared to 2 and 3 inch. The interactions of all the three factors with each other and among themselves were non-significant. Table 4 presents data recorded after 96 h on mortality of larvae embedded in soil taken from various horizontal and vertical distances from the point of release of IJs. IJs were intercepted up to 4 inch horizontally as well as vertically after 96 hrs, but they were more concentrated at a horizontal distance of 3 inch compared to 2 and 4 inches from the point of release and is revealed by pooled mean (A) for horizontal migration. Significantly maximum mortality at a horizontal distance of 3 inches from the point of release was recorded compared to 2 and 4 inch distances. Pooled mean (A) for vertical migration revealed that highest number of IJs migrated up to 4 inch vertically causing maximum mortality (48.57%) as compared to 2 and 3 inch. The interactions of all the three factors with each other and among themselves were non-significant.

Table 5.1.2.1: Horizontal and vertical migration of *Heterorhabditis indica* in the soil post application low pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae (24 hours)

Distance (inch)	Pipe diameter (mm)	% Mortality of *G. mellonella* larvae as parameter of horizontal and vertical migration at various distances											
		Horizontal migration						Vertical migration					
		Head (m) 0	1	1.5	2	Control	Mean	Head (m) 0	1	1.5	2	Control	Mean
2	16	66.67 (54.86)	57.14 (49.26)	61.90 (52.62)	66.67 (54.86)	0.00 (0.00)	**50.48 (42.32**	30.05 (41.80)	38.09 (37.93)	38.10 (38.05)	38.10 (38.05)	0.00 (0.00)	**30.67 (31.20)**
	20	61.90 (51.99)	47.62 (43.65)	57.14 (49.25)	61.90 (51.99)	0.00 (0.00)	**45.71 (39.38**	32.05 (34.60)	23.81 (28.96)	33.33 (35.19)	33.33 (35.19)	0.00 (0.00)	**24.50 (29.79)**
	Mean	**64.29 (53.43)**	**52.38 (46.45)**	**59.52 (50.94)**	**64.29 (53.43)**	**0.00 (0.00)**	**48.09 (40.85)**	**35.56 (38.2)**	**30.95 (33.45)**	**35.72 (36.62)**	**35.72 (36.62)**	**0.00 (0.00)**	**27.59 (28.98)**
3	16	14.29 (18.18)	14.29 (18.18)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	**11.43 (14.54)**	52.38 (46.39)	57.14 (49.25)	66.66 (55.36)	66.67 (54.86)	0.00 (0.00)	**48.57 (41.17)**
	20	9.53 (14.81)	14.29 (18.18)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	**10.48 (13.87)**	47.62 (43.65)	52.38 (46.39)	61.90 (51.99)	52.38 (46.39)	0.00 (0.00)	**42.86 (37.68)**
	Mean	**11.91 (16.50)**	**14.29 (18.18)**	**14.29 (18.18)**	**14.29 (18.18)**	**0.00 (0.00)**	**10.96 (14.21)**	**50.00 (45.02)**	**54.76 (47.82)**	**64.28 (53.68)**	**59.52 (50.62)**	**0.00 (0.00)**	**45.71 (39.43)**
4	16	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	14.29 (18.18)	14.29 (18.18)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	**11.43 (14.55)**
	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	14.29 (18.18)	14.29 (18.18)	9.53 (14.81)	9.53 (14.81)	(0.00 (0.00)	**9.53 (13.20)**
	Mean	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**14.29 (18.18)**	**14.29 (18.18)**	**11.91 (16.50)**	**11.91 (16.50)**	**0.00 (0.00)**	**10.48 (13.87)**

Factors	SE(m)	C.D. at 5%	Factors	SE(m)	C.D. at 5%
Distance (A)	1.46	4.12	Distance (A)	0.87	2.45
Pipe diameter (B)	1.19	N/A	Pipe diameter (B)	0.71	N/A
A X B	2.06	N/A	A X B	1.23	N/A
Head (C)	1.06	NS	Head (C)	0.12	NS
A X C	1.26	NS	A X C	0.94	NS
B X C	1.66	N/A	B X C	1.58	N/A
A X B X C	2.61	N/A	A X B X C	2.74	N/A

Values in parentheses are *arc sine* transformed. SE(m): Standard Error of Mean; CD: Critical difference.

