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Response of native entomopathogenic nematode, *Steinernema* spp. (TFRIEPN-57) isolated from central India to variation in temperature and soil moisture

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Abstract

The paper reports effect of temperature and soil moisture on horizontal or vertical dispersal by infective juveniles (IJs) of native entomopathogenic isolate, *Steinernema* sp. (TFRIEPN-57) from central India. The observations recorded 72 hrs after exposure of IJs to eleven temperature regimes (0, 5, 10, 15, 27, 30, 32, 34, 36, 38 and 40 ^{0}C) revealed survival of IJs from 10 to 36^{0}C temperature, below and above which, there was 100% mortality. Within the above range, IJs exposed to 10^{0}C and 36^{0}C showed 88.59% and 55.41% survival, respectively. Exposure of IJs to 0^{0}C revealed no survival at and above 12 hrs, whereas 5^{0}C allowed survival up to 48 hrs. IJs exposed to temperature extreme of 38^{0}C exhibited 38.98% survival up to 8 hrs. Soil moisture of 10% proved suitable for horizontal dispersal of IJs towards the *G. mellonella* larvae embedded in soil 30 cm away from the point of release in 72 hrs. There was no horizontal dispersal beyond 30cm. However, the IJs exposed to soil moisture of 10 and 15% displayed vertical dispersal up to 40 cm towards embedded *G. mellonella* larvae in 72 hrs. Multiple regression analysis of the data showed that infectivity in embedded *G. mellonella* larvae at various horizontal and vertical distances from the point of release of IJs had significant positive correlation with soil moisture and exposure time and significant negative correlation with distance.

Key words

Dispersal, EPN, Forest insect pests, *Galleria mellonella*, Infective juveniles, Native populations, *Steinernema*

Introduction

Heterorhabditid and Steinernematid nematodes, by virtue of being obligate parasites of insects, are widely being experimented as biological control agents for the management of insect pests (Bedding, 2006; Kaya *et al*., 2006). Nearly 24 species of Heterorhabditid and 66 species of Steinernematid entomopathogenic nematodes (EPNs) are known world-wide (Anon, 2012).While most of them have been experimented against agricultural pests, their potential as biological control agent in management of forest insect pests has recently been realized and experimented (Shapiro-Ilan, 2005; Kulkarni *et al.,* 2008; Kulkarni, 2014).

An economical *in vivo* mass-multiplication capability on the fictitious host is a key for successful establishment of any biological control agent (Kulkarni *et al*., 2012). The Greater waxmoth, *Galleria mellonella* larvae, by virtue of being susceptible, has been used as standard insect for evaluating infectivity under varied experimental conditions by any new isolate of entomopathogenic nematode (Kavanagh and Reeves, 2004).

Entomopathogenic nematodes are essentially aquatic organisms moving on water films through soil pore spaces whose widths are determined by soil texture and structure (Koppenhöfer and Fuzy, 2007).Thus, soil moisture and

temperature play species-specific role in their survival, movement, infectivity and persistence (Ganguly and Gavas, 2004, Yadav and Lalramliana, 2012). This effect of abiotic factors may differ among different EPN species (Grant and Villani, 2003). While choice of nematode species or population in relation to the target host takes care of biotic factor (Shapiro-Ilan *et al*., 2006), application under favorable soil moisture levels as abiotic factor is equally critical (Kaya and Gaugler, 1993). It is due to the effect on IJs movement through interstitial spaces coated by water film or through water-filled pores, which vary in diameter in relation to the nematodes' body. Infectivity of many EPN species is highest at moderate soil moisture (Wallace, 1958), with thickness of water film being approximately half the thickness of the nematodes' body. Water film becomes thinner with drying of soil and larger pores drain of water which increasingly restricts nematode movements. Infective juveniles cannot survive rapid desiccation in laboratory experiments under low RH regimes (Womersley, 1990), but they can persist considerable lengths of time in dry soil (Kung *et al*., 1991), which also indicate capability to adapt to low moisture conditions. On the other hand, nematode movement can also be restricted if the interstitial spaces are completely filled with water (in water saturated soil) when the pore's diameter is much greater than that of the nematodes, *i.e.*, >200 μm (Quénéhervé and Chotte, 1996). As nematode species may differ in their ability to adjust to low soil moisture, the effect of soil moisture on nematode persistence may also differ among nematode species (Grant and Villani, 2003). Needless to say, data generated on response of native isolatein terms of survival and infectivity under different temperature regimes and the soil moisture in relation to the soil depth will not only help in developing protocol for its economical mass-multiplication, but also in bioassays for efficacy against the target soil pests. These aspects have been experimented and results discussed for this native population of *Steinernema* sp. (TFRIEPN-57) isolated from central India, for which such dispersal mechanism with respect to the temperature and soil moisture was not known. Results can be utilized in planning management of soil insect pests in forest nurseries.

