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EFFECT OF SOIL TEXTURE, SOIL MOISTURE AND DEPTH ON SURVIVAL AND INFECTIVITY OF INDIGENOUS ISOLATE OF ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA DHARANAII* (NEMATODA: RHABDITIDA: STEINERNEMATIDAE) TO INFECT WAXMOTH, *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Entomopathogenic nematodes (EPNs) are effective biological control agents against many soil-inhibiting insect pests. The different soil related factors have influence on the survival, reproduction, movements, penetration and infectivity of these nematodes. This work aimed at evaluating the effect of soil texture, moisture, depths, movement, penetration and foraging capacity on the survival and infectivity of new species of EPN, *Steinernema dharanaii* against fifth-instars of *Galleria mellonella*. To compare the efficiency of these indigenous isolates at different soil texture (sand, sandy loam, clay, gravel), moistures (0.0%, 5.0%, 10.0%, 15.0%, 20.0%, 25.0%, 30.0% and 40.0%), depths (9, 18, 21 and 30 cm), movement, penetration and foraging capacity (3.5cm and 6cm distance) in the laboratory.

The result showed that the sand proved the best with obtained 100.0% mortality at and above moisture level of 5% upto 30.0%, whereas, sandy loam, though, required minimum moisture level of 10.0% with 80.0% mortality, moisture levels of 15 to 25.0% were best with 100.0% mortality obtained. In all the soil moisture levels 2cm distance was covered by the IJs in 72 hrs, evident by 100.0% mortality of waxmoth larvae, except 40.0% moisture level, at which no mortality was recorded. The IJs could move easily at soil moisture level of 10 to 20.0% up the distance of 3.5 cm, evident by 100.0% mortality in waxmoth larvae, whereas, increasing the moisture level to 40.0% did not allow IJ movement and thus no mortality was obtained after 72 hrs of experimental exposure. The IJs exposed to soil columns with waxmoth larvae placed at different depths for assessing time required by IJs for vertical movement, it was noted that silt required significantly less time, as compared to others (P<0.05). It took 86.6 ± 13.14, 91.2 ± 10.73, 100.8 ± 10.73 and 124.8 ± 20.07 hrs to reach respectively at 9, 18, 21 and 30 cm depth.

These finding can be utilized in planning management of soil insect pests in forest nurseries.

Key words: Biological control, entomopathogenic nematodes, *Steinernema dharanaii*, Abiotic factors, Soil texture, Soil moisture, Soil depth

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1. Introduction

Entomopathogenic nematodes (EPNs) in the families Heterorhabditidae and Steinernematidae have a mutualistic-symbiotic association with enteric γ-Proteobacteria, *Steinernema-Xenorhabdus and Heterorhabditis-Photorhabdus* (Kaya and Gaugler, 1993; Bedding, 2006) They are soil-dwelling parasites, which confer high virulence against soil insect pests and widely commercially used against many economically important insect pests worldwide (Gaugler and Kaya, 1990;Grewal et al., 2005; Kulkarni et al., 2008; Paunikar and Kulkarni, 2020). EPNs have been studied intensively because of their role as a natural mortality factor for soil-dwelling arthropods and their potential as biological control agents for below ground insect pests (Klein, 1990; Lacy and Georgis 2012).They has been particularly successful against certain insect species that spend a large portion of their life cycle in the soil, particularly the termites, white grub, some lepidopteran insects and others (Kaya, 1990; Shapiro-Ilan et al., 2002; Hussaini et al., 2003; Toepfer, et al., 2010; Paunikar and Kulkarni, 2019a).

The biocontrol potentials of EPNs are therefore influenced by different abiotic and biotic factors, beside others (Gaugler, 1988; Toledo et al., 2009; Paunikar, 2014; Stuart et al., 2015; Sharmila et al., 2018). It has been reported that the soil texture, soil moisture, soil depth, foraging capacity, movement and penetration, temperature and relative humidity play an important role in affecting the infectivity, time of death, development, reproduction and storage of entomopathogenic nematodes (Schroeder and Beavers, 1987; Shapiro-Ilan, et al., 2006;Kulkarni et al., 2016; Hartley and Wallace, 2017; Paunikar and Kulkarni, 2019b). EPNs differ in their ecological and behavioural traits with regard to their persistence and survival in the soil. It is for these reasons that for their use in biological control locally adapted species or isolates from native habitats need a characterization in terms of their optimum biological requirements (Koppenhoffer, 2000; Shelmith, et al., 2007; Ramalingam et al., 2011).

