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Standard Knapsack Sprayer's Nozzles and Pressures does not affect Viability and Infectivity of Entomopathogenic nematode, *Heterorhabditis indica*

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Abstract: Exposure to hydrodynamic stresses during flow through nozzles can cause permanent damage to *Heterorhabditis indica* during spray application. Aqueous suspensions of *Heterorhabditis indica* were passed through four different nozzles [standard flat fan (model FFP/ 95/ 900; Flood jet (model XLP/ WP/ 40), hollow cone (model HCN/ PB) and duro mist (model NM/DS 450) fitted on standard knapsack sprayer (Manufacturer: ASPEE Model: Vibrant, Code: AVB16, 16 L capacity). The sprayer was set up in the laboratory to examine the effect of passage through different nozzles at various pressure ranges on the viability and infectivity of the nematodes. As a control, just before spraying, a control sample was taken directly from the sprayer tank. Four operating pressure ranges administered were (0-20, 20-40, 40-60 and 60-80 psi). Overall, the Flat fan nozzle performed better than the other nozzles and it resulted in maximum mean % survival (86.88) for all the range of operating pressures. Percent survival after flow through the nozzles increased as we lowered the operating pressure range, being maximum at lowest operating pressure range of less than 20 psi (84.08). Infectivity of *H. indica* IJs on *Galleria. mellonella* larvae reduced with an increase in pressure range after 24, 48 or 72 h. The total infectivity increased with time from 24h to 48h and to 72h. Difference in infectivity of IJs after 24, 48 or 72 h with respect to type of nozzle was found to be insignificant. Maximum infectivity after 24, 48 and 72 hours was observed at lowest pressure range (0-20 psi) and as we increased the operating pressure range, there was a decrease in infectivity of IJs, being maximum reduction at highest pressure range (60-80 psi). The bioassay provides consistent results of 64 to71% mortality of *G. mellonella* within 72 h for *H. indica*. The differences in EPN damage were due to the distinct characteristics of each nozzle's flow field. Overall, common

sprayer nozzles were found to be acceptable for spray application of EPNs following the manufacturer's recommendations.

Keywords: *Heterorhabditis indica*, Nozzle, Spray, Pressure.

Heterorhabditis indica (Rhabditida: Heterorhabditidae) is an environment friendly bioagents that have become an important tool for management of pests. This nematode has the ability to disperse in the environment and pose no risk to plants and other animals. EPNs can be applied using conventional spraying systems and irrigation systems (Grewal, 2002). A major obstacle for field level application of *H. indica* under Indian scenario is the lack of information on the delivery system and post-application survival and infectivity. In a conventional knapsack sprayer, the liquid suspension in the tank is pumped and forced under pressure through the orifice of the nozzle to the atmosphere. During flow through the sprayer system, a variety of physical stresses acts on the biological agents. A detailed study on effect of these physical forces operating within the spray system on the survival and efficacy of biological agents being applied is a primary requirement for the identification of operating conditions that are crucial to the biological agents. Broadly, there are two types of nozzles, flat-fan and cone-type, which are commonly used in conventional knapsack sprayer. Standard fan nozzles have a narrow elliptical orifice through which it produces a sheet of liquid, which develops into various sizes of droplets through atomization. The flat-fan nozzle is typically used for broadcast spraying herbicides and fertilizers on targets such as bare soil, field crops or weeds. The cone nozzle gives the liquid a high rotational velocity inside its cavity of the nozzle before the liquid passes through a circular orifice and as a result, produces a cone-shaped spray pattern. The cone nozzle is typically used for spraying contact materials and foliage where good coverage is required, mainly to control insects and fungal diseases. Keeping in view the existing gaps in our knowledge regarding application technology of *H. indica*, present investigation was undertaken with the objective to standardize the delivery system for *Heterorhabditis indica* using different spray nozzles at various pressure ranges.

MATERIALS AND METHODS

Pure population of *H. indica* was cultured in the laboratory on the greater wax moth larvae, *Galleria mellonella* following the standard procedures (Kaya and Stock, 1997). Infective juveniles (IJs) emerging from the wax moth larval cadavers were collected in sterilized distilled water using White traps (White, 1927) and stored in BOD incubator (Sandeep Instruments and Chemicals (SANCO), New Delhi, India) at 15°C. The IJs from this suspension were used for subsequent studies within 2-4 weeks.