Table 5.1.2.2: Horizontal and vertical migration of *Heterorhabditis indica* in the soil post application low pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae (48 hours)

Distance (inch)	Pipe Diameter (mm)	% Mortality of *G. mellonella* larvae as parameter of horizontal and vertical migration at various distances — Horizontal migration, Head (m) 0	Horizontal 1	Horizontal 1.5	Horizontal 2	Horizontal Control	Horizontal Mean	Vertical migration, Head (m) 0	Vertical 1	Vertical 1.5	Vertical 2	Vertical Control	Vertical Mean
2	16	33.81 (35.45)	33.33 (35.19)	28.57 (31.82)	28.57 (31.82)	0.00 (0.00)	**30.07 (26.86)**	61.90 (51.99)	61.43 (51.89)	57.14 (49.25)	61.90 (51.99)	0.00 (0.00)	**48.43 (41.02)**
	20	29.05 (32.59)	28.57 (32.33)	29.05 (32.59)	28.81 (32.56)	0.00 (0.00)	**23.01 (26.01)**	52.38 (46.39)	66.67 (54.86)	54.67 (48.86)	57.14 (49.13)	0.00 (0.00)	**43.31 (38.15)**
	Mean	**31.43 (34.02)**	**30.95 (33.76)**	**28.81 (32.21)**	**28.69 (32.19)**	**0.00 (0.00)**	**23.98 (26.44)**	**57.14 (49.19)**	**59.05 (50.04)**	**54.76 (47.82)**	**59.52 (50.56)**	**0.00 (0.00)**	**45.87 (39.59)**
3	16	20.29 (26.18)	19.05 (25.59)	19.53 (25.71)	19.05 (21.55)	0.00 (0.00)	**15.58 (19.81)**	33.34 (34.68)	33.81 (34.84)	33.33 (35.19)	32.81 (33.96)	0.00 (0.00)	**26.66 (27.60)**
	20	19.79 (25.68)	19.05 (25.59)	16.53 (25.71)	19.05 (21.55)	0.00 (0.00)	**15.38 (19.71)**	28.57 (31.82)	23.81 (28.96)	23.81 (28.96)	23.81 (28.96)	0.00 (0.00)	**20.00 (23.74)**
	Mean	**19.79 (25.93)**	**19.05 (25.59)**	**19.53 (25.71)**	**19.05 (21.55)**	**0.00 (0.00)**	15.48 (19.78)	**30.96 (33.25)**	**28.81 (31.9)**	**28.57 (32.08)**	**28.31 (31.46)**	**0.00 (0.00)**	23.33 (25.67)
4	16	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
	Mean	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**

Factors	SE(m)	C.D. at 5%
Distance (A)	1.49	4.22
Pipe diameter (B)	1.22	N/A
A X B	2.11	N/A
Head (C)	1.23	NS
A X C	1.34	NS
B X C	2.73	N/A
A X B X C	4.72	N/A

Values in parentheses are *arc sine* transformed. SE(m): Standard Error of Mean; CD: Critical difference.

Table 5.1.2.3: Horizontal and vertical migration of *Heterorhabditis indica* in the soil post application low pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae (72 hours)

