Survival of Infective Juveniles of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) in open field and protected cultivation after their foliar application

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Abstract: *The survival of infective juveniles of* Heterorhabditis indica *(Rhabditida: Heterorhabditidae) was investigated after foliar application in open field and protected cultivation on soybean and tomato crops, respectively. The concentration used was 2500 infective juveniles (IJs)/ml water. On soybean, the survival of IJs was 46.61, 3.89 and 1.13% after 30, 60 and 90 min of spray, respectively in the morning hours; and 42.89, 13.20 and 4.48% after 30, 60 and 90 min of spray, respectively in the evening hours. Addition of adjuvant resulted in better survival of IJs. On tomato in protected cultivation, the survival of IJs of* H. indica *was 37.38, 15.13 and 3.26% at 30, 60, and 90 min post-spray.*

INTRODUCTION

Soybean (Glycine max (L.) Merr.) and tomato (*Solanum lycopersicum* L.) are important crops in India and provide nutritious food, feed and fodder and constitute an integral component of subsistence farming system of the country. Among the various biotic constraints, the infestation and damage caused by insect pests is one of the major constraints towards their low production. The insect-associated nematodes commonly known as entomopathogenic nematodes (EPN) are widely used as biological control agents. The EPN are highly pathogenic to a multitude of insects belonging to various orders and are commercially employed for insect pest management because of their ability to rapidly kill the insects (Kaya and Gaugler, 1993). A number of attributes of EPN, such as host-ûnding ability, rapid death of host insect after infection (24 – 48 h), nontoxicity to vertebrates and environmental safety, have generated interest in their use as biopesticides. Soil application of EPN through irrigation is a successful method against soil insect pests (Feaster & Steinkraus 1996), however, use of EPN to manage insect pests feeding on aerial parts poses a considerable challenge as aboveground conditions are detrimental to nematodes (Arthurs *et al*. 2004). Infective juveniles (IJs) get inactivated quickly and are sensitive to extremes of physical environment, particularly rapid desiccation (Womersley 1990), high temperature (Grewal *et al*. 1994), lethal UV radiation (Gaugler *et al*. 1992), and difûculty in establishing attraction gradients (Glazer 1992). Particularly, foliar application of EPN against aerial insect pests at 35 – 40 deg. C needs to be resolved by improving their survival and efûcacy. Efforts have been made to increase the survival of EPN through addition of adjuvant to minimize the above mentioned detrimental factors. An attempt was made to study the survival of *Heterorhabditis indica* on soybean and tomato in open field and protected cultivation system after foliar spray at flowering stage

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MATERIALS AND METHODS

Nematodes and insect culture

Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), required for in vivo production of EPN, was reared on a semi-synthetic diet as per procedure described by Ali *et al*. (2005). *H. indica* was multiplied on the last instar larvae of *G. mellonella* and freshly harvested IJs were used in the present study.

Foliar application of *H. indica* in open field

This experiment was conducted in the Division of Entomology field, ICAR-IARI, New Delhi on soybean (Variety: JS 335, Spacing: R×R: 4.5 cm. P×P: 15 cm.). Two formulations were tested: 1. EPN alone (Liquid EPN suspension containing 2500 IJs/ml) and 2. EPN + adjuvant (Liquid EPN suspension containing 2500 IJs/ml+ Sticker 99@1%). There were two spray schedules: Morning (8 AM) and evening (6 PM).Spraying was done with a standard knapsack sprayer (16 l capacity) fitted with a flat fan nozzle. Number of plants per treatment were 5 and 10 leaves per plant were cut with scissors after 0, 30, 60 and 90 minutes post-spray and dipped in 100 ml distilled water and left for 1 hour in the morning spray and overnight in case of evening spray before the final counts were made under a binocular microscope.

There were eight treatments viz., 2 formulations, 4 observation periods (0, 30, 60 and 90min), 2 spray schedules (morning and evening) and these were replicated five times. Temperature and relative humidity were also recorded simultaneously.

