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Application of Infective Juveniles of Entomopathogenic nematode, *Heterorhabditis indica,* through Low Pressure Drip Irrigation System under Protected Cultivation System

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Abstract: Low Pressure Drip Irrigation System may be used for application of infective juveniles (IJs) of entomopathogenic nematode, *Heterorhabditis indica* in protected cultivation. The hydrodynamic forces exerted during flow through laterals and emitters may affect survival and infectivity of nematodes. Three heads (1.0, 1.5 and 2.0) and two types of pipes having diameters of 16 mm and 20 mm were evaluated. The IJs were injected at the point where the drip line left the main line. A 100 ml dilution of one lakh *H. Indica* IJs was injected at the point where the drip line left the filter. Drip water was collected under opening of each of the six laterals for 30 min. and nematodes were allowed to settle to the bottom of each plastic container and were used for further studies on survival and infectivity. Viability of IJs was evaluated under the microscope after the IJ passed through the drip irrigation systems. Infectivity on *G. mellonella* larvae as evidenced by larval mortality were evaluated under lab conditions *in vitro*. Results showed that survival and infectivity of infective juveniles of *H. indica* is not affected with an increase in head up to 2 m compared to control. Use of pipe having diameter 20 mm showed infectivity at par to pipe having diameter 16 mm. Therefore, for IJs of *H. indica,* the use of both types of pipe, having diameter 16 and 20 mm, and head upto 2m is perfectly tolerable and does not affect the viability.

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

INTRODUCTION

Heterorhabditis indica is highly virulent against soil insect pests and have been used widely against many economically important insect pests of crops [4, 7]. EPNs can be applied with standard pesticide equipments (hand pumps, spray cannons etc.) as well as through different irrigation systems and other application types such as cadaver application [8, 9]. As EPNs are tolerant to shear stress, they can survive under high pressure [2].

In the current agricultural scenario, 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. Because of the negative sides of pesticide spraying tools, a more suitable application method should be developed for EPN application. Thus, our study aimed to examine the performance of low pressure drip irrigation system for delivering EPNs with three different heads and pipes having diameters 16 and 20 mm. The objective of this study was to evaluate the survival and infectivity of infective juveniles of *H. indica* after their passing through Low Pressure Drip

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Irrigation System. It is expected that this study will sort out useful knowledge about use of drip irrigation optimization for EPN application.

MATERIALS AND METHODS

The experiment was conducted at field of CPCT, ICAR-IARI, New Delhi during 2020.

Entomopathogenic Nematode

Entomopathogenic nematode, *Heterorhabditis indica,* was used in experiments. EPNs were reproduced using in vivo method with greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) [5]. Freshly emerged infective juveniles from the wax moth larval cadavers were collected in sterilized distilled water using White traps [10] and were used for subsequent studies within 2-4 weeks. Mortality of the populations was generally lower than 1%.

Drip irrigation system

The field dimensions were 100 m × 30 m. 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 30 cm. A filter after the control valve was installed to prevent blockages.

Application

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.

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 nematode distribution along the lines, drip water was collected under opening 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. These IJs were used for further studies on survival and infectivity. 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. *H. indica* IJs viability and infectivity was quantified

Viability of *H. indica* IJs

Numbers of live and dead nematodes within a defined sample are counted using a light microscope. For each experimental trial, the treated suspension was thoroughly mixed and a 1000µl 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 were used). The plates were sealed and incubated at 25 °C. After 24, 48 and 72 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).

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 survival and infectivity of infective juveniles of *Heterorhabditis indica* released via low pressure drip irrigation system operated at 3 heads (1 m, 1.5 m and 2.0 m) is presented in tables 1 and 2, respectively.