Materials and Methods

Collection and maintenance of EPN population : The population of *Steinernema* sp. (TFRIEPN-57) was isolated under the environmental conditions of 28 to 36ºC and relative humidity 40-78%, as existing in nature during the month of June, 2010. The habitat of collection was soil of forest floor of dense teak (*Tectona grandis* L.) plantation. The soil sample collections were made from 10-15 cm depth, baited with the mature last instar larvae of *G.mellonella* (Bedding and Akhurst, 1975). The recovered infective juveniles (IJs) of EPN were multiplied in laboratory on *G. mellonella* larvae *in vivo* (Dutky *et al*.,1964). Freshly emerged IJs of population were used for experimental purpose.

Effect of temperature : The counted number of IJs were suspended in 2 ml sterile distilled water in 5 ml beakers and exposed to varied range of temperature : 0, 5,10,15, 27, 30, 32, 34, 36, 38 and 40 ºC maintained in BOD continuously all through the experimental period. Additional set of experiments were set up and observations were taken after every 2 hrs interval up to 72 hrs for the experimental temperature ranges which did not allow survival even up to 72 hrs. Ten such sets were kept for each temperature. The whole experimental set-up was repeated thrice and data recorded in terms of number of surviving IJs, every 24 hrs till 72 hrs, and was pooled. Care was taken to leave the experimental set up in room temperature for 30 min before recording observations and the IJs without any mobility were considered dead. The temperature of 27 ºC, as existed in nature during the experimental period, was considered as control.

Effect of soil moisture: Effect of soil moisture on horizontal and vertical distribution of native EPN isolate TFRIEPN-57 (*Steinernema* spp.) was experimented in laboratory. The experiment was carried out in 50 x 25 cm fibre tray filled with pre-sterilized (heated at 80 ºC for 48 hrs) soil. Soil was sandy loam with bulk density of 1.177g cm^3 and porosity of 0.556 (calculated following the method suggested by Hsiao, 1996). The moisture level of 5, 10, 15 and 20 % was maintained by adding sterilized distilled water in soil (w/v). Each moisture set up was accompanied with control, in which no EPNs were released. Additionally, a master check (control) of 0% moisture was also taken. This arrangement was replicated thrice. The larvae of *G. mellonella w*ere exposed by placing caged larvae (7 mature larvae per cage) embedded 20.0 mm deep in soil at a distance of 10, 20, 30 and 40 cm from the point of EPN release. Each replication was released with 1000 number of freshly harvested IJs of TFRIEPN-57, which were released at the centre of Petri dish. The trays were covered with fine polythene sheet and maintained at 27.0 ºC. Mortality of larvae was closely observed every 24 hr, by which the percentage mortality was calculated.

Statistical analyses: The percentage data on larval mortality under different experimental temperature and soil moisture were transformed using Angular Transformation (arc sin $\sqrt{n}$), before subjecting to ANOVA by factorial method. Soil moisture data was further subjected to multiple regression analysis to statistically validate the effect of soil moisture and distance of movement by the IJs separately for horizontal and vertical movement, with respect to period of exposure.

Results and Discussion

The observations recorded 72 hrs after exposure of IJs of *Steinernema* sp. (TFRIEPN-57) to eleven temperature