Foliar application of *H. indica* in protected cultivation

Test site: This experiment was conducted at CPCT, ICAR-IARI, New Delhi on tomato (Variety: GS 600-335). Two formulations were tested: 1. EPN alone (Liquid EPN suspension containing 2500 IJs/ml) and 2. EPN + adjuvant (Liquid EPN suspension containing 2500 IJs/ml+ Sticker 99@1%). Spraying was done in the evening hours (03.00 PM) with a standard knapsack sprayer (16 L capacity) fitted with a flat fan nozzle. Number of plants per treatment were 5 and 10 leaves per plant were cut with scissors after 0, 30, 60 and 90 minutes post-spray and dipped in 100 ml distilled water and left for overnight before the final counts were made under a binocular microscope. All the treatments were replicated five times. Temperature and relative humidity were also recorded simultaneously.

Statistical analysis: Data on the survival of the nematode were analyzed using factorial ANOVA and means were separated using LSD. Differences among means in experiments were considered significant at p= 0.05.

RESULTS AND DISCUSSION

Survival of *H. indica* infective juveniles on leaves in open field

The data obtained on survival of *H. indica* infective juveniles at different time intervals (30, 60 and 90 min) post spray on soybean grown in open field is presented in table 1.

Table 1. Per cent survival of infective juveniles of *Heterorhabditis indica* after spray on soybean

Spraying schedule (A)	EPN formulation (B)	% survival of IJs after spray					
		Time (min) (C)				Mean (A×B)	Pooled mean (A)
		0	30	60	90		
Morning	EPN alone	100.00(90.05)	45.30(42.32)	4.75(12.46)	1.47(6.79)	37.88(37.91)	37.91(37.54)
	EPN + adjuvant	100.00(90.05)	47.93(43.84)	3.03(9.88)	0.79(4.92)	37.94(37.17)	
	Mean (A×C)	100.00(90.05)	46.61(43.08)	3.89(11.17)	1.13(5.86)		
Evening	EPN alone	100.00(90.05)	44.73(42.00)	6.18(14.38)	3.05(10.03)	38.49(39.11)	40.14(40.89)
	EPN + adjuvant	100.00(90.05)	41.05(39.87)	20.22(26.72)	5.90(14.00)	41.79(42.66)	
	Mean (A×C)	100.00(90.05)	42.89(40.93)	13.20(20.55)	4.48(12.01)		

	Mean (B×C)				
	0	30	60	90	**Pooled mean B**
EPN alone	100.00(90.05)	45.02(42.16)	5.47(13.42)	2.26(8.41)	38.19(38.51)
EPN+Adjuvant	100.00(90.05)	44.49(41.86)	11.63(18.30)	3.35(9.46)	39.87(39.92)
Pooled mean C	100.00(90.05)	44.75(42.01)	8.55(15.86)	2.80(8.94)	

Factors	SE(m)	C.D. at 5%
Spraying schedule (S)	0.219	0.623
EPN formulation (N)	0.219	0.623
S×N	0.309	0.881
Time elapsed post-spray (T)	0.309	0.881
S×T	0.437	1.246
N×T	0.437	1.246
S×N×T	0.619	1.763

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

Weather parameters on the day of spray:

Temp.: Maximum: 31˚ C and minimum: 20.6˚ C

Relative humidity: Maximum 83% and minimum 52%

Irrespective of EPN formulation and time elapsed post spray, pooled mean (A) revealed that spraying during evening hours was superior with respect to the EPN survival over the morning spray schedule. Considering the effect of EPN formulation alone (irrespective of spraying schedule and time elapsed post spray), EPN+adjuvant was significantly better compared to EPN alone. Addition of adjuvant resulted in better survival compared to treatments where adjuvant was not added in the aqueous solution of IJs. Pooled mean (C) depicted that the survival of EPNs post spray significantly reduced at each successive observation time. The interactions of all the three factors with each other and among themselves were also significant. In the morning sprays, the *H. indica* IJs population reduced drastically to 45.3% within 30 min and only a few nematodes could survive after 90 min (1.47%).

