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Influence of soil charaters on attachment of the bacterial parasite, *Pasteuria penetrans* to the root knot nematode, *Meloidogyne incognita*

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ABSTRACT: Effect of abiotic factors *viz,* as soil type, soil pH and soil moisture on attachment of endospores of *Pasteuria penetrans* to second stage juveniles of root knot nematode, *Meloidogyne incognita* was studied. Spore attachment and number of spores attached per juvenile to be increased when soil moisture was high (90%). Sandy loam soil showed 97.5 per cent spore attachment and was found more suitable than other soil types. Soil pH of 5.0-7.0 was suitable for spore attachment on juveniles and extreme alkaline and acidic pH were found to reduce spore attachment on juveniles.

Keywords: *Pasteuria penetrans*, soil pH, soil moisture, soil type

INTRODUCTION

Root knot nematode, *Meloidogyne* spp. is the most prevalent nematode causing heavy damage to many crops. Even though chemical nematicides reduce nematode problems substantially they may cause toxic effects to human and other vertebrates. Accordingly, there is a need to exploit biocontrol agents to manage serious pests (Chand and Gill, 1994). Among various biocontrol agents, *Pasteuria penetrans,* a mycelial endospore forming hyperparasitic bacterium is a highly potential bacterial parasite and can be used as an alternative agent to manage *Meloidogyne* spp. (Mankau and Prasad, 1972; Sayre, 1984; Stirling, 1984; Brown and Smart 1984; Maheswari *et al.,* 1988). One of the important requirements for an organism to be used as a biocontrol agent is to determine the host specificity, influence of external factors on that organism and compatibility with other organisms. Hence, the present study was undertaken to study the influence of various edaphic factors such as soil moisture, soil pH and soil type on attachment of *P. penetrans* endospores on nematode cuticle.

MATERIALS AND METHODS

Maintenance of pure culture of *Pasteuria penetrans*

Egg masses of root knot nematode, *M. incognita* were collected from infested roots and incubated at room temperature (27±1°C) in water for hatching. After egg hatching, 500 J2 of *M. incognita* /plant were inoculated to tomato plant (variety of PKM 1) maintained in pots with pot mixture (Sand: Red soil: Sand at the ratio of 1:2:2) under glasshouse. After 30 days, tomato plants were pulled out from earthen pot to observe gall formation and egg production of *M. incognita* on root system.

The infected *M. incognita* was extracted from tomato root samples from Mathampatti village of Coimbatore. *P. penetrans* infested gravid female of root knot nematode was dissected from tomato plant and it was crushed between glassslide and presence of endospores was confirmed under compound microscope (Leica - 020-518500). Endospores quantity was counted in haemocytometer (Neibular). Second stage juvenile (J2) of *M. incognita* were taken in a beaker containing water and endospores of *P. penetrans* were inoculated. This solution was agitated using fish tank agitator continuously for encumbrance of endospores on J2 cuticle. After 72h, 6-8 spores were attached was on juveniles and these spore encumbered juveniles were inoculated in to tomato plants for further culture maintenance.

Influence of soil pH on *P. penetrans* spore attachment

Effect of soil pH on *P. penetrans* endospores and attachment on juveniles of *M. incognita* was studied at different pH level *viz*., 4, 5, 8 and 9 and a check was maintained with normal tap water (pH 7.5). Sandy loam soil was taken to test the effect of soil pH on spore attachment on *M. incognita* juveniles. Soil slurry was prepared and different soil pH was adjusted by adding acid (HCl) and base (NaOH) solutions and soil was air dried for 3 days to get optimum moisture level. Dried soil was filled in 150g holding plastic cups. Spores of *P. penetrans* were extracted from infected *M. incognita* gravid females by mechanical crushing method and spore load was confirmed in haemocytometer. One million endospores were incubated in different soil pH for fifteen days. After that 100IJ of *M. incognita* juveniles were inoculated in each cup and whole set up was incubated in room temperature for 48h to observe the spore attachment on juvenile nematodes. Each treatment was replicated four times.