regimes revealed survival in IJs from 10 to 36 ºC temperature, below and above which, there was 100% mortality. Within the above range, IJs exposed to 10 ºC and 36 ºC showed 88.59% and 55.41% survival. There was 100% survival in IJs exposed to 27 ºC as control. The survival percentage was maintained above 95% in IJs exposed to temperature up to 32 ^{0}C (P<0.05)($F_{(0.001)}$ = 516.38, df = 88, $SE_{(d)}\pm$ = 0.904, $LSD_{(P<0.05)}$ = 1.78) (Fig. 1). Exposure of IJs to 0^0 C revealed no survival at and above 12 hrs (P<0.05). There was significant reduction in survival (93.64%) as compared to control at 27^{0}C, even after first 2 hrs of exposure. There was significant mortality every 2 hrs, which allowed only 0.79% IJs to survive at 12hrs interval)($F_{(0.001)}$ = 497.99, df = 44, $SE_{(d)}\pm$ = 1.24, $LSD_{(P<0.05)}$ = 2.51) (Fig. 2). Infective juveniles exposed to 5^{0}C did not survive beyond 48 hrs, with significantly less survival (86.89%) even after 24 hrs as compared to control maintained at 27^{0}C (P<0.05)($F_{(0.001)}$ = 422.60, df = 26, $SE_{(d)}\pm$ = 0.81, $LSD_{(P<0.05)}$ = 1.63) (Fig. 3). The IJs exposed to 38^{0}C exhibited 38.98% survival after 8 hrs of exposure ($F_{(0.001)}$ = 272.18, df = 44, $SE_{(d)}\pm$ = 1.49, $LSD_{(P<0.05)}$ = 3.01)(Fig. 4).

Table 1 presents data recorded at 24 hrs intervals till 72 hrs on mortality in caged *G. mellonella* larvae embedded at various horizontal distances from the point of release of IJs as a parameter for horizontal foraging by *Steinernema* sp. (TFRIEPN-57). Results revealed no movement by IJs, evident by no mortality recorded in *G. mellonella* larvae kept embedded in soil without moisture. Soil moisture of 5% allowed distribution of IJs up to 30 cm after 72 hrs, but number of IJs were less as evident by only 28.57% mortality. However, at 10 cm distance, significant mortality was obtained under similar conditions. Soil moisture of 10% proved suitable as maximum number of IJs seem to have migrated towards *G. mellonella* larvae causing 71.43, 61.90

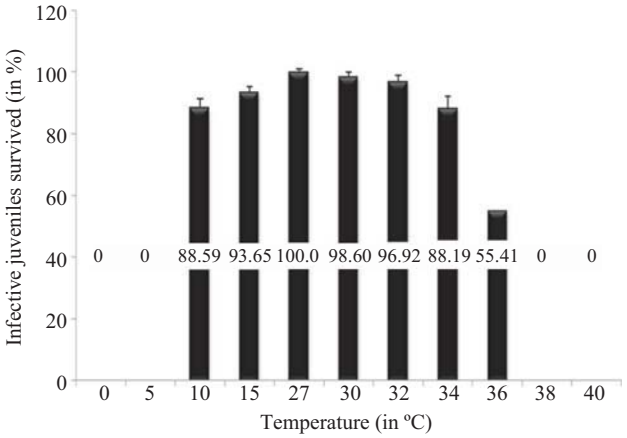

Fig. 1 : Survival of infective juveniles of *Steinernema* sp. (TFRIEPN-57) after exposure to 0ºC temperature (ANOVA included initial population of IJs as a covariate, Error bars represent ±SD value) ($F_{(0.001)}$ = 516.38, df = 88, $SE_{(0)}\pm$ = 0.904, $LSD_{(P-0.05)}$ = 1.797).

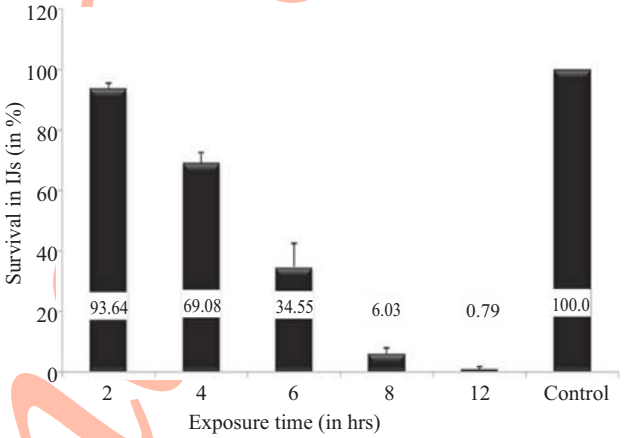

Fig. 2 : Survival of infective juveniles of *Steinernema* sp. (TFRIEPN-57) after exposure to 0ºC temperature (ANOVA included initial population of IJs as a covariate, Error bars represent ±SD value) ($F_{(0.001)}$ = 497.99, df = 44, $SE_{(0)}\pm$ = 1.24, $LSD_{(P-0.05)}$ = 2.51).