Distance (inch)	Pipe diameter (mm)	% Mortality of *G. mellonella* larvae as parameter of horizontal and vertical migration at various distances											
		Horizontal migration						Vertical migration					
		Head (m)						Head (m)					
		0	1	1.5	2	Control	Mean	0	1	1.5	2	Control	Mean
2	16	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	**8.57 (11.72)**	9.52 (10.78)	14.29 (18.18)	19.05 (21.04)	19.05 (25.59)	0.00 (0.00)	**12.38 (15.12)**
	20	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	**8.57 (11.72)**	9.52 (10.78)	14.29 (18.18)	14.29 (18.18)	19.05 (25.59)	0.00 (0.00)	**11.43 (14.55)**
	Mean	**9.53 (14.81)**	**14.29 (18.18)**	**9.52 (10.78)**	**9.53 (14.81)**	**0.00 (0.00)**	8.57 (11.72)	**9.52 (10.78)**	**14.29 (18.18)**	**16.67 (19.61)**	**19.05 (25.59)**	**0.00 (0.00))**	11.91 (14.83)
3	16	28.57 (31.82)	14.29 (18.18)	28.57 (31.82)	28.57 (31.82)	0.00 (0.00)	**20.00 (22.73)**	23.81 (28.96)	38.10 (38.05)	38.10 (38.05)	38.10 (38.05)	0.00 (0.00)	**27.62 (28.62)**
	20	28.57 (32.33)	14.29 (18.18)	23.81 (28.96)	23.81 (28.96)	0.00 (0.00)	**18.10 (21.69)**	23.81 (28.96)	33.34 (34.68)	33.33 (35.19)	38.10 (38.05)	0.00 (0.00)	**25.72 (27.38)**
	Mean	**28.57 (32.08)**	**14.29 (18.18)**	**26.19 (30.39)**	**26.19 (30.39)**	**0.00 (0.00)**	19.05 (22.21)	**23.81 (28.96)**	**35.72 (36.37)**	**35.72 (36.62)**	**38.10 (38.05)**	**0.00 (0.00)**	26.67 (28.00)
4	16	14.29 (18.18)	4.76 (7.41)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	**9.52 (12.39)**	57.14 (49.13)	57.14 (49.25)	66.66 (55.36)	76.19 (61.09)	0.00 (0.00)	**51.43 (42.97)**
	20	9.53 (14.81)	4.76 (7.41)	14.29 (18.18)	9.53 (14.81)	0.00 (0.00)	**7.62 (11.04)**	52.38 (46.39)	47.62 (43.65)	61.90 (51.99)	66.67 (54.86)	0.00 (0.00)	**45.71 (39.38)**
	Mean	**11.91 (16.50)**	**4.76 (7.41)**	**14.29 (18.18)**	**11.91 (16.50)**	**0.00 (0.00)**	8.57 (11.72)	**54.76 (47.76)**	**52.38 (46.45)**	**64.28 (53.68)**	**71.43 (57.97)**	**0.00 (0.00)**	48.57 (41.17)

Factors	SE(m)	C.D. at 5%	Factors	SE(m)	C.D. at 5%
Distance (A)	2.24	6.35	Distance (A)	1.71	4.84
Pipe diameter (B)	1.83	N/A	Pipe diameter (B)	1.40	N/A
A X B	3.17	N/A	A X B	2.42	N/A
Head (C)	1.90	NS	Head (C)	1.21	NS
A X C	5.02	N/A	A X C	3.02	NS
B X C	4.10	N/A	B X C	3.12	N/A
A X B X C	7.10	N/A	A X B X C	5.40	N/A

Values in parentheses are *arc sine* transformed. SE(m): Standard Error of Mean; CD: Critical difference.

Table 5.1.2.4: Horizontal and vertical migration of *Heterorhabditis indica* in the soil post application low pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae (96 hours)

Distance (inch)	Pipe diameter (mm)	% Mortality of *G. mellonella* larvae as parameter of horizontal and vertical migration at various distances											
		Horizontal migration						Vertical migration					
		Head (m)						Head (m)					
		0	1	1.5	2	Control	Mean	0	1	1.5	2	Control	Mean
2	16	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	**8.57 (11.72)**	9.52 (10.78)	14.29 (18.18)	19.05 (21.04)	19.05 (25.59)	0.00 (0.00)	**12.38 (15.12)**
	20	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	**8.57 (11.72)**	9.52 (10.78)	14.29 (18.18)	14.29 (18.18)	19.05 (25.59)	0.00 (0.00)	**11.43 (14.55)**
	Mean	**9.53 (14.81)**	**14.29 (18.18)**	**9.52 (10.78)**	**9.53 (14.81)**	**0.00 (0.00)**	8.57 (11.72)	**9.52 (10.78)**	**14.29 (18.18)**	**16.67 (19.61)**	**19.05 (25.59)**	**0.00 (0.00)**	11.91 (14.83)
3	16	28.57 (31.82)	14.29 (18.18)	28.57 (31.82)	28.57 (31.82)	0.00 (0.00)	**20.00 (22.73)**	23.81 (28.96)	38.10 (38.05)	38.10 (38.05)	38.10 (38.05)	0.00 (0.00)	**27.62 (28.62)**
	20	28.57 (32.33)	14.29 (18.18)	23.81 (28.96)	23.81 (28.96)	0.00 (0.00)	**18.10 (21.69)**	23.81 (28.96)	33.34 (34.68)	33.33 (35.19)	38.10 (38.05)	0.00 (0.00)	**25.72 (27.38)**
	Mean	**28.57 (32.08)**	**14.29 (18.18)**	**26.19 (30.39)**	**26.19 (30.39)**	**0.00 (0.00)**	19.05 (22.21)	**23.81 (28.96)**	**35.72 (36.37)**	**35.72 (36.62)**	**38.10 (38.05)**	**0.00 (0.00)**	26.67 (28.00)
4	16	14.29 (18.18)	4.76 (7.41)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	**9.52 (12.39)**	57.14 (49.13)	57.14 (49.25)	66.66 (55.36)	76.19 (61.09)	0.00 (0.00)	**51.43 (42.97)**
	20	9.53 (14.81)	4.76 (7.41)	14.29 (18.18)	9.53 (14.81)	0.00 (0.00)	**7.62 (11.04)**	52.38 (46.39)	47.62 (43.65)	61.90 (51.99)	66.67 (54.86)	0.00 (0.00)	**45.71 (39.38)**
	Mean	**11.91 (16.50)**	**4.76 (7.41)**	**14.29 (18.18)**	**11.91 (16.50)**	**0.00 (0.00)**	8.57 (11.72)	**54.76 (47.76)**	**52.38 (46.45)**	**64.28 (53.68)**	**71.43 (57.97)**	**0.00 (0.00)**	48.57 (41.17)