Standard knapsack sprayer (Manufacturer: ASPEE, Model: Vibrant, Code: AVB16 (16 L) was used for this study. Details of various nozzle models used in the study are as follows:

Table 1: Details of various spray nozzles used in the study

Name of nozzle	Code no.	Pressure(kg/ cm^2)	Spray angle	Discharge (cc/min)
Flat fan	FFP/ 95/ 900	2.8	95°	900
Flood jet	XLP/ WP/ 40	0.7	60°	500
Hollow cone	HCN/ PB	2.8	80°	450
Duro mist	NM/DS 450	3.0	80°	450

Manufacturer: ASPEE (Head office: 4th Floor, Aspee House, Aspee Enclave, P.O. Box No. 7602, Opp. I.O.B. Bank, Marve Road, Malad (West), Mumbai - 400064, Maharashtra, India; Plate No. 3.2)

The sprayer was set up in the laboratory to examine the effect of passage through different nozzles at various pressure ranges on the viability and infectivity of the nematodes. Approximately 30 min before every test, 2.5 million IJs were suspended in 200 ml of water before it was added to 10 L of water in the sprayer tank to obtain a suspension of approximately2500 IJs/ml and the suspension was mixed for 5 min using a wooden rod to ensure an even distribution of IJs in the spray tank.

A sample was taken directly from the sprayer tank just before spraying, to serve as a control. Four operating pressure ranges administered were: 0-20, 20-40, 40-60 and 60-80 psi). Pressure was monitored by a Calibro brand glycerine filled pressure gauge with full scale between 0 and 100 PSI (0-7 kg, cm^2) fitted on the delivery pipe connecting lance with the sprayer. The experiment was conducted in a randomized complete design with 17 treatments, being 4 models of nozzles, 4 operating pressure range and one control. For each treatment, six repetitions were performed. The control was used to determine the effect of the nozzle model and pressure range on viability and infectivity of EPNs.

The pressure inside the sprayer was increased from atmospheric pressure to the desired pressure by pressing the piston lever with hand. The suspension was then released through the nozzle outlet orifice by operating the trigger valve. Samples were taken at the outlet of the nozzles in a 500 ml glass beaker lined with two layers of Whatman No.1 filter paper at successive intervals corresponding to decreasing pressure in the spray tank. The experiment was replicated three times each with a different IJs suspension.

In each test and for each treatment, a sample of 100 ml was taken for analysis. After each trial, until EPN counting, samples were stored in plastic test tubes in a BOD incubator at a temperature of 14-16 °C for a maximum of 24 hrs. Three sub-samples of nematodes (1 ml each) were extracted from each of the 100 ml sample using a calibrated pipette. The sub-samples, diluted with 10 ml of distilled water, were placed in Petri dishes with a grid base, and the nematodes were observed using a binocular microscope. IJs were considered dead if they did not respond to prodding. Relative nematode viability was calculated as the percentage of living nematodes.

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 1000il 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.

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 4 different nozzles operated at 4 pressure ranges (60-80, 40-60, 20-40 and 0-20 psi) on the survival and infectivity of IJs of *Heterorhabditis indica* are presented in tables 2 and 3, respectively.

Table 2. Per cent survival of *Heterorhabditis indica* IJs after flow through different nozzles operated at various pressure ranges in a standard knapsack sprayer

(Mean of 3 replications)

Nozzle (N)	Nematode survival (%) Pressure range ([a]psi) (P)					
	60-80	40-60	20-40	0-20	Control	Mean (N)
Flat fan	97.98(84.37)	99.50(87.72)	98.52(85.37)	100.00(90.05)	100.00(90.05)	99.20(87.51)
Flood Jet	93.17(75.25)	95.50(78.06)	96.17(79.08)	98.00(84.40)	100.00(90.05)	96.57(81.37)
Hollow cone	92.33(74.16)	93.00(74.84)	95.33(77.79)	97.17(81.38)	100.00(90.05)	95.57(79.64)
Mist	87.67(69.49)	90.83(72.46)	93.17(74.98)	96.50(80.49)	100.00(90.05)	93.63(77.49)
Mean (P)	92.79(75.82)	94.71(78.27)	95.80(79.31)	97.92(84.08)	100.00(90.05)	96.24(81.50)

Factor	SE (m)	C.D. at 5%
Nozzle	0.80	2.25
Pressure	0.80	2.25
N×P	1.60	N.S.

