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Effect of soil properties and temperature on the nematode antagonistic potential of bacterial bioagent, *Pasteuria penetrans* against root knot nematode, *Meloidogyne incognita*

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ABSTRACT: The nematode antagonistic bacterium, *Pasteuria penetrans* is one of the proven and highly efficient biocontrol agents. A study was conducted to understand the influence of various abiotic factors. Five different soil types *viz*., alluvial, black, red, laterite and sandy were filled in paper cups (200g/cup) and freshly hatched second stage juvenile (J2) of root knot nematode, *M. incognita* (1000 J2/cup) along with 10 mg of root powder (1.84 x 10^6 spores/g) was thoroughly mixed and incubated at 25°C for 48 h. The parasitization was higher (95.5%) in sandy soil with a clay content of 6.0 per cent. Spore attachment of *P. penetrans* at four levels of temperatures *viz*., 15, 20, 25 and 30°C in red loamy soil was tested. Significant influence of temperature was observed in parasitization of second stage juvenile under 25^0C (90.2%). A glasshouse experiment was conducted to study the influence of nematicides. Application of carbofuran along with *P. penetrans* increased the rate of parasitisation which was 83.1 per cent as against 61.0 per cent in *P. penetrans* treatment only. Influence of root exudates of host and non-host plants of *M. incognita* was tested on the parasitizing ability. The parasitizing ability significantly increased by 4.5 per cent in tomato root exudates and by 4.0 per cent in coleus. The host plant had influence over the number of spore attachment on J2. The results of these experiments paved way to exploit the nematode bacterial hyper parasite, *P. penetrans* for managing plant parasitic nematodes under varied soil environmental conditions.

Keywords: Root knot nematode, *Pasteuria penetrans*, soil type, temperature, nematicides, spore attachment, root exudates

INTRODUCTION

Plant parasitic nematodes are one of the limiting factors in crop productivity. Most of the horticultural crops are susceptible to nematodes and cause 10 – 15% yield loss. Chemical nematicides are widely used for nematode management. Some of the major nematicides were withdrawn from market due to their harmful effects to soil and environment. *Pasteuria penetrans* is a potential bacterial hyper parasite of nematodes. Biological control potential of this bacterium has been proved by many Nematologists in India (5, 11) and abroad. The endospores of *P. penetrans* can tolerate various temperature regimes, moisture levels, pH and chemicals. This research paper describes the parasitisation potential of *P. penetrans* on root knot nematode, *Meloidogyne incognita* under various abiotic stresses.

MATERIALS AND METHODS

Soil Type

Influence of soil type on the activity of *P. penetrans* was carried out in five different type *viz*., alluvial, black, red, laterite and sandy. Plastic cups (250g capacity) were filled with sterilized soils (200g/cup) of the above mentioned types separately. Freshly hatched juveniles (J2) of *M. incognita* (1000 J2/cup) along with 10 mg of root powder (*P. penetrans* @ 1.84 x 10^6 spores / g) was thoroughly mixed with soil and incubated at 25ºC for 48 h. Soil was maintained with 25 per cent moisture level by adding 50 ml water. Each treatment was replicated six times. The nematodes in each replicate were extracted by using a combination of sieving and Baermann funnel technique and the number of spores attached/J2 and percentage of parasitisation were assessed. The soil texture, field capacity, pH, EC and organic matter were analysed as per standard methods.

Temperature

Interaction between *P. penetrans* and *M. incognita* was determined under four levels of temperatures *viz*., 15, 20, 25 and 30ºC on red loamy soil. Plastic cups were filled with sterilized soil (200 g/cup) and thoroughly mixed with 100 J2 of *M. incognita* along with 10 mg root power / cup (1.84 x 10^6 spores/g) and incubated at the above mentioned temperatures for 48 h. Each level of temperature was replicated eight times. The spore attachment percentages on J2 were recorded after extraction of nematodes from soil.

Nematicides

Glasshouse experiment

A glasshouse experiment was conducted to study the influence of nematicides on *P. penetrans*. Sterilized soil was filled in 2.5 kg clay pots and 3-week-old tomato cv. Co 3 seedlings were transplanted. The following treatments were given three days after transplanting.