Entomopathogenic nematodes also require adequate soil moisture levels for their survival, infectivity and locomotion, which may vary among nematode species and isolates and among different soil types. The high and low soil moisture levels can be lethal to these entomopathogenic nematodes. However, some species develop survival strategies under water stress conditions, by reducing the body surface area exposed to the air and their cell metabolism. This process, known as anhydrobiosis, allows the nematode to become resistant to desiccation, and it can be reversible when the soil becomes wet again. On the other hand, high soil moisture levels can cause oxygen depletion and restrict the entomopathogens mobility (Koppenhöfer et al.,1995, Patel et al.,1997; Rhode et al., 2010; Sharmila and Subramanian 2015; Bal et al., 2017).

Soil characteristics can contribute to variations in nematode efficacy (Kaya 1990, Barbercheck and Miller 1999; Bal and Grewal, 2015). Soil moisture is a critical factor influencing nematode survival, movement, and infectivity (Molyneux and Bedding 1984; Kung et al., 1991, Koppenhoffer and Fuzy, 2006, 2007; Salame and Glazer 2015; Abarami et al., 2017). Soil texture can also influence nematode efficacy. As clay content increases, nematode dispersal (Georgis and Poinar 1983ab) and survival (Kung, 1990; Kung et al., 1990) has been reported to decrease (Shapiro-Ilan et al., 2000; Lankin,et al., 2020).

The objective of this study was to evaluate the effect of soil texture, moisture, depth, foraging capacity, movement and penetration on the survival and infectivity of indigenous isolate from tropical forest area species of EPN, *Steinernema dharanaii* Kulkarni et al.,2012a to *G. mellonella* larvae, and to compare the virulence of these isolates.

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2. Materials and Methods

2.1. Collection and maintenance of EPN population:

The population of *Steinernema* spp.was isolated under the environmental conditions of 28 to 36ºC and relative humidity 40-78%, as existing in nature during the month of June. The habitat of collection was soil of forest floor of dense teak (L.) plantation. The soil sample collections were made from 10-15 cm depth, baited with the mature last instar larvae of (Bedding and Akhurst, 1975). The recovered infective juveniles (IJs) of EPN were multiplied in laboratory on larvae (Dutky et al., 1964; Kulkarni et al., 2012b). Later, the species of EPN was identified from their adult male and molecular characterisation and described as a new species by Kulkarni et al., 2012a under genera and species *S. dharanaii* (TFRIEPN-15). The White trap technique as described by White (1927) was used for harvesting nematodes progeny (Infective Juveniles "IJs") at 27±1 ^{0}C. Freshly emerged IJs of population were used for experimental purpose.

2.2. Soil Collection from Forest areas:

The different Soil type *viz*; sand, sandy loam, clay, gravel were collected from Tropical Forest Research Institute campus, Jabalpur, Madhya Pradesh and surrounding areas. The collected soil was exposed to 80^{0}C constantly for 48 hrs in Hot Air Oven. The sterilized soil was allowed to normalize at room temperature and used for all the following three experiments.

2.3. Experiment-1. Testing of Effect of soil texture and soil moisture on survival and infectivity of *Steinernemadharanaii*

A uniform quantity (20 gm) of the same i.e. was taken in a Petri dish (5 cm dia x 1.5 cm depth). Different moisture regimes v*iz*; 0.0%, 5.0%, 10.0%, 15.0%, 20.0%, 25.0%, 30.0% and 40.0% were maintained by w/w basis. The freshly harvested IJs (1000 IJs) were loaded on the top of sand layer and Petri dish was covered with the lid. Whole experimental set up was placed in BOD at 27±1 ^{0}C temperature with 60-70.0% RH for 3-10 period of time. The mature waxmoth larvae were released and observed after 3-10 days. Each treatment including control without moisture was replicated five times. The experiment was repeated thrice and data pooled before subjecting to statistical analysis.