Table 1: Survival of *Heterorhabditis indica* applied via low pressure drip irrigation

(Mean of 3 replications)

Pipe diameter (mm)	*Per cent survival*				
	Head (m)				*Mean (Diameter)*
	1.0	*1.5*	*2.0*	*Control*	
16	96.33 (79.20)	96.33 (80.31)	96.67 (80.68)	97.00 (80.33)	96.58 (80.13)
20	97.17 (80.73)	98.33 (84.06)	97.67 (82.26)	97.00 (80.34)	97.54 (81.85)
Mean (Head)	96.75 (79.96)	97.33 (82.18)	97.17 (81.47)	**97.00 (80.33)**	

Factors	*SE (m)*	*C.D. at 5%*
Pipe diameter (A)	0.834	NS
Head (B)	1.18	NS
A×B	1.669	NS

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

Table 2: *In vitro* mortality of *Galleria mellonella* larvae by *Heterorhabditis indica* infective juveniles after passing through a low pressure drip irrigation system at 27°C ±1°C

(Mean of 3 replications)

Time (T)	*Pipe diameter (D)*	*Per cent mortality of Galleria mellonella*					*Pooled mean (T)*
		Head (H)				*Mean (T×D)*	
		1.0 m	*1.5 m*	*2.0 m*	*Control*		
24 h	16 mm	45.50 (42.70)	45.00 (42.25)	47.50 (43.59)	47.50 (43.59)	46.38 (43.03)	46.69 (43.02)
	20 mm	47.50 (43.59)	47.50 (43.59)	45.50 (42.70)	47.50 (43.59)	47.00 (43.37)	
	Mean (T×H)	46.50 (43.59)	46.25 (42.92)	46.50 (43.15)	47.50 (43.59)		
48 h	16 mm	82.50 (65.36)	82.50 (65.36)	80.00 (64.02)	82.50 (65.36)	81.88 (65.03)	80.94 (64.28)
	20 mm	82.50 (65.36)	77.50 (61.75)	77.50 (61.75)	82.50 (65.36)	80.00 (63.56)	
	Mean (T×H)	82.50 (65.36)	80.00 (63.56)	78.75 (62.89)	82.50 (65.36)		
72 h	16 mm	100.00 (90.05)	95.00 (77.12)	100.00 (90.05)	100.00 (90.05)	98.75 (84.89)	99.38 (89.80)
	20 mm	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	
	Mean (T×H)	100.00 (90.05)	97.50 (83.59)	100.00 (90.05)	100.00 (90.05)		

Mean (D×H)					
	1	*1.5*	*2*	*Control*	*Pooled mean D*
16	76.00 (66.04)	74.17 (61.58)	75.83 (65.89)	76.67 (66.33)	75.67 (64.96)
20	76.67 (66.33)	75.00 (65.13)	74.33 (64.83)	76.67 (66.33)	75.67 (65.66)
Pooled mean H	76.33 (66.19)	74.58 (63.35)	75.08 (65.36)	76.67 (66.33)	

Factors	*SE (m)*	*C.D. at 5%*
Time (T)	0.536	1.565
Pipe diameter (D)	0.438	NS
T×D	0.758	NS
Head (H)	0.619	NS
T×H	1.072	NS
D×H	0.876	NS
T×D×H	1.516	NS

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

There were no differences in the means of viability of IJs applied via Low Pressure drip irrigation using any of the tested pipe diameters and at all the three tested heads compared to the control (Table 1). Therefore, for IJs of *H. indica,* the use of both types of pipe, having diameter 16 and 20 mm, and head upto 2m is perfectly tolerable and does not affect the viability.

Table 2 shows the mean infectivity of IJs of *H. indica* passed through low pressure drip irrigation system against *G. mellonella* larvae after 24, 48 and 72 h of inoculation. Pooled mean (T) revealed that per cent mortality increased with time from 24h to 72 h in all the treatments including control. A perusal of data indicated that *H. indica* infectivity is not affected with an increase in head up to 2 m compared to control. Use of pipe having diameter 20 mm showed infectivity at par to pipe having diameter 16 mm. The results suggest that the *H. indica* infective juveniles that survived the passing through drip irrigation were able to maintain infectivity rates equivalent to those that had not been passed through the drip irrigation system.

Fife *et al.* [2] 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.* [3] established that *S. glaseri* retained its viability at 1379 MPa. Cleyton Batista de Alvarenga *et al.* [1] 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 equipments, 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 the EPNs that survived the hydrodynamic conditions of low pressure drip irrigation system were not damaged and were able to maintain their infectivity equivalent to those EPNs that had not been applied through low pressure drip irrigation. The results of present study are only indicative of the survival and infectivity potential of IJs of *H. indica* applied via low pressure drip irrigation system.

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