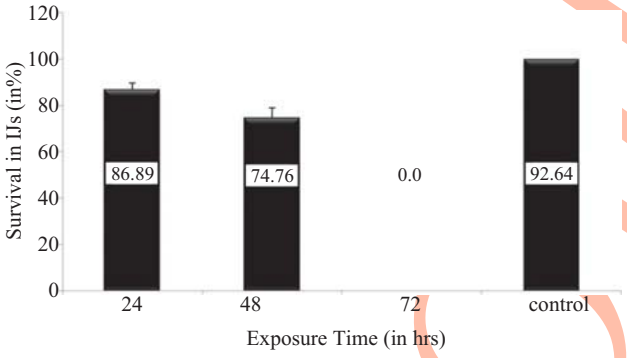

Fig. 3 : Survival of infective juveniles of *Steinernema* sp. (TFRIEPN-57) after exposure to 5ºC temperature (ANOVA included initial population of IJs as a covariate, Error bars represent ±SD value) ($F_{(0.001)}$ = 422.60, df = 26, $SE_{(0)}\pm$ = 0.89, $LSD_{(P-0.05)}$ = 1.63).

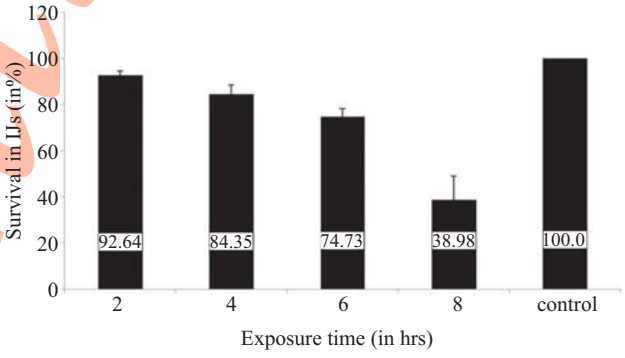

Fig. 4 : Survival of infective juveniles of *Steinernema* sp. (TFRIEPN-57) after exposure to 38ºC temperature (ANOVA included initial population of IJs as a covariate, Error bars represent ±SD value) ($F_{(0.001)}$ = 272.18, df = 44, $SE_{(0)}\pm$ = 1.49, $LSD_{(P-0.05)}$ = 3.01).

and 47.62% morality, respectively, at 10, 20 and 30 cm distance after 72 hrs of exposure as compared to other soil moisture treatments and control and differed significantly from distance to distance of exposure ($F_{(P<0.001)}$=88.44, *df* = 78, $SE_{(d)}$±=2.54, $LSD_{(P<0.05)}$=5.06).While IJs could reach 10 cm distance within 24 hrs above 5% soil moisture, there was time and soil moisture dependent relationship in distances covered by the IJs (Table 1). The movement of IJs was reduced with increasing soil moisture upto 20%, proven by significantly lower mortality (P<0.05) in *G. mellonella* larvae due to of less number of IJs reaching to embedded larvae over 24, 48 and 72 hrs as compared to corresponding data in other soil moisture. Thus, mortality obtained was inversely proportional to soil moisture all distance and time of observations. The IJs of *Steinernema* sp. (TFRIEPN-57) could not move beyond 30 cm even after 72 hrs, irrespective of experimental soil moisture.

Multiple regression analysis for correlating the predictor variable mortalities (mortality at 24hr, 48 hr and 72 hr with respect to explanatory variables, moisture and distance, presented in table 2 indicates that effect of intercept value after 72 hrs is maximum rather than on 24 and 48hrs. It indicates time taken for IJs to move up the experimental distances. Effect of moisture on all the mortalities showed significant positive correlation having the highest after 72 hrs and followed by 48 and 24 hrs, respectively, which confirm the relationship of time of exposure with that of the mortalities obtained and thus the movement of IJ numbers to the targeted distances. This was further proven by the negative effect exhibited by distance as parameter for all the mortalities, highest negative effect being at longest distance with inverse relationship with time of exposure, since at lower percentages, less mortality was caused due to movement of less number of IJs up to caged *G. mellonella* larvae (Table 2).