Tap water pH was adjusted by adding base (NaOH) and acid (HCl) solution to test the effect of *P. penetrans* spore attachment on *M. incognita* juveniles. The females of *M. incognita* which were infested with *P. penetrans* were pressed gently using a sterile dissection needle tip. The spore load per female was assessed using haemocytometer by drawing 1 ml from spores containing microfuge tube. One million spores were incubated in pH adjusted solution for seven days after incubation, *M. incognita* juveniles (50J2/ml) were inoculated to test the influence of spore on juvenile attachment. Each treatment was replicated four times. Endospore in different pH solution was incubated at 25°C for 15 days. After 15 days incubation, observation was taken at every 24 h interval.

Influence of soil types on *P. penetrans* spore attachment

Influence of soil types on *P. penetrans* endospore attachment was conducted in varied soil type such as sandy loam, loam, sandy clay loam, clay loam and sandy soil. Each treatment was replicated four times. To test the effect of soil type on endospore attachment 50 g of each soil was taken. Soil was filled in plastic cups and $4x10^6$ endospores/ml of distilled water were inoculated in each cup. Whole set up was incubated at 25°C for 15 days. After 15 days of incubation, 50 J2 of *M. incognita*/ml of water were inoculated into cups. After 72 h of incubation at room temperature, attached nematodes were extracted by Cobb's sieving technique and modified Baermann technique (Schindler, 1961). pH of each soil type was recorded using pH meter (Elico, deep vision -161).

Influence of soil moisture on spore attachment

Sandy loam soil was sterilized and mixed with sterile water to maintain moisture level of 0, 20,40, 50 and 70 per cent. These soils with different moisture levels were filled in plastic cups (100g/cup) to test the effect of soil moisture on endospores of *P. penetrans* attachment on *M. incognita* juveniles. Initially, field capacity of the loamy sand soil was tested through pressure plate apparatus (Richards and Milton, 1943) and different moisture levels were maintained by adding water at 0, 2.9, 5.9, 11.91, 14.89 and 20.84ml to get 0, 10, 20, 40, 50 and 70 per cent moisture level. One million endospores were inoculated at each soil moisture level and incubated for ten days 1 ml of distilled water was added every day to avoid evaporation of water content in each treatment. After ten days and *M. incognita* juveniles (100J2/ml) were inoculated in each treatment. Each treatment was replicated four times.

Statistical analysis

The data obtained from above mentioned experiments were subjected to statistical analysis following the methods formulated by ANOVA using the statistical package SPSS v.8.0.

RESULTS AND DISCUSSION

Influence of soil pH on *P. penetrans* spore attachment

The observation of juveniles encumbrance and number of endospores indicated that encumbrance of endospore on juveniles was highest in soil pH 5 (8.25 per cent) and number of spore attached were 8 spores per juvenile. The encumbrance of endospores on juveniles was 6.75 and 6.00 per cent at pH 7 and 8 respectively. Number of endospores per juvenile was higher in pH 7 (12 endospores/ juvenile) followed by pH 8 (6 endospores/J2) (Table 1). Extreme acidic and alkaline pH were not suitable for both encumbrance of endospores on juvenile and number of endospores attached per juvenile. The results of effects of soil pH clearly indicated that extreme alkaline and acidic soils are not suitable for spore attachment on juveniles of root knot nematode. In contrast, Javed *et al* (2002) reported that endospore attachment on juvenile was increased by increasing soil pH in alkaline condition (pH 9.0).

Influence of soil types on *P. penetrans* spore attachment

Table 1. Influence of soil pH on *P. penetrans* endospore encumbrance on *M. incognita*

Level of soil pH	Encumbered juveniles (%)*	Number of spores attached per J2**
pH 4	0.25[b]	0.00[b]
	(4.81)	(0.20)
pH 5	82.5[a]	8.00[a]
	(65.83)	(0.64)
pH 7	72.50[a]	12.00[a]
	(62.22)	(0.97)
pH 8	60.0[a]	6.00[a]
	(50.82)	(0.67)
pH 9	20.0[ab]	3.00[b]
	(27.10)	(0.50)
SEd	8.41	0.12
CD(p=0.01)	24.80	0.36

Values with same alphabets are not significantly different by DMRT. Figures in parentheses are *arc sin and ** log transformed values.