2.4. Experiment- 2. Testing of effect of soil texture and soil moisture on foraging capacity

Three early last instar of wax moth larvae were caged in piece of brass net to restrict their movement and placed deep in the sand layer in 3.5cm and 6cm distance from the center of the Petri dish. At the center 1000 freshly harvested IJs were loaded on the top of sand layer and Petri dish was covered with the lid. Whole experimental set up was placed in BOD/ temperature controlled room at 27±1 ^{0}C temperature with 60-70.0% RH for one week. Each treatment including control without moisture level was replicated five times. Observations on larval mortality were taken in 24 hour intervals without disturbing the overall set up for one week. The experiment was repeated thrice and data pooled before compilation and statistical analysis.

2.5. Experiment- 3. Testing of effect of different soil types and depth on movement and penetration of population of *S. dharanaii*

The 35 cm height specimen jars were taken for the experiments. A uniform quantity of sterilized soil was taken in glass specimen jar filled up to 35 cm depth. The soil was moistened by adding sterile distilled water on w/v basis. The early last instar 5 larvae of waxmoth were caged in brass wire mesh net to restrict their movement and placed in the different depth *viz*; 9, 18, 21 and 30 cm of the jar. The freshly IJs 1000 of

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EPNs in quantity were released and covered with the lid. The observations were taken after 3 to 10 days till the termination of the experiments. The dead larvae (cadaver) were placed in the BOD for incubation and emergence of IJs for recovery of IJs. The experiment was repeated thrice and data pooled before compilation and statistical analysis.

2.6. Statistical analyses:

The percentage data on larval mortality under different experimental soil moisture, depth and distance were transformed data (if required) were subjected to Analysis of Variance (ANOVA) (Gomez and Gomez, 1984). The multiple comparison of means was done using the Ryan, Eniot-Gabriel &Welsch (REGW) procedure (Quinn and Keough, 2002), using statistical software GenStat Discovery Version3.

3. RESULTS

3.1. Effect of soil moisture and soil texture on infectivity of *S. dharanaii* (TFRIEPN-15) against *G. mellonella*

Results indicated that clay and silt required more moisture, i.e., above 20.0% to get the required infectivity by EPN to waxmoth larvae. Gravel allowed foraging by the IJs causing infectivity at and above moisture level of 10 to 30.0%. Sand proved the best with obtained 100.0% mortality at and above moisture level of 5% upto 30.0%. Sandy loam, though, required minimum moisture level of 10.0% with 80.0% mortality, moisture levels of 15 to 25.0% were best with 100.0% mortality obtained. At 30.0% moisture level mortality again reduced to 60.0%. It can be noted that there was no mortality at 0.0% moisture level in any probably because the movement of the IJs was restricted (Table 1).

3.2. Effect of different distances and moisture level on foraging capacity

Results indicated that in all the soil moisture levels 2cm distance was covered by the IJs in 72 hrs, evident by 100.0% mortality of waxmoth larvae, except 40.0% moisture level, at which no mortality was recorded. The IJs could move easily at soil moisture level of 10 to 20.0% up the distance of 3.5 cm, evident by 100.0% mortality in waxmoth larvae, whereas, increasing the moisture level to 40.0% did not allow IJ movement and thus no mortality was obtained after 72 hrs of experimental exposure. At 5.0% moisture level, 86.11% mortality was recorded at 3 cm distance. When the larvae were kept at 6 cm distance from the point of IJ release, mortality of 79.16, 91.66, 87.50 and 70.16% was recorded respectively at 5, 10, 15 and 20.0% moisture levels. There was no mortality at 40.0% level, as the soil becomes completely drenched allowing no movement of IJs. Larvae mortality under this condition is due to suffocation and not due to IJs, evident by non-recovery of the IJs of the next generations (Table 2).