Factors	SE(m)	C.D. at 5%	Factors	SE(m)	C.D. at 5%
Distance (A)	2.24	6.35	Distance (A)	1.71	4.84
Pipe diameter (B)	1.83	N/A	Pipe diameter (B)	1.40	N/A
A X B	3.17	N/A	A X B	2.42	N/A
Head (C)	1.90	NS	Head (C)	1.21	NS
A X C	5.02	N/A	A X C	3.02	NS
B X C	4.10	N/A	B X C	3.12	N/A
A X B X C	7.10	N/A	A X B X C	5.40	N/A

Values in parentheses are *arc sine* transformed. SE(m): Standard Error of Mean; CD: Critical difference.

Table 4: Horizontal and vertical migration of *Heterorhabditis indica* in the soil after 98 h of application via fully automated high pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae after

(Mean of 3 replications)

Distance (inch) (A)	Pipe diameter (mm) (B)	Per cent mortality of *G. mellonella* larvae as parameter of horizontal and vertical migration at various distances														
		Horizontal migration							Vertical migration							
		Pressure (akPa) (C)				Control	Mean (A×B)	Pooled mean (A)	Pressure (kPa) (C)				Control	Mean (A×B)	Pooled mean (A)
		0	200	300	400				0	200	300	400			
2	16	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	8.57 (11.72)		9.52 (10.78)	14.29 (18.18)	19.05 (21.04)	19.05 (25.59)	0.00 (0.00)	12.38 (15.12)	
	20	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	8.57 (11.72)	8.57 (11.72)	9.52 (10.78)	14.29 (18.18)	14.29 (18.18)	19.05 (25.59)	0.00 (0.00)	11.43 (14.55)	11.91 (14.83)
	Mean (A×C)	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.810	0.00 (0.00)			9.52 (10.78)	14.29 (18.18)	16.67 (19.61)	19.05 (25.59)	0.00 (0.00)		
3	16	28.57 (31.82)	14.29 (18.18)	28.57 (31.82)	28.57 (31.82)	0.00 (0.00)	20.00 (22.73)		23.81 (28.96)	38.10 (38.05)	38.10 (38.05)	38.10 (38.05)	0.00 (0.00)	27.62 (28.62)	
	20	28.57 (32.33)	14.29 (18.18)	23.81 (28.96)	23.81 (28.96)	0.00 (0.00)	18.10 (21.69)	19.05 (22.21)	23.81 (28.96)	33.34 (34.68)	33.33 (35.19)	38.10 (38.05)	0.00 (0.00)	25.72 (27.38)	26.67 (28.00)
	Mean (A×C)	28.57 (32.08)	14.29 (18.18)	26.19 (30.39)	26.19 (30.39)	0.00 (0.00)			23.81 (28.96)	35.72 (36.37)	35.72 (36.62)	38.10 (38.05)	0.00 (0.00)		
4	16	14.29 (18.18)	4.76 (7.41)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	9.52 (12.39)		57.14 (49.13)	57.14 (49.25)	66.66 (55.36)	76.19 (61.09)	0.00 (0.00)	51.43 (42.97)	
	20	9.53 (14.81)	4.76 (7.41)	14.29 (18.18)	9.53 (14.81)	0.00 (0.00)	7.62 (11.04)	8.57 (11.72)	52.38 (46.39)	47.62 (43.65)	61.90 (51.99)	66.67 (54.86)	0.00 (0.00)	45.71 (39.38)	48.57 (41.18)
	Mean (A×C)	11.91 (16.50)	4.76 (7.41)	14.29 (18.18)	11.91 (16.50)	0.00 (0.00)			54.76 (49.26)	52.38 (46.45)	64.28 (53.68)	71.43 (57.98)	0.00 (0.00)		