Values in parentheses are *arc sine* transformed.

[a]Pounds per square inch; SE (m): Standard Error of mean; C.D.: Critical difference

Table 2 summarizes the observed percent survival of IJs of *Heterorhabditis indica* with respect to the type of nozzle and operating pressure range used for delivery of IJs using a standard knapsack sprayer. The differences in mean nematode survival among nozzle types (P=0.05) and for different operating pressure ranges (P=0.05) were statistically significant, whereas their interaction (nozzle × pressure) was non-significant.

Comparison of individual treatment means provides further insight. Overall, the Flat fan nozzle performed better than the other nozzles and it resulted in significantly maximum mean survival (99%) for all the range of operating pressures. Per cent survival after flow through the nozzles increased as the operating pressure range was lowered, being maximum at lowest operating pressure range of less than 20 psi (97.92%) which was at par with 20-40 psi range. Overall, *H. indica* IJs experienced only a little reduction in survival after passing through various tested nozzles and could withstand the pressure differentials and hydrodynamic conditions of a knapsack sprayer operated up to 80 psi pressure.

Table 3. Infectivity of *Heterorhabditis indica* infective juveniles after flow through different nozzles at various pressures ranges in a standard knapsack sprayer on *Galleria mellonella* larvae *in vitro* (at 27°C ±1°C)

(Mean of 3 replications)

Time (T)	Nozzle (N)	Per cent mortality of *Galleria mellonella*							
		Pressure range ([a]psi) (P)					Control	Mean (T×N)	Pooled mean (T)
		[b]Initial	60-80	40-60	20-40	0-20			
24 h	Flat fan	62.50 (52.30)	41.50 (40.56)	52.50 (46.14)	62.30 (52.50)	37.50 (37.77)	0.00 (0.00)	42.72 (38.21)	42.10 (37.38)
	Flood Jet	62.50 (52.30)	42.50 (40.70)	45.00 (42.10)	50.00 (45.00)	57.50 (49.34)	0.00 (0.00)	42.92 (38.24)	
	Hollow cone	62.50 (52.30)	35.00 (36.20)	41.00 (39.30)	57.50 (49.30)	57.50 (49.30)	0.00 (0.00)	42.25 (37.73)	
	Mist	62.50 (52.30)	33.50 (34.80)	52.50 (36.50)	42.50 (42.70)	52.00 (45.60)	0.00 (0.00)	40.50 (35.32)	
	Mean (T×P)	62.50 (52.30)	38.13 (38.07)	47.75 (41.01)	53.08 (47.38)	51.13 (45.50)	0.00 (0.00)		
48 h	Flat fan	82.50 (65.40)	80.00 (63.60)	77.50 (62.04)	80.00 (63.50)	82.50 (65.40)	0.00 (0.00)	67.08 (53.32)	66.59 (53.04)
	Flood Jet	82.50 (65.40)	75.00 (60.20)	75.00 (60.20)	80.00 (63.50)	82.50 (65.40)	0.00 (0.00)	65.83 (52.45)	
	Hollow cone	82.50 (65.40)	75.50 (61.80)	80.00 (63.50)	77.60 (62.00)	82.50 (65.40)	0.00 (0.00)	66.35 (53.02)	
	Mist	82.50 (65.40)	80.00 (63.50)	77.50 (62.00)	77.50 (62.00)	85.00 (67.25)	0.00 (0.00)	67.08 (53.36)	
	Mean(T×P)	82.50 (65.40)	77.63 (62.28)	77.50 (61.94)	78.78 (62.75)	83.13 (65.86)	0.00 (0.00)		
72 h	Flat fan	90.00 (71.60)	82.00 (65.80)	85.00 (67.30)	85.00 (67.30)	90.50 (71.60)	0.00 (0.00)	72.08 (57.27)	68.58 (57.60)
	Flood Jet	90.00 (71.60)	80.00 (64.50)	85.00 (67.60)	90.00 (71.60)	90.00 (71.60)	0.00 (0.00)	72.50 (57.82)	
	Hollow cone	90.00 (71.60)	80.50 (64.40)	90.00 (71.60)	88.00 (72.30)	86.00 (70.50)	0.00 (0.00)	72.42 (58.40)	
	Mist	90.00 (71.60)	75.00 (61.60)	87.50 (69.40)	85.00 (67.30)	90.00 (71.60)	0.00 (0.00)	71.25 (56.92)	
	Mean(T×P)	90.00 (71.60)	81.90 (64.08)	86.90 (68.98)	86.25 (69.63)	88.90 (71.33)	0.00 (0.00)		