Results showed that 5% soil moisture allowed movement of IJs up to 10 cm distance with only 28.57% mortality in *G. mellonella* larvae even after 72 hrs of exposure (Table 3). While considerable number of IJs of *Steinernema* sp. (TFRIEPN-57) moved up to 40 cm after 72 hrs of exposure at 10 and 15% soil moisture evident by 100% mortality at 10 cm distance after 48 hrs and up to 30 cm after 72 hrs (P<0.05) ($F_{(P<0.001)}$=88.44, *df.*= 78, $SE_{(d)}$±=2.54, $LSD_{(P<0.05)}$=5.06). For vertical distribution also, significant relationship between soil moisture distance and time of exposure was obtained. Soil moisture of 15% proved superior for vertical movement of the IJs (P<0.05) ($F_{(P<0.001)}$=33.18, *df.* 78, $SE_{(d)}$±=3.19, $LSD_{(P<0.05)}$=6.35) . However, movement was negatively affected by further increasing the soil moisture (Table 3).

Table 1 : Effect of soil moisture on horizontal foraging by *Steinernema* sp. (TFRIEPN–57)

Mortality of *G. mellonella* larvae as parameter of horizontal foraging at various distances															
Soil	**24 hrs**				**Control**	**48 hrs**				**Control**	**72 hrs**				**Control**
Moisture %	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**
0	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
5	14.29 (18.18)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	23.81 (28.96)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	42.86 (40.79)	28.57 (31.82)	28.57 (31.82)	0.0 (0.00)	0.0 (0.00)
10	38.10 (37.42)	28.57 (27.15)	14.29 (18.18)	0.0 (0.00)	0.0 (0.00)	57.14 (49.25)	52.38 (46.52)	28.57 (31.82)	0.0 (0.00)	0.0 (0.00)	71.43 (58.23)	61.90 (52.62)	47.62 (43.65)	0.0 (0.00)	0.0 (0.00)
15	28.57 (27.15)	14.29 (22.22)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	57.14 (49.13)	28.57 (32.33)	28.57 (27.15)	0.0 (0.00)	0.0 (0.00)	66.67 (54.86)	61.90 (52.62)	42.86 (40.91)	0.0 (0.00)	0.0 (0.00)
20	14.29 (22.22)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	28.57 (32.33)	14.29 (18.18)	14.29 (22.22)	0.0 (0.00)	0.0 (0.00)	57.14 (49.25)	28.57 (27.28)	19.05 (25.59)	0.0 (0.00)	0.0 (0.00)
$F_{(P<0.001)}$	Moisture				11.72			64.50					88.44		
	Distance				12.51			44.02					59.90		
	Moisture x Distance				151.25			10.04					11.13		
Df					78			78					78		
$SE_{(d)}$±	Moisture				2.06			2.215					2.54		
	Distance				1.30			1.40					1.60		
	Moisture x Distance				4.11			4.43					5.09		
$LSD_{(P<0.005)}$	Moisture				5.79			4.41					5.068		
	Distance				3.66			2.79					3.206		
	Moisture x Distance				11.58			8.82					10.137		

Table 2 : Multiple regression analysis (horizontal) with predictor variable: mortality, response variables: moisture and distance

Morality at 24 hr			
	Coefficients	***Standard error***	***P-value***
Intercept	10.47619	5.432190384	0.058774
Moisture	0.285714	0.271609519	0.297271
Distance	-0.22857	0.171780943	0.18862
Multiple R Square		**0.40**	
Morality at 48 hr			
	Coefficients	***Standard error***	***P-value***
Intercept	19.28571	7.220299	0.009838
Moisture	1.02381	0.361015	0.006314
Distance	-0.51429	0.228326	0.028163
Multiple R Square		**0.20**	
Morality at 72 hr			
	Coefficients	***Standard error***	***P-value***
Intercept	28.80952	9.539127	0.003777
Moisture	1.404762	0.476956	0.004665
Distance	-0.6	0.301654	0.051505
Multiple R Square		**0.20**	

Multiple regression analysis for vertical movement of IJs is presented in Table 4. Higher intercepts values for maximum time of exposure, *i.e.*, 72 hrs confirmed positive correlation of distance travelled by the IJs in different exposure periods with respect to soil moisture increase. For vertical movement also, the effect of moisture on all the mortalities is showed significant positive correlation having highest after 72 hrs and followed by 48 and 24 hrs, respectively, which confirmed the relationship of time of exposure with that of mortalities obtained, and thus the movement of IJ numbers to the targeted distances. Here also negative effect exhibited by distance as parameter for all the mortalities, highest negative effect being at longest distance with inverse relationship with time of exposure, since at lower percentages, less mortality was caused due to movement of less number of IJs up to caged *G. mellonella* larvae (Table 4).

