Infective juveniles of entomopathogenic nematode, *Heterorhabditis indica* can be applied through fully automated high pressure micro sprinkler irrigation system

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Abstract: *Application of infective juveniles (IJs) of entomopathogenic nematode,* Heterorhabditis indica *in the field through fully automated high pressure micro sprinkler irrigation system may affect survival and infectivity 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 Mini-sprinklers with 2.3mm fan nozzles (2-3 bar), outflow 355 L/h and 16 m wetted diameter were used for the study. Three release pressures(200, 300 and 400 kPa) and two types of pipes having diameters of 16 mm and 20 mm were evaluated. There were no differences in the means of viability and infectivity of IJs applied via fully automated high pressure micro sprinkler irrigation system using any of the tested pipe diameters and at all the three tested heads compared to the control.*

Keywords: *EPN, Sprinkler,* 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 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 survival and infectivity of infective juveniles of *H. indica* after

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their passing through fully automated high pressure micro sprinkler irrigation system in the field to facilitate *H. indica* to establish itself as a potent bio-control agent under Indian conditions.

MATERIALS AND METHODS

This 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. The field dimensions were100 m×30 m. Empty plots used for small scale injection of IJs consisted of six drip laterals each 10 m long (Netafim Irrigation Co.). Mini-sprinklers with 2.3 mm fan nozzles (2-3 bar), outflow 355 L/ h and 16 m wetted diameter were used for the study. The pressure compensating emitters were 1.0 m apart. Three release pressures (200, 300 and 400 kPa) and two types of pipes having diameters of 16 mm and 20 mm were evaluated.Treatments were assigned in a completely randomized design and were replicated three times. Before injecting IJs, the drip lines were flushed. The irrigation water was checked for the presence of nematodes before the application. 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 million *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 main line. The injection port was then sealed to avoid nematode leakage. The irrigation pump was stopped during the nematode injection process and the lines were pressurized after application. Each line was a replicate. To evaluate nematode distribution along the lines, drip water was collected under emitters of each of the six laterals for 30 min. Nematodes were allowed to settle to the bottom of each plastic container in the lab for 12 h before the water was siphoned off, leaving around 100 ml containing the nematodes. Five aliquots of 1ml each were taken to estimate number of IJs using a zoom binocular microscope. The percent of nematodes recovered from the drip system was determined by multiplying the mean number of nematodes recovered per emitter by the total number of emitters in a line, dividing by the number of nematodes injected, and multiplying by 100.

Viability of *H. indica* IJs

The procedure for quantifying nematode viability is based on the commonly used practice, where the numbers of live and dead nematodes within a defined sample are counted using a light microscope. Live and dead EPNs of the IJ stage are easily distinguishable from one another. All EPN species move in a sinusoidal manner and will budge when prodded. Dead nematodes appear straight and do not move. For each experimental trial, the treated suspension was thoroughly mixed and a 1000ìl subsample was removed with a micro-dispenser and added to approximately 10 ml of water in a Petri dish with grid base to allow easy viewing with the light microscope.

Quantification of *H. Indica* infectivity

Infectivity profile of IJs of *H. indica* was developed following a filter paper technique reported by Miller (1989). Twenty five randomly picked live EPNs were transferred using a micro-dispenser into each plate well lined with double Whatman No.1 filter paper containing one larva of *G. mellonella* (12-well sterile polystyrene tissue culture plates with 15.6 mm diameter wells was used). The plates were sealed and incubated at 25 °C. After 24, 48, and 72 and hours, dead larvae were collected to determine nematode infectivity. The dead larvae were dissected under stereomicroscope in order to prove whether the larva has been killed by nematodes. For each replication, a plate with water but no EPNs was included as a control for *G. mellonella*. The average percent infectivity of EPNs against *G. mellonella* for each treatment was determined by taking the average number of dead *G. mellonella* larvae for the treatment, subtracting

the average dead *G. mellonella* larvae for the control, and dividing by 12 (the number of wells per plate).

Statistical Analysis

Data were arcsine transformed and analyzed by a completely randomized, factorial ANOVA and means were compared at the P= 0.05 level.

RESULTS AND DISCUSSION

The data obtained on the effect of fully automated high pressure micro sprinkler irrigation system operated at three pressures (200 kPa, 300 kPa and 400 kPa) on the survival and infectivity of infective juveniles of *Heterorhabditis indica* is presented in tables 1 and 2, respectively.

Table 1: Survival of *H. indica* applied via Fully Automated High Pressure micro sprinkler irrigation system (Mean of 3 replications)

Pipe diameter (D)	Per cent survival				
	Pressure ([a]kPa) (P)				Mean (D)
	400	300	200	Control	
16 mm	93.50(75.50)	96.00(79.74)	96.17(79.22)	97.00(80.33)	**95.67(78.70)**
20 mm	94.67(76.93)	95.17(77.97)	96.67(80.10)	97.00(80.33)	**95.88(78.83)**
Mean (P)	94.08(76.22)	95.58(78.85)	96.42(79.66)	97.00(80.33)	

Factors	SE (m)	C.D. at 5%
D	0.763	NS
P	1.079	NS
D×P	1.526	NS

[a] Kilo Pascal

Values in parentheses are arc sine transformed.