	Mean (N×P)						
	Initial	**60-80**	**40-60**	**20-40**	**0-20**	**Control**	**Pooled mean (N)**
Flat fan	78.33(63.10)	67.83(56.65)	71.67(58.49)	75.77(61.10)	70.17(58.26)	0.00(0.00)	60.63(49.60)
Flood Jet	78.33(63.10)	65.83(55.13)	68.33(56.63)	73.33(60.03)	76.67(62.11)	0.00(0.00)	60.42(49.50)
Hollow cone	78.33(63.10)	63.67(54.13)	70.33(58.13)	74.37(61.20)	75.33(61.73)	0.00(0.00)	60.34(49.72)
Mist	78.33(63.10)	62.83(53.30)	72.50(55.97)	68.33(57.33)	75.67(61.48)	0.00(0.00)	59.61(48.53)
Pooled mean (P)	78.33(63.10)	65.04(54.81)	70.71(57.31)	72.95(59.92)	74.46(60.90)	0.00(0.00)	

Factors	SE (m) ±	C.D. at 5%
Time (T)	0.77	2.19
Nozzle (N)	0.89	N/A
T×N	1.55	N/A
Pressure range (P)	1.00	2.83
P×T	1.73	4.90
P×N	2.00	NS
T×N×P	3.46	NS

Values in parentheses are *arc sine* transformed.

[a]Pounds per square inch; [b] IJs were taken directly from the sprayer tank.

SE (m): Standard Error of mean; C.D.: Critical difference

A perusal of data in table 3 indicated that irrespective of nozzles and pressure ranges, pooled mean (T) revealed that 72 h (68.58% mortality) and 48 h (66.59% mortality) were most effective and at par in terms of *Galleria* mortality. The *Galleria* mortality was significantly less (42.1%) at 24 h.

Considering the effect of pressure ranges alone (irrespective of nozzles and time), maximum infectivity was observed at lowest pressure range (0-20 psi) and as the operating pressure range was increased, there was a decrease in infectivity of IJs, maximum reduction being at highest pressure range (60-80 psi). Type of nozzle alone at all pressure ranges and time did not cause significantly more mortality than what resulted without use of nozzle. The interaction of pressure range and time was also significant. After 24 h, significantly less mortality was recorded in case where the IJs were passed through nozzles at all pressure ranges tested compared to control. After 48 or 72 h, significantly less mortality was recorded only in case of highest pressure range (60-80 psi). The interaction of time with nozzle, pressure with nozzle and all three factors taken together (pressure×time×nozzle) was non-significant.

Among the tested nozzles, the flat fan nozzle performed better than the other nozzle types and it resulted in significantly maximum survival (99%) at all ranges of operating pressures. Per cent survival after flow through the nozzles increased as the operating pressure range was lowered; it was maximum at lowest operating pressure range of less than 20 psi (97.92% survival) which was at par with 20-40 psi range. Overall, *H. indica* experienced reduction in survival after passing through various tested nozzles of a knapsack sprayer operated up to 80 psi pressure. Significantly maximum infectivity after 24, 48 and 72 h was observed at lowest pressure range (0-20 psi) and it reduced with increase in the operating pressure range; maximum reduction was recorded at highest pressure range (60-80 psi).

The decrease in viability and infectivity of IJs was probably due to mechanical stresses from the nozzle and the rise in temperature in the suspension due to high pressure. Consequently, nozzle type and operating pressure range are important factor to consider when defining spray operating conditions. The general recommendation for EPN application has been "common agricultural sprayers fitted with nozzles with exit orifices larger than 50 ìm and operating at pressures less than 2000 kPa (290 psi) (Georgis, 1990; Shetlar, 1999); however, no studies were cited to support these recommendations. Evaluation of different physical phenomenon occurring within a common knapsack sprayer is an important step for optimizing the performance of EPNs applied through it. During passage through a common knapsack sprayer, IJs are confronted with a sharp pressure drop from the sprayer operating pressure to the atmospheric pressure as the suspension is released through the nozzle exit orifice. This may cause the stress on nematode body, thus reducing their potential efficacy. Direct influence of application equipment on EPNs has been studied earlier by different workers as discussed below.