Effect of temperature on survival and infectivity of entomopathogenic nematode isolates from India has earlier been reported (Ganguly and Gavas, 2004). The EPN strain DD136 (Rajeshwari *et al*., 1984) and *S. thermophilum* (Ganguly and Gavas, 2004) were stored better in temperature range of 25 to 30^{0}C and 10 to 35^{0}C, respectively. Hussaini *et al*. (2003) experimented lower temperature and reported survival to be unaffected after storage of *H. indica* (PDBC strain 13.3) and *S. abbasi* (PDBC strain 2.1) at 8 ºC up to 6 weeks. Higher temperature of 35^{0}C did not exhibit significant mortality in IJs of *S. carpocapsae, S. tami, S. feltiae* and *S. abbasi* up to 10 hrs, whereas, storage of *S. bicornutum* at even 30^{0}C resulted in 50% survival (Hussaini *et al*., 2005). Sunanda *et al*. (2012) reported highest survival of *S. abbasi*

Table 3 : Effect of soil moisture on vertical foraging by *Steinernema* sp. (TFRIEPN–57)

Mortality of *G. mellonella* larvae as parameter of vertical foraging at various distances															
Soil	**24 hrs**				**Control**	**48 hrs**				**Control**	**72 hrs**				**Control**
Moisture %	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**	**10 cm**	**20 cm**	**30 cm**	**40 cm**	**(without IJs)**
0	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
5	14.29 (22.22)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	14.29 (22.22)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	28.57 (32.33)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
10	95.24 (82.64)	71.43 (62.77)	71.43 (62.77)	14.29 (22.22)	0.0 (0.00)	100.00 (90.05)	90.48 (75.23)	80.95 (69.00)	14.29 (22.22)	0.0 (0.00)	100.00 (90.05)	100.00 (90.05)	100.00 (49.13)	57.14 (0.00)	0.0 0.00
15	100.00 (90.05)	95.24 (82.64)	90.48 (75.23)	14.29 (22.22)	0.0 (0.00)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	42.86 (40.91)	0.0 (0.00)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	42.86 (40.91)	0.0 (0.00)
20	57.14 (49.13)	57.14 (49.13)	28.57 (32.33)	0.0 (0.00)	0.0 (0.00)	66.67 (54.86)	61.90 (51.99)	28.57 (32.33)	0.0 (0.00)	0.0 (0.00)	71.43 (57.72)	71.43 (57.72)	28.57 (32.33)	0.0 (0.00)	0.0 (0.00)
$F_{(P<0.001)}$	Moisture					213.73				705.44			NS		
	Distance					55.08				138.24			-		
	Moisture x Distance					12.97				33.18			-		
Df						78				78			-		
$SE_{(d)}\pm$	Moisture					2.56				1.59			-		
	Distance					1.62				1.01			-		
	Moisture x Distance					5.12				3.19			-		
$LSD_{(P<0.005)}$	Moisture					5.098				3.17			-		
	Distance					3.224				2.01			-		
	Moisture x Distance					10.197				6.35			-		

Table 4 : Multiple regression analysis (vertical) with predictor variable: mortality, response variables: moisture and distance

Morality at 24 hr			
	Coefficients	***SE***	***P-value***
Intercept	22.38095	12.18272	0.071409
Moisture	2.857143	0.609136	1.75E-05
Distance	-0.61905	0.385251	0.11361
Multiple R Square		**0.301322**	
Morality at 48 hr			
	Coefficients	***Standard error***	***P-value***
Intercept	21.19048	12.83850253	0.104332
Moisture	3.214286	0.641925127	5.65E-06
Distance	-0.53333	0.405989097	0.194222
Multiple R Square		**0.319796**	
Morality at 72 hr			
	Coefficients	***Standard error***	***P-value***
Intercept	22.85714	13.29389	0.090973
Moisture	3.285714	0.664695	7.11E-06
Distance	-0.42857	0.42039	0.312291
Multiple R Square		**0.308877**	