Survival of *H. indica* infective juveniles on leaves in protected cultivation

The data obtained on the survival of *H. indica* IJs at different time intervals (30, 60 and 90 min) post spray on tomato grown under greenhouse conditions is presented in table 2.

Table 2. Per cent survival of infective juveniles of *Heterorhabditis indica* after spray on tomato

EPN formulation (N)	**Per cent survival of IJs after spray**				
	Time (minutes) (T)				
	0	30	60	90	Mean (N)
EPN alone	100.00(90.05)	40.24(39.39)	13.48(21.52)	3.78(10.78)	32.64(32.62)
EPN + adjuvant	100.00(90.05)	34.52(36.00)	16.79(24.18)	2.73(9.22)	33.78(33.69)
Mean (T)	77.07(62.09)	37.38(37.69)	15.13(22.85)	3.26(10.00)	

Factors	SE(m)	C.D. at 5%
EPN formulation (N)	1.172	NS
Time elapsed post-spray (T)	1.657	5.075
N×T	2.344	NS

Values in parentheses are *arc sine* transformed.

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

Weather parameters at the time of spray: Temp.: 23.4° C; Relative humidity: 49.4%

A perusal of data in table 2 showed that there was a significant reduction in population of *H. indica* IJs after 30, 60 and 90 min post spray. *H. indica* IJs population reduced drastically to 40.24% within 30 min and only a few nematodes could survive after 90 min (3.78%) when EPN alone were sprayed. Differences between the two formulations used were non-significant and addition of adjuvant was found at par with EPN alone formulation with respect to the EPN survival. Smits (1996) reported 40 to 80% mortality of heterorhabditid nematodes after application due to ultraviolet radiation and dehydration. In the present study, the nematode survival on soybean foliage was lower in the morning spray schedule compared to evening spray schedule. This may be due to increase in solar radiation and temperature and decrease in relative humidity with passage of time in case of morning spray schedule. The reverse was the case with the evening spray schedule where higher per cent survival was recorded. Both the factors acted negatively on nematode survival. Addition of adjuvant resulted in better survival of IJs irrespective of time elapsed between spray and observation. Glazer (1991) reported that the survival of IJs of *S. carpocapsae* at 50-70% relative humidity reduced to 20% after 4 h and to nil after 8 h. Similarly, Gaugler *et al*. (1992) reported that exposure to ultra violet radiation resulted in a significant delay in progeny emergence, a decline in reproductive capacity and loss of pathogenicity in *H. bacteriophora*. The present study suggests that the effects of sunlight, harmful UV rays or high temperature can be minimized by applying the nematodes in the evening hours. However, maintaining a high relative humidity is more difficult to achieve, especially in open field conditions.

The results of present study are in accordance with Prabhuraj *et al.* (2005) who recorded glycerol at 0.1% as the most appropriate anti-desiccant resulting in 81.2% survival of *H. indica* after 2 h of foliar spray on chickpea foliage but survival reduced drastically after 4 h under field conditions (12-26.8° C with 5-60% RH). In the present study, with the passing of time, there was a drastic reduction in the survival of IJs population in all the treatments on tomato in protected cultivation indicating that Sticker 99 was not very effective in improving the nematode survival in this system. Hence some other chemical adjuvants need to be studied for their appropriateness in the protected cultivation systems. Similar results were reported by Ahmad *et al.* (2009) who studied the survival of *Steinernema masoodi* and *S. carpocapsae* after foliar application on pigeonpea and chickpea twigs. Nematode survival in case of evening spray was higher compared to morning spray at a given time. Addition of UV retardant improved the survival of nematode. The nematodes remained viable up to 3 h post spray. Similar results were obtained by Akhtar *et al.* (2016) who studied survival of *Steinernema seemae* and *Oscheius nadarajni* on chickpea leaves.

CONCLUSION

Results indicated that survival rate of IJs decreased fast and viability remained up to 90 min. Serious attempts are needed to improve the survival of nematodes after foliar spray by adding efficient adjuvant, humectant, anti-desiccant and/ or UV retardant for the management of aerial insect pests.

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