Klein and Georgis (1994) studied effects on application of *Steinernema* spp. and *Heterorhabditis bacteriophora* involving different pumps (piston, centrifugal, roller, and diaphragm), nozzle types (Spraying Systems XR8001VS, TK-VS2, FL-5VS), and strainers (100 mesh, 50 mesh, and 50 slotted) and reported no adverse effects. However, they did not report any data explicitly and the criteria for determining survival and infectivity.

The results of present study are in conformity with Nilsson and Gripwall (1999) who investigated the effect of application technique on the viability of *Steinernema feltiae* with a backpack sprayer operated at 200 kPa, fitted with diaphragm pump and Hardi 4110-12 fan nozzle, and a high-pressure sprayer with 1000 and 2000 kPa pressure fitted with piston pump and 1.2 mm Wanjet pressure swirl solid cone; reported that all high-pressure sprayer treatments significantly reduced viability of the nematodes.

Fife *et al.* (2003) studied the extent of damage to *H. bacteriophora*, *H. megidis and S. carpocapsae*, in aqueous suspension due to exposure to pressure differential; they reported that the magnitude of the pressure differential has an effect on the relative viability of EPNs and the effect is species dependent. Operating pressures less than 200 psi for *H. megidis* and less than 290 psi for *H. bacteriophora* and *S. carpocapsae* resulted in a viability of above 85%.

Fife *et al.* (2005) investigated the effects of two types of nozzles, flat fan and cone type, on damage to four EPN species (*H. bacteriophora*, *H. megidis*, *S. carpocapsae*, and *S. glaseri*). No damage to *H. bacteriophora* IJs was observed when sprayed at 60 psi from an air-pressurized canister, using either of these nozzles. Hence, both nozzles are acceptable for spray application when the manufacturer's recommendations are followed. When the flow rates were considerably higher than those suggested by the manufacturer, greater reductions in relative viability were observed for the flat fan nozzle compared to the hollow cone due to the distinct characteristics of each nozzle's internal flow field. However, hydrodynamic damage can be avoided by using a nozzle which is larger in size than the organism for spray application. Larger capacity flat fan nozzles resulted in zero nematode damage for the same range of experimental conditions. Larger capacity nozzles are more desired for applying EPNs in the soil where a high volume of water is necessary to get the nematodes beyond the soil surface. The results of present investigation support these findings.

The results of present study are in agreement with that obtained by Lanzoni *et al.* (2014); they demonstrated that a static pressure up to 14 bars

(203 psi) causes no significant damage to *S. carpocapsae* and that the passage of the nematode through the flat fan nozzles does not affect their viability.

Moreira *et al.* (2013) evaluated application of *S. feltiae* with backpack sprayer using three models of flat air induction spray nozzles (AI 11003, TTI 11003 and AD-IA 11004), three spray pressures (207, 413 and 720 kPa), four different additives for tank mix compatibility (cane molasses, mineral oil, vegetable oil and glycerin) and the influence of tank mixture stirring time. All nozzle models at pressures of up to 620 kPa were compatible with *S. feltiae*. Vegetable oil, mineral oil and molasses were compatible adjuvants for *S. feltiae*, and stirring in a motorized backpack sprayer for 30 min did not impact the viability or pathogenicity of this nematode.

Cleyton Batista de Alvarenga *et al.* (2018) evaluated the effects of 34 different hydraulic spray nozzles on the viability and infectivity of the IJs of *Heterorhabditis amazonensis* MC01. All nozzles were operated at working pressure of 400 kPa and an aqueous suspension of the nematode at a concentration of 400 IJs/ml was sprayed. Nematode viability was influenced by the nozzles and the nematodes which were live after spray were able to infect *Tenebrio molitor* larvae.

Based on the results found in this study, use of common spray nozzles for application of entomopathogenic nematodes using backpack sprayers in crop field may be advantageous to the growers.

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