3.3. Effect of soil types and depth on vertical movement, penetration and infectivity

The IJs exposed to soil columns with waxmoth larvae placed at different depths for assessing time required by IJs for vertical movement, it was noted that silt required significantly less time, as compared to others (P<0.05). It took 86.6 ± 13.14, 91.2 ± 10.73, 100.8 ± 10.73 and 124.8 ± 20.07 hrs to reach respectively at 9, 18, 21 and 30 cm depth. It was followed by sand with 92.1 ± 10.73, 120 ± 16.97, 1139.2 ± 20.07 and 147.2 ± 18.41 for the depths mentioned earlier. Further sandy loam facilitated the IJ movement better than gravel and clay. Sandy loam required 110.4±13.14, 120±16.97, 120.6±21.46 and 153.6±21.46 hrs for 9, 18,21,30 cm, respectively. Gravel took the maximum time as compared to all other soil types and the clay did not allow movement beyond 18 cm, for 18 cm also it took 225±13.14 hrs (Table 3).

Table 1: Effect of soil texture and soil moisture on infectivity of *S. dharanaii* against *G. mellonella*

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Soil Texture	Soil Moisture (in %)							
	0	5	10	15	20	25	30	40
Gravel	0.00[a]	0.00	60.00[a]	100.00[a]	100.00[a]	100.00[a]	80.00[ab]	0.00[b]
	(0.00)	(0.00)	(54.00)	(90.04)	(90.04)	(90.04)	(72.03)	(0.00)
Sand	0.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	40.00[b]	0.00[b]
	(0.00)	(90.04)	(90.04)	(90.04)	(90.04)	(90.04)	(36.01)	(0.00)
Sandy Loam	0.00[a]	0.00	80.00[a]	100.00[a]	100.00[a]	100.00[a]	60.00[ab]	0.00[b]
	(0.00)	(0.00)	(72.03)	(90.04)	(90.04)	(90.04)	(54.00)	(0.00)
Clay	0.00[a]	0.00	0.00[b]	0.00[b]	80.00[a]	100.00[a]	100.00[a]	0.00[b]
	(0.00)	(0.00)	(0.00)	(0.00)	(72.03)	(90.04)	(90.04)	(0.00)
Silt	0.00[a]	0.00	0.00[b]	0.00[b]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	(0.00)	(0.00)	(0.00)	(0.00)	(90.04)	(90.04)	(90.04)	(90.04)
$F_{(P<0.001)}$	-	*NS*	9.64	*NS*	1.00	*NS*	1.79	*NS*
df	16	--	16	--	16	--	16	--
$SE_{(d)}\pm$	-	-	18.87	-	11.34	-	24.81	-
$LSD_{(P<0.05)}$	-	-	40.02	-	24.13	-	52.59	-

*Data in paranthesis are Arc SinV n transformation of percentage values.

[a,b]Values followed by similar alphabets do not differ significantly with each other (P>0.05).

Table 2: Foraging capacity of *S. dharanaii* in different distance and moisture level in sandy loam soil.

Treatments (cm)	5% Moisture	10% Moisture	15% Moisture	20% Moisture	40% Moisture
2	100.00[a]	100.00[a]	100.00[a]	100.00[a]	0.00
	(90.04)	(90.04)	(90.04)	(90.04)	(0.00)
3.5	86.11[b]	100.0[a]	100.0[a]	100.00[a]	0.00
	(68.38)	(90.04)	(90.04)	(90.04)	(0.00)
6	79.16[c]	91.66[b]	87.50[b]	79.16[b]	0.00
	(62.98)	(77.99)	(69.59)	(62.98)	(0.00)
Control (IJs but 0% moisture)	0.00[d] (0.00)	0.00[c] (0.00)	0.00[c] (0.00)	0.00[c] (0.00)	0.0 (0.00)
Control (Moisture but no IJs)	0.00[d] (0.00)	0.00[c] (0.00)	0.00[c] (0.00)	0.00[c] (0.00)	0.00 (0.00)
$F_{(P<0.001)}$	213.78	386.33	408.06	591.62	*NS*
Df	20	20	20	20	-
$SE_{(d)}\pm$	1.28	3.40	1.03	0.83	-
$LSD_{(P<0.05)}$	2.68	7.11	2.15	1.74	-

*Data in paranthesis are Arc SinV n transformation of percentage values.

[a,b] Values followed by similar alphabets do not differ significantly with each other (P>0.05).