and *H. indica* at 30 ºC up to 15 days (85.76 % survival in *S. abbasi* and 88.09 5 in *H. indica*). Further, storage up to 90 days resulted in to 70.22% in *S. abbasi* and 72.71% in *H. indica*. Fragmentary information on mortality in IJs was also reported when stored below 30 ºC and above 48 ºC. In the present investigation, IJs of *Steinernema* sp. (TFRIEPN-57) survived at temperature 10ºC (88.59% survival) to 36^{0}C (55.41% survival), below and above which, there was 100% mortality within 72 hrs. The native isolate in the present investigation appeared to be at par in thermo-tolerance with *S. thermophilum*. Exposure of IJs to 0 ºC revealed no survival at and above 12hrs. Infective juveniles exposed to 5 ºC did not survive beyond 48 hrs. The IJs exposed to 38 ºC exhibited 38.98% survival after 8 hrs of exposure.

Steinernematid and heterorhabditid nematodes are exclusively soil organisms and ubiquitous, having been isolated from every inhabited continent from a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts, and even ocean beaches (Hominick, 2002). This can be attributed to their capacity to adapt to conditions by avoiding damaging effects of temperature extremes or lack of humidity (Villani and Wright 1990). The reports indicated that lack of moisture cannot induce movements even in response to the host cues, even in sandy loam soil, which is considered best for EPNs (Mwaniki *et al*., 2010), irrespective of particular EPN population (Yadav and Lalramliana, 2012). However their response to gradient of soil moisture is population-specific,depending upon the isolate (Wennemann *et al*., 2004). Soil moisture of 4 - 5% for establishment of *S. carpocapsae* and *S. glaseri* (Koppenhofer *et al*.,1995) and soil moisture above 3% (even 3.5%) for *S. monticolum* has been found suitable with peak results at 6%. In both the cases, establishment declined upon increasing soil moisture to 19% (Koppenhofer *et al*., 2000).Without taking soil moisture as parameter, Gavas and Ganguly (2002) reported *S. thermophilum* to be more efficient than *S. glaseri* in causing mortality of *G. mellonella* (placed in soil column) larvae up to 10 cm soil depth, while the latter could do so up to 5 cm depth only. Ganguly and Gavas (2004) reported that IJs of *S. thermophilum* could enter and infect the host at soil moisture levels varying from 1 – 19% (w/w), with infectivity initiated from 3% onwards, increased up to 9% moisture level, followed by decline, if soil moisture is further increased. Yadav and Lalramliana (2012), while working on *S. thermophilum* in a sandy loam soil, reported *S. thermophilum* to establish at 4% and above soil moisture, *H. indica* and *S. glaseri* at 5% and above soil moistures. The optimum soil moisture for different nematode species were noted as: *H. indica* 8–18%, *S. thermophilum* 6–20%, and *S. glaseri* 8–25%. Further, a minimum of 6% soil moisture was noted to be essential for achieving 100% host mortality for all the three nematode species. There is no earlier report available on the effect of soil moisture in relation to horizontal and vertical movement of IJs and time of exposure, as the present investigation. Recently, Singh *et al*. (2015), while carrying out diversity analysis of entomopathogenic nematodes from Tarai Region of India, reported vertical distribution of a few species of nematodes, but the report does not refer to any soil and temperature conditions as compare to the present results and is actually only a field isolation study.

The present results revealed that in absence of any moisture the IJs did not make any horizontal or vertical migration from the place of release. This is evident by no mortality recorded in embedded *G. mellonella* larvae at various distances in soil from the point of release of IJs. Soil moisture of 5% allowed horizontal movement of IJs up to 30 cm in 72 hrs, but number of IJs were less. It did not facilitate vertical migration of IJs beyond 10 cm distance even after 72 hrs. Soil moisture of 10% proved suitable as maximum number of IJs seems to have displayed horizontal movement towards *G. mellonella* larvae. However, vertical migration was up to 40 cm after 72 hrs of exposure at 10 and 15% soil moistures evident by 100% mortality.Soil moisture of 15% proved superior for vertical movement of the IJs. However, movement was negatively affected by further increase in soil moisture.The movement of IJs was reduced with increasing soil moisture to 20%, proven by significantly lower mortality in *G. mellonella* larvae because of less number of IJs reaching to embedded larvae over 24, 48 and 72 hrs.

The IJs of *Steinernema* sp. (TFRIEPN-57) could not migrate horizontally beyond 30 cm even after 72 hrs, irrespective of the experimental soil moisture. The

information generated will be used in maintaining laboratory stock of the EPN population in soil and developing field application strategy against soil insect pests.

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