Table 2. Influence of soil types on *P. penetrans* endospore encumbrance on *M. incognita*

Soil type	Encumbered juveniles (%)*	Number of spores attached per J2**
Sandy loam	97.5[a] (85.17)	15.0 (1.17)
Loam	60.0[b] (51.11)	6.0 (0.61)
Sandy clay loam	67.50[b] (55.44)	9.75 (0.97)
Clay loam	60.0[b] (50.89)	4.25 (0.71)
Sandy	80.0[b] (63.80)	12.0 (1.05)
SEd	5.87	0.27
CD (p=0.01)	17.3	14.54 (NS)

Table 3. Influence of soil moisture on *P. penetrans* endospore encumbrance on *M. incognita*

Moisture level	Encumbered juveniles* (%)	Number of spores attached per J2**
10%	5.0[c] (9.36)	2.0[c] (0.44)
20%	37.5[bc] (33.82)	5.0[bc] (0.80)
40%	77.5[ab] (62.89)	12.0[b] (0.86)
50%	80.0[ab] (65.17)	18.0[b] (0.97)
60%	90.0[a] (74.07)	24.0[a] (1.17)
Control	2.5 (4.82)	1.0[c] (0.37)
SEd	10.36	0.15
CD(p= 0.01)	35.16	0.45

Values with same alphabets are not significantly different by DMRT. Figures in parentheses are *arc sin and ** log transformed values.

Encumbrance of endospores on juveniles was more in sandy loam (97.5 per cent) followed by sandy soil (80.0 per cent). In sandy clay loam and clay loam soils, 67.5 per cent and 60.0 per cent encumbrance were recorded, respectively. Endospore encumbrance was in soil. However, it was on par with wet land soil. Number of spore attachment on juvenile was high in sandy loam soil (15 endospores/juvenile) followed by sandy soil (12 endospores / J2). Nine spores per juvenile were recorded in sandy clay loam but loamy soil had only 6 spores attachment on juvenile (Table 1).

The results of current study revealed that endospore attachment on juveniles was more in sandy loam soil followed by sandy soil and less in clay soil. This may be due to the higher water holding capacity of sandy loam soil compared to sandy soil and available water in soil is an important factor for nematode survival and movement. Similarly, more clay content will arrest spore dispersal and it may lead to suffocation of juveniles which in turn reduced spore attachment. The results of present study is in agreement with the findings of Verdjo-Lucus (1992) who stated that uniform distribution of endospores and movement of nematodes were restricted when the clay content was more in soil. The findings of Talavera and Mizukubo (2003) are in contrast to current results who stated that spore attachment was directly proportional to sand particle present in soil. Mean while, Sigh and Dhawan (1996) reported that the reproduction of nematodes was restricted to spore attached juvenile in sandy loam soil.

Influence of soil moisture on spore attachment

Percentage of spore attachment on juveniles was gradually increased with soil moisture. When soil moisture was low (10%), only 5 per cent attachment was observed and number of spores was also 2 endospores per J2. Highest spore attachment (90 per cent) was recorded when the soil moisture was 60% followed by 50, 40 and 20 per cent soil moisture which recorded 80, 77.5 and 37.5 per cent attachment respectively. Meanwhile, number of spores attached per juvenile was optimum (12 endospores/ J2) in 40 per cent moisture while it was more in 50 and 60 per cent soil moisture as 18 and 24 endospores/ juvenile respectively (Table 1-3).

Lower moisture level in soil may lead to reduction in

nematode movement and spore attachment on juveniles, which was reported by Talavera and Mizukubo (2003). The result of current study is in agreement with above findings. The present study indicated that the moisture level of 50-60% in was more suitable for spore attachment and further development of bacteria in nematode and low moisture level caused an reduce nematode movement in soil. This result was similar to that of Antoniou (1989) who reported that storage of root knot nematode juvenile in water for prolonged time may induce quiescent stage in nematodes which in turn reduced nematode movement.

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