Factors	**SE(m)**	**C.D. at 5%**	Factors	SE(m)	C.D. at 5%
Distance (A)	2.24	6.35	Distance (A)	1.71	4.84
Pipe diameter (B)	1.83	NS	Pipe diameter (B)	1.40	NS
A × B	3.17	NS	A × B	2.42	NS
Pressure (C)	3.90	NS	Pressure (C)	3.21	NS
A × C	5.02	NS	A × C	4.82	NS
B × C	4.10	NS	B × C	3.12	NS
A × B × C	7.10	NS	A × B × C	5.40	NS

[a] kilo Pascal. Values in parentheses are *arc sine* transformed. SE (m): Standard Error of Mean; CD: Critical difference; NS: Non-significant.

A perusal of literature reveals that no such studies have been conducted earlier. There is no published report on the horizontal and vertical movement of IJs applied in the field via drip irrigation.

Nematodes need a thin water film in soil pore spaces for movement. If thickness of the water film is approximately half the thickness of the nematodes' body, it is optimum for nematode movement (Wallace, 1958). To escape negative effects of solar radiation and desiccation, the nematodes applied on soil must move quickly (Gaugler, 1988). Only a small proportion of EPN populations disperse when placed on the soil surface (Kaya, 1990).Inactivity of EPNs in the soil is a behavioral strategy for their persistence in the soil. They become active in response to various mechanical and chemical stimuli. Migration of EPNs in the soil increases their chances for encountering a susceptible host as well as permits them escape from unfavourable habitats (Ishibashi and Kondo, 1990). Choo and Kaya (1991) demonstrated that shape and size of the root system of the plant influence the distribution of target insects as well as migration and host finding of nematodes. Paunikar and Kulkarni (2020) evaluated the effect of soil texture, moisture and depths on survival and infectivity of *Steinernema dharanaii* against *G. mellonella*. The IJs could move easily at soil moisture level of 10 to 20% up the distance of 3.5 cm, whereas at moisture level 40.0%, there was no IJ movement. Since the physical edaphic properties (temperature, moisture content etc.) affect the nematode migration behaviour to a large extent, further in depth studies are warranted on the impact of various edaphic factors for comparison with the available information on the topic (Koppenhöfer and Fuzy, 2007; Manimaran *et al.,* 2012; Kulkarni *et al.,* 2015).

It can be concluded that after 96 h, horizontally maximum number of IJs were concentrated at 3 inch distance compared to 2 and 4 inch distance; while vertically, maximum concentration IJs was recorded at 4 inch distance compared to 2 and 3 inch distance as evident by maximum mortality of *G. mellonella* larvae at these points. Also, movement of IJs was positively correlated with time. The rate of downward migration was faster compared to lateral movement, suggesting that IJs showed positive geotropism. Regulating pressure of drip irrigation system or use of pipes having diameters of 16 or 20 mm had no effect on the migration of *H. indica* IJs post application in the field.

CONCLUSION

It is, therefore, concluded from this study that the EPNs that survived the hydrodynamic conditions of drip irrigation system were not damaged and were able to maintain their migration equivalent to those EPNs that had not been applied through drip irrigation besides the use and relevance of application of their formulations through drip irrigation. The results of present study are only indicative of the migration potential of IJs of *H. indica* applied via drip irrigation system. Nematode migration in field conditions depends on a plethora of other factors such as the presence of the host insect, physical and chemical properties of the soil, soil temperature, and characteristic of root systems etc.

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