T1- *M. incognita* 5000 J2 / pot
T2 - *M. incognita* 5000 J2 / pot + *P. penetrans* root power 10 mg /pot @1.84 x 10^6 spores / g
T3 - *M. incognita* 5000 J2 / pot + carbofuran 3 mg / pot
T4 - *M. incognita* 5000 J2 / pot + *P. penetrans* root power 10 mg @1.84 x 10^6 spores / g+ carbofuran 3 mg/ pot

Each treatment was replicated five times and completely randomised. Plant biometric observations, soil and root nematode population, number of J2 parasitized and number of spores attached per J2 were recorded at 60 days after transplanting.

Root exudates

Root exudates of tomato *(Lycopersicon esculentum* Mill), Chilli *(Capsicum annuum L.),* Cowpea *(Vigna unglciculata L.)***,** coleus (*Coleus blumi,* Benth)**,** marigold (*Tagetes patula* L.), maize *(Zea mays L.)., (Sorghum vulgare L.)* and sunflower *(Helianthus annus L.)* were collected 35-40 days after germination. Root exudates of each plant species was taken individually in 5 cm Petr idish (5 m l / dish). Freshly hatched 500 J2 of *M. incognita* and 10 mg root powder (1.84 x 10^6 Pp spores / g) per dish were added and gently mixed with the root exudates and incubated at 25º C for 48 h. Tap water was used as control. A completely randomised block design with eight replicates was adopted. The percentage of individual J2 parasitized and the numbers of spores attached per J2 were recorded.

RESULTS AND DISCUSSION

Influence of different abiotic factors and root exudates on parasitization of bacterial parasite, *P. penetrans* is elaborated below.

Soil type

The texture of soils varied from sandy to clay loam with the clay content varying from 6 per cent to 25.9 per cent. The percentage of parasitisation was high in sandy soil (95.5) with a clay content of only 6.0 per cent and the least in laterite soil (71.0) with a clay content of 25.9 per cent (Table 1 & 2). The relationship

Table 1. Properties of different types of soil used for parasitisation study

Soil types	Coarse sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	Field Capacity	pH	EC dSm-1	OM (%)
Alluvial (sandy loam)	20.9	32	26	19.5	28.5	9.1	1.05	0.83
Black cotton (clay loam)	20.2	43.8	3.5	22.5	30.2	8.3	0.52	1.1
Red (sandy loam)	41.6	26.4	17	12	14.7	7.4	0.48	0.38
Laterite (sandy clay loam)	32.1	22.7	18.2	25.9	20	4.9	0.24	2.18
Sandy	40.3	51.2	2.5	6	5	8	0.05	-

Table 2. Effect of Different soil types on the interaction of *M. incognita* and *P. penetrans*

Soil Types	Parasitization (%)	Number of spores/J2
Alluvial	86.9 (68.78)^c	8.0^c
Black	81.3 (64.38)^d	7.0^d
Red	90.7 (72.24)^b	9.5^b
Laterite	71.9 (57.99)^e	6.3^e
Sandy	95.5 (77.75)^a	10.7^a
CD (P=0.05)	3.5	0.47

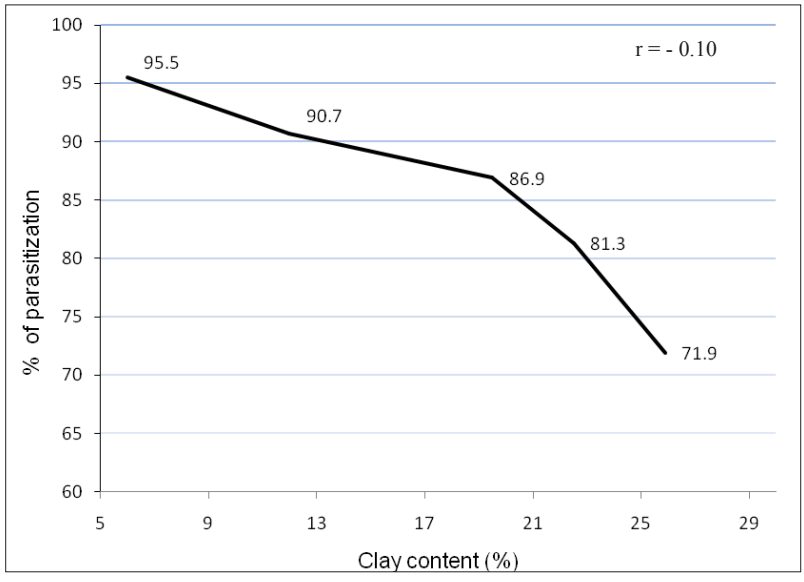

Fig. 1. Influence of clay content on percentage of parasitization of *P. penetrans* on *M. incognita*