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Table 3: Time required by IJs for vertical movement /penetration in different soil depth

Soil Type	Time (in hrs.) taken for movement and penetration of IJs in different depth (in cm)			
	9 cm	18 cm	21 cm	30 cm
Gravel	115 ± 20.07	124.8 ±10.73	147.2± 18.19	168±16.97
Sand	92.1±10.73	120±16.97	139.2±20.07	147.2±18.41
Sandy Loam	110.4±13.14	120±16.97	129.6±21.46	153.6±21.46
Clay	158.4±13.14	225.6±13.14	0.00±0.00	0.00±0.00
Silt	86.4±13.14	91.2±10.73	100.8±10.73	124.8±20.07
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
$F_{(P<0.001)}$	Soil type	256.65		
	Soil depth	20.11		
	Soil type x Soil depth	69.86		
df	Soil type	92		
	Soil depth	92		
	Soil type x Soil depth	92		
$SE_{(d)}\pm$	Soil type	4.48		
	Soil depth	3.66		
	Soil type x Soil depth	8.97		
$LSD_{(P<0.05)}$	Soil type	8.91		
	Soil type x Soil depth	7.27		
	Soil depth	17.81		

*Data in paranthesis are Arc Sinv n transformation of percentage values.
[a,b] Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

4. DISCUSSION

Results indicated that clay and silt required more moisture, i.e., above 20.0% to get the required infectivity by EPN to waxmoth larvae. Gravel allowed foraging by the IJs causing infectivity at and above moisture level of 10.0% to 30.0%. Sand proved the best with mortality obtained 100.0% mortality at and above moisture level of 5.0% till 30.0%. Sandy loam, though, required minimum moisture level of 10.0% with 80.0% mortality. Moisture levels of 15.0% to 25.0% were best with 100.0% mortality obtained. At 30.0% moisture level mortality again reduced to 60.0%. It can be noted that there was no mortality at 0.0% moisture level, probably because the movement of the IJs was restricted. Less mortality at higher moisture levels could be due to the restricted movements, as reported by Koppenhoffer and Fuzy (2006), who studied the effect of soil type on infectivity and persistence of the entomopathogenic nematodes *S. scarabaei*, *S. glaseri*, *H. zealandica* and *H. bacteriophora*. Hussaini and Sankaranarayanan (2001) reported activity of six populations of *Steinernema* and one population of *Heterorhabditis* was higher in sandy loam than sand against *G. mellonella* at 5 and 10 cm. Toledo et al., (2009) examined effect of soil texture on the activity of *S. carpocapsae* by applying the corresponding LC_{50} concentrations of nematodes to sand, sandy-clay and loamy-sandy soils. For 6 days old larvae, soil type had a highly significant effect on infection with the highest percentages of infection observed

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in the sandy-clay mixture (60-80* depending the depth) compared to 45-64.0% infection in sand and 23-30.0% infection in loamy-sand soil. Mwaniki et al. (2010) studied the effect of different soil types and depth on movement and penetration of EPN species. They reported the highest *H. indica* induced percent mean *Galleria* mortality occurred in clay loam soil (80.0%), and median mortality of 65.0% in sandy clay loam soils and the lowest level of 40.0% in the clay soil. The differences between the means were significantly different (P< 0.05). *S. karii* induced percent mean *Galleria* mortalities were similar (56-60.0%) for all the soil (P>0.05).

Results indicated that in all the experimental moisture levels IJs required 72 hrs to move 2 cm distance, except 40.0% moisture level. The IJs could move easily at soil moisture level of 10 to 20% up the distance of 3.5 cm, whereas, increasing the moisture level to 40.0% did not allow IJ movement. The silt required significantly less time, with 86.6 ± 13.14, 91.2 ± 10.73, 100.8 ± 10.73 and 124.8 ± 20.07 hrs to reach respectively at 9, 18, 21 and 30 cm depth. It was followed by sand with 92.1 ± 10.73, 120 ± 16.97, 1139.2 ± 20.07 and 147.2 **±** 18.41 for the depths mentioned earlierFurther sandy loam facilitated the IJ movement better than gravel and clay. Sandy loam required 110.4±13.14, 120±16.97, 120.6±21.46 and 153.6±21.46 hrs for 9, 18,21,30 cm, respectively Gravel took the maximum time as compared to all other soil types and the clay did not allow movement beyond 18 cm, for 18 cm also it took 225.6±13.14 hrs.