SE(m): Standard Error of Mean; CD: Critical difference; NS: non-significant.

There were no significant differences in the means of viability of IJs applied via Fully Automated High Pressure micro sprinkler irrigation system compared to the control (Table 1). Therefore, for IJs of *H. indica,* the use of both pipes having diameter 16 and 20 mm and pressure up to 400 kPa is tolerable and does not affect the viability when applied via Fully Automated High Pressure micro sprinkler irrigation system.

Table 2: *In vitro* mortality of *Galleria mellonella* larvae by *Heterorhabditis indica* infective juveniles after passing through Fully Automated High Pressure micro sprinkler irrigation system (at 27°C ±1°C)

(Mean of 3 replications)

Time (T)	Pipe diameter (D)	Per cent mortality				Mean (T×D)	Pooled mean (T)
		Pressure ([a]kPa) (P)					
		400	300	200	Control		
24 h	16 mm	37.50(37.77)	37.50(37.77)	40.00(39.25)	47.50(43.59)	40.63(39.59)	40.31(39.41)
	20 mm	37.50(37.77)	35.00(36.29)	40.00(39.25)	47.50(43.59)	40.00(39.22)	
	Mean (T×P)	37.50(37.77)	36.25(37.03)	40.00(39.25)	47.50(43.59)		
48 h	16 mm	50.00(45.03)	72.50(58.43)	75.00(60.15)	80.00(63.47)	69.38(56.77)	74.38(59.92)
	20 mm	77.50(61.75)	80.00(63.47)	80.00(63.64)	80.00(63.47)	79.38(63.08)	
	Mean (T×P)	63.75(53.39)	76.25(60.95)	77.50(61.89)	80.00(63.47)		
72 h	16 mm	82.50(65.36)	90.00(71.60)	100.00(90.05)	100.00(90.05)	93.13(79.27)	95.63(82.70)
	20 mm	92.50(74.36)	100.00(90.05)	100.00(90.05)	100.00(90.05)	98.13(86.13)	
	Mean (T×P)	87.50(69.90)	95.00(80.83)	100.00(90.05)	100.00(90.05)		

	Mena (P×D)				
	400	300	200	Control	Pooled mean D
16	56.67(49.39)	66.67(55.93)	71.67(63.15	75.83(65.70)	67.71(58.54)
20	69.17(57.96)	71.67(63.27)	73.33(64.31	75.83(65.70)	72.50(62.81)
Pooled mean P	62.92(53.67)	69.17(59.60)	72.50(63.73	75.83(65.70)	

Factors	SE (m)	C.D. at 5%
T	1.2	3.51
D	0.98	2.86
T ×D	1.698	NS
P	1.386	4.05
T × P	2.401	7.01
D × P	1.96	5.72
T × D × P	3.395	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 data in table 2 indicated that *H. indica* infectivity was slightly reduced with an increase in pressure compared to control. Irrespective of pipe diameter and pressure, pooled mean (T) revealed that infectivity was significantly maximum after 72 h compared to 24 or 48 h. Irrespective of pipe diameter and time, a pressure of 200 kPa was statistically at par with control, while pressures of 300 and 400 kPa caused significantly less mortality than control. Pooled mean (D) revealed that use of pipe having diameter 20 mm showed better infectivity compared to that of 16 mm. Interaction of pipe diameter with pressure time with pressure were also significant, whereas, interaction of time with pipe diameter and interaction of all the three factors was non-significant.

Fife *et al.* (2003) studied viability and infectivity differences among various EPN species in relation to pressure differential treatments and recommended 1380 kPa (200 psi) for *H. megidis* and 2000 kPa (290 psi) for *S. carpocapsae* and *H. bacteriophora*. Garcia *et al.* (2005) established that *S. glaseri* retained its viability at 1379 MPa. Cleyton Batista de Alvarenga *et al.* (2018) evaluated the effects of hydraulic spray nozzles operated at working pressure of 400 kPa on the viability and infectivity of the *H. amazonensis* IJs and found that the nematodes which were live after spray were able to infect *Tenebrio molitor* larvae. These data suggest that low pressure equipment, in general, do not affect the viability and infectivity of IJs. Also, each nematode species/ strain might have its own recommended pressure.

CONCLUSION

It is, therefore, concluded from this study that in case of fully automated high pressure micro-sprinkler irrigation system, no differences were recorded on the viability of the IJs applied at 400 kPa but *H. indica* infectivity was slightly reduced with an increase in pressure. Pressure of 200 kPa was statistically at par with control, while pressure of 300 and 400 kPa caused less mortality than control. Use of pipe having 20 mm diameter showed better infectivity compared to pipe having 16 mm diameter.

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