between the clay content and extent of parasitization was statistically negatively correlated (r = - 0.10) (Fig. 1) The clay content also influenced the spore load carried by individual J2 which varied from 6.3 in laterite soil to 10.7 in sandy soil. Textural and physical characters of soils were studied for interaction of *M. incognita* and *P. penetrans*.

The spore attachment on *M. incognita* and the percentage of parasitization varied significantly in soils of different textures. Soil texture and other factors such as salinity, pH, organic content etc., that influence the nematode movement in soil (5) are likely to influence the *P. penetrans* activity. The parasitizing ability of *P. penetrans* was high in sandy soil and low in laterite soil. The higher parasitizing ability of the hyper-parasite is thus attributable to the more rapid and easy dispersal of nematode in sandy and sandy loam soils when compared to heavy laterite soil. Spaull (7) observed that *P. penetrans* occurred more frequently in sand and loamy sand than in other types in sugarcane fields of South Africa. The soil structure and EC have also been reported to affect the dispersal of *P. penetrans* spores in soil with loose texture (2). Physical and chemical soil properties might also affect the bacterial establishment and dispersal as has been shown for other nematode-antagonist systems. Small soil pores existed in soil containing high percentage of clay which may restrict the movement of *P. penetrans* spores. Verdjo-Lucas (12) discussed that spore may adhere to clay particles and would not be free to attach themselves to nematodes. This factor also could have influenced the reduced level of parasitization observed in the heavy soils in the present investigation.

Temperature

Significant influence of temperature was observed in the extent of parasitisation of J2 by *P. penetrans* which was 90.5 per cent under 25ºC and 20.7 per cent under 15 ºC. The same trend was seen in the spore load carried by individual J2 which ranged from 3.4 under 15ºC to 7.5 under 25ºC (Table 3). The results of present experiment agreed with the findings of Anjukamra and Dhavan (1). Greater spore attachment was observed on *M. javanica* at

Table 3. Influence of temperature on per cent parasitization of *P. penetrans*

Temperature	Percentage of parasitization	Number of spores attached/J2
15^0C	20.7^{d} (26.0)	3.4^{d}
20^0C	64.4^{b} (53.4)	4.3c
25^0C	90.59^{a} (71.9)	7.5^{a}
30^0C	55.6^{c} (48.0)	5.8^{b}
CD (P=0.05)	0.7	0.2

Table 4. Effect of nematicides on the interaction of *M. incognita* and *P. penetrans* in tomato

Treatments	Number of nematodes /200 g soil	Number of galls /g root	Number of egg masses/ 2g root	Percentage of J2 parasitized/ 200 g soil	Number of spores attached/ J2	Fresh weight of shoot (g)	Fresh weight of root (g)
M. incognita alone	320.0[a]	28.8[a]	40.4[a]	-	-	31.4[c]	19.2[abc]
M. incognita + *P. penetrans* (10mg)	147.4[b]	22.6[b]	18.6[b]	61	8.3	41.6[b]	19.8[abc]
M. incognita + carbofuran 6 mg	122.3[c]	20.6[bc]	16.0[bc]	-	-	47.6[b]	21.2[ab]
M. incognita + *P. penetrans* (10mg) + carbofuran 6 mg	107.0[d]	18.0[bc]	11.4[c]	83.1	11	57.4[a]	22.8[a]
CD (P=0.05)	14	4.8	5.4			7.9	7.0