Molyneux and Bedding (1984) discussed inhibition of nematode mobility and decreased survival in very saturated soil.Georgis and Poinar (1983ab) reported the effect of soil texture on the distribution and infectivity of *Neoaplectanaglaseri.* Movement of the nematode was least in clay soil and limited in silty clay loam soil. The number of migrating nematodes was greatest when wax moth pupae were present. Kung et al. (1990) investigated that the best soil for retaining *S. carpocapsae* ability to kill insects is sandy loam as compared to sand for *S. glaseri*. Townsend et al. (1998) monitored mortality of green June beetles, *Cotinis nitida* larvae by *S. carpocapsae* and reported the optimum soil moisture by weigh as 30.0%. Present observations do not conform to Koppenhoffer et al. (2000) who have reported IJs of *S. monticolum* not to infect waxmoth larvae at 2 and 3.0% soil moistures. In this case, the establishment started at 3.5% and reached to its peak at 6.0% moisture, with very low establishment at 19.0%. Blackshaw and Senthamizhselvan (1991) showed that the foraging efficiency of *S. feltiae*is sensitive to particle grain size, with maximum activity occurring in sandy soils of intermediate grain size (700– 800 m). The reduced pore space could hinder the transmission of carbon dioxide or volatile exudates which the nematodes use as host-finding cues. Koppenhoffer et al. (1995) have observed that considerable established of IJs of *S. carpocapsae* and *S. glaseri* occurred at 4-5% moistures, however, nematodes establishment declined at the highest soil moisture studied (19.0%). Duncan et al. (1996) demonstrated that *S. riobrave* survived best at 2 to 4.0% moisture compared with lower (0.5 01.0%) and higher (4.0- 12.0%) moisture level. However, effect of moisture level mearly for survival was not aimed under the current work against *S. dharanaii* (TFRIEPN-15). Gouge et al. (2000) studied the effect of soil moisture on the distribution of *S. riobrave* in a sand column was determined. They found that EPN, *S. riobrave* infective juveniles (IJ) in each 2.5-cm section of 30-cm-long soil columns. Soil moisture was determined for each section and related to the numbers of nematodes recovered from infected insect baits. Infective juveniles of *S. riobrave* applied on the sand column surface showed some degree of positive geotaxis. IJ in soil columns with a consistent moisture gradient grouped in the upper 12.7 cm within a water potential range of -40 to -0.0055 MPa (2% to 14% moisture). Nematodes in sand columns that were gradually dehydrating moved down the soil column, aggregating on the 28th day between 15-23 cm in depth. Nematode redistribution over time allowed IJ to remain within a water potential range of -0.1 to -0.012 MPa (5.2% to 9.5% moisture).

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Hussaini et al. (2000) investigated infectivity of four populations of *Steinernema* spp. and *Heterorhabditis indica* alone and in combinations were evaluated against *Agrotis ipsilon* in sand and sandy loam soil columns. *H. indica* PDBCEN 13.22 outcompeted *Steinernema* spp. irrespective of soil type, depth and time in terms of *A. ipsilon* mortality. *S. bicornutum* PDBCEN 3.1 was found to be promising against *A. ipsilon*. The performance of all the nematode populations was better in sandy loam soil than in sand at 5 cm depth. The mortality of *A. ipsilon* was enhanced by increasing the time of exposure. A combination of *S. carpocapsae* PDBCEN 6.11 and *H. indica* PBBCEN 13.22 had an additive effect over the control achieved by their individual populations in both soil types at 10 cm depth. Grant and Villani (2003) while investigating effect of soil moisture on five isolates of entomopathogenic nematodes: *H. bacteriophora* Poinar (Oswego and Tuscarora strains), *Steinernema glaseri* (Steiner) (NC1 strain), *S. feltiae* (Filipjev) (Bioassays 369 strain), and *S. carpocapsae* (Weiser) (NY001 strain) demonstrated that insect mortality was generally low in low-moisture, nematode-infested soils before rehydration, but increased to high levels post-hydration. The current observations partially conform to the report on IJs of *S. thermophilum* (Ganguly and Gavas, 2004; Yadav and Lalramliana 2012) that soil moisture levels varying from 1 to 19.0% (w/w) is the most effective. Insect mortality increased significantly at 3.0% moisture and mortality gradually increased up to the 9.0% moisture level, and thereafter, it declined. Yadavand Lalramliana (2012) have studied soil moisture effect on the activity of three entomopathogenic nematodes isolated from Meghalaya. They noted optimum soil moisture for different nematodes species, *S. thermophilum* 6-20.0% and *S. glaseri* 8-25.0%. Further, a minimum of 6.0% soil moisture was noted to be essential for achieving 100.0% host mortality for the above nematodes species. Kaspi et al. (2010) have tested the foraging efficacy (movement and host finding) of *S. riobrave* in these soils at three different soil depths; 100, 250 and 500 mm. Soil textures and physical characteristics. *S. riobrave* ability to infect hosts varied among soil types and depths. They were also found significant correlations between soil characteristics (texture and physical characteristics) and *S. riobrave* foraging efficacy.