Table 5. Effect of root exudates on the parasitizing ability of *P. penetrans*

Treatments	Percentage of parasitization	Number of Spores/ J2
Tomato	97.2 (80.37)[a]	180[a]
Coleus	96.7 (79.53)[b]	17.8[b]
Cowpea	91.1 (72.64)[d]	14.1[c]
Sorghum	50.5 (45.29)[f]	5.0[e]
Maize	47.3 (43.45)[g]	4.6[ef]
Sunflower	69.8 (56.66)[e]	9.0[d]
Tagetes	26.0 (29.33)[h]	3.0[ef]
Tap water	92.8 (74.44)[c]	15.5[c]
CD P=0.05)	1.14	2.2

22.5 – 30.0°C (8). Wallace (13) reported that the optimum temperature for *M. incognita* J2 migration is 25°C. Thus the optimum temperature for spore attachment corresponds with the optimum temperature for migration of *M. incognita* J2 in soil. The data further substantiates that the higher parasitizing ability of *P. penetrans* was probably due to greater nematode mobility at 25°C.

Nematicides

Glasshouse experiment

The nematicide and *P. penetrans* when applied together reduced the number of galls, egg masses and soil population to the highest level, which was followed by individual application of carbofuran and *P. penetrans* (Table 4). Application of carbofuran along with *P. penetrans* increased the rate of parasitisation which was 83.1 per cent as against 61.0 per cent in *P. penetrans* treatment only. The number of spores encumbered was also higher when *P. penetrans* was applied along with carbofuran (Table 4).The growth of plant assessed through fresh weight of shoot and root indicated that the application of the bio control agent along with the nematicides gave the best results than the application of either carbofuran or *P. penetrans* alone (Table 5). Application of a combination treatment consisting of

nematicide and P. *penetrans* reduced the number of galls, egg and soil nematode population in a significantly better manner than either component applied individually.

The nematicides carbofuran and aldicarb had no detrimental effect on *P. penetrans* (9). The possibility of increased spore attachment by *P. penetrans* in the presence of nematicide resulting in synergistic reduction of root galling by *M. javanica* was suggested by Brown *et al*. (3). Enhanced activity of the juvenile due to toxic effect of carbamate and organophorphate nematicides resulting in more frequent encounter with the spores has been suggested as reason for increased spore encumbrance and level of parasitism by *P. penetrans* (14).

Root exudates

The influence of root exudates of host and non-host plants of *M. incognita* was tested on the parasitizing ability of *P. penetrans***.** The parasitizing ability significantly increased over control in tap water in tomato by 4.5 per cent and coleus by 4.0 per cent. In the other plants tested namely cowpea, sunflower, maize and tagetes, the parasitizing ability decreased and ranged from 91.1 per cent to 26.0 per cent, while in control it was 92.8 per cent. The difference between exudates of different host plants on the degree of parasitisation by P. penetrans was statistically significant. The host plant had influence over the number of spores which attach to the J2 In tap water control it was 15.5/J2 while in tomato and coleus it was 18.0 and 17.8/J2, respectively. In the exudates of other plant species tested the spore load varied from 3.0 tagetes to 14.1 in cowpea (Table 6). Madula *et al*. (4) reported a high level of infection (75 per cent) of *M. javanica* with *P. penetrans* occurred under continuous cropping with tomato and lower level (25 per cent) under continuous cropping with tobacco and suggested that the action of root exudates could increase the multiplication of *P. penetrans*. The chemical components of root exudates probably influenced the differences in *P. penetrans* parasitization. O'Brien (6) reported that the carbohydrate related lectins are primary factors for spore attachment. So that the carbohydrates present in root exudates may be involved in spore adherence on juvenile cuticle. The parasitization by *P. penetrans* was very low in the exudates of the nematode antagonistic plant, tagetes. Nematicidal property of tagetes also reduce mobility of juveniles and this probably resulted in lower parasitization of J2 under this treatment, since the attachment of spores on J2 cuticle depends on chance contact which increases with nematode migration (8).

CONCLUSION

The bacterial hyper parasite, *Pasteuria penetrans* is a potential bio control agent for nematode management. The findings of the present research work proved that *P. penetrans* can withstand wide range of temperatures and pH ranges. Similarly, the bacterium is compatible with nematicides. Root exudates of nematode host plants enhance the parasitisation per cent of *P. penetrans*.

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