Raheel, et al. (2015) evaluated the infectivity of four species of EPNs i.e. *Heterorhabditis bacteriophora*, *H. indica*, *S. feltiae* and *S. asiaticum* in different soil textures (loamy sand, sandy loam and clay loam). The *in vitro* assessment of the infectivity was done by exposing last larval instar of *Galleria mellonella* to nematodes. The results revealed that the infectivity was the greatest in sandy loam soil (71.42%) followed by clay loam (54.75%), while it was the lowest in loamy sand (41.63%). Among all species examined, *H. bacteriophora* showed maximum infectivity (69.82%), followed by *H. indica* (52.36%), *S. feltiae* (52.36%) and *S. asiaticum* (49.19%) showing similar trends. Sharmila et al. (2015) studied the effect different soil moisture levels had effect on the survival of nematodes in soil medium. The survival of infective juveniles for *H. indica* was at 4-25 % moisture level and for *S. glaser i*was at 5-25 %. *H. indica* showed an increased mortality of *C. cephlonica* at increasing soil moisture conditions. The highest mortality of 100 per cent observed at 14 to 25 % of soil moisture content and lowest larval mortality of 6.66 % at 5 per cent moisture content. *S. glaseri* showed highest mortality of 100 per cent at 16 to 25 % soil moisture content and lowest mortality 6.66 per cent at 5 % soil moisture. The highest mortality of insect larvae was observed for *H. indica* followed by *S. glaseri*. The soil moisture of 6 % and above caused 100 % larval mortality for all nematodes. The infectivity of *H. indica* and *S. glaseri* could takes place only at 6 % and above soil moisture.

Kulkarni et al. (2016) studied the effect of soil moisture on horizontal or vertical dispersal by infective juveniles (IJs) of native entomopathogenic isolate, *Steinernema* sp. (TFRIEPN-57) from central India. They found that the 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

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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. Abirami et al. (2017) reported the sandy soil was more suitable for survival of both *S. carpocapsae* and *S. abbasi* which recorded highest survival per cent of 96 and 92 per cent in sandy soil, followed by red soil more suitable for both species which showed 84 and 95 per cent. Least survival of both species were recorded in black cotton soil, due to reduced space between soil particles and high water holding capacity, which affects the nematode movement and survival.

Dzięgielewska and Skwiercz (2018) investigated the impact of soil texture on the infectivity of different species of entomopathogenic nematodes against greater wax moth (*Galleria mellonella* L.). *S. silvaticum* and *H. bacteriophora* were found only in sands, and *H. megidis* predominantly in clay. Nematodes were found in soils of varying pH levels, although individual species preferred a certain degree of acidity. *S. bicornutum* and *H. megidis* were found only in alkaline soils, while others, such as *S. silvaticum,* only in acidic environments (pH<4.5).

Recently, Modic et al. (2020) suggested that the sandy loam soil to be more favourable compared to silty loam soil for EPNs treatment with alcohol ethoxylate.

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.

5. CONCLUSION

To conclude, the findings of present results revealed that the native entomopathogenic nematode, have isolated, being maintained in Tropical Forest Research Institute, Jabalpur, Madhya Pradesh *Steinernema dharanaii* n.r. (TFRIEPN-15) have survived and potential infectivity against wax moth larvae, *Galleria mellonella* in different soil texture, soil moisture and soil depth. These, can be used in management against of wide range of soil-inhabiting insect pests of forestry as well as agricultural importance.

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