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BIOCONTROL POTENTIAL OF PASTEURIA PENETRANS AGAINST MELOIDOGYNE INCOGNITA

NEMATOLOGY

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ABSTRACT

Nematode parasitic bacteria Pasteuria penetrans has been studied for its biocontrol potential against root-knot nematode Meloidogyne incognita. Two isolates of P. penetrans viz., Assam isolate (AM) and Delhi isolate (DL) was taken for this purpose. Study on the spore encumbrance of P. penetrans on root-knot J2 revealed that AM isolate of P. penetrans is more effective than DL isolate. Significantly higher numbers of encumbered J2 and higher numbers of spores per J2 (second stage juvenile) was recorded in the highest level of P. penetrans inoculums in both the isolates. Management of root-knot population by Pasteuria isolates showed that, both the isolates were effective in reducing nematode population in tomato roots, thereby improving the plant growth parameters in accordance with the increase in inoculum levels. Likewise, number of galls, egg masses and nematode population also declined with corresponding increase in inoculum levels of Pasteuria. Assam isolate (AM) performed better then Delhi isolate (DL) in managing root-knot nematode in Assam agroclimatic condition.

KEYWORDS:

Assam isolate, Delhi isolate, *Meloidigyne incognita* and *Pasteuria penetrans*

INTRODUCTION

Root-knot nematode, *Meloidogyne incognita*, is a sedentary endoparasitic nematode with a wide host range. It includes more than 2,000 plant species, causesing substantial yield loss that may go up to 50 percent. Root-knot nematode damage results in poor growth, a decline in qualitative and quantitative yield of the crop and reduced resistance to other stresses. For the management of root-knot nematode, about 48% of total available nematicides are being used in different parts of the world (Whitehead, 1998). It is well known fact that these nematicides have been identified as the major cause of environmental pollution and ground water contamination. Many of these have been banned due to their harmful effects on human, live stocks, environment and ground water contamination. Further the cost benefit ratio, except for the cash crops has not been always favorable for the commercial growers. On the other hand the use of a resistant cultivar in managing these abnoxious nematodes is ideal but breeding nematode resistant variety takes very long time. Hence the emphasis on chemicals has been gradually shifting towards other eco friendly approaches. In this scenario Nematologists have been exploring the natural enemies to manage nematodes. Among the various biological control agents identified till date, *Pasteuria penetrans* has been found to have potential effect in suppressing some plant parasitic nematodes including root knot nematode. It is an obligate, endospore-forming bacteria that parasitise the host nematode. The most important fact regarding this bacterial bio agent is its capacity to adhere or encumber on the J_2 of the root-knot nematode, that determines the pathogenicity on the host nematode. Somasekhar and Gill (1990) reported that larval density in soil decreased with an increase in spore density level. Both the organisms being soil borne, they are continuously subjected to biotic and abiotic factors in soil, that influence spore encumbrance (Gogoi and Gill, 2003). Likewise performance of *P. penetrans* population reported from different places with varied agroclimatic condition may also have a bearing on its effectiveness. *P. penetrans* was reported from Assam by Gogoi and Neog (2001). This Assam isolate performed very well in pot as well as field conditions in suppressing root-knot population (Gogoi and Neog, 2004).

With these considerations in mind, this piece of research work was undertaken to compare the efficacy of two different isolate i.e., the Delhi isolate (D_L) and Assam isolate (A_M) of *P. penetrans* in managing *M. incognita,* in Assam condition.

Materials and Methods

The study on biocontrol potential of *Pasteuria penetrans* against *Meloidogyne incognita*, was carried out in the Department of Nematology, Assam Agricultural University, Jorhat, Assam. The experiments were carried out in Completely Randomized Design, replicated 5 times. Root-knot susceptible tomato variety Pusa Ruby was used for the purpose.

Raising of pure nematode culture

A single egg mass of *M. incognita* was collected from pure culture maintained in tomato plants in the Green house, Department of Nematology, AAU, Jorhat. Newly hatched fresh active second stage juvenile ($\mathbf{J_2}$) of *M. incognita* were inoculated to a series of tomato seedlings grown in sterilized soil in pots. These plants were used as a source of inoculums for subsequent experiments.

Infested plants were uprooted carefully and soaked in water for few minutes and then carefully washed in tap water. Egg masses were collected with the help of forceps from the infested roots and placed in a cavity block in filter water. Then the egg masses were transferred to double layered tissue paper supported on a wire gauge kept on a petriplate containing filter water. After 24 hours, active J_2 were collected. The nematode counting was done by using Hawkslay's nematode counting dish under stereoscopic binocular microscope.

Culturing of *P. penetrans*:

P. penetrans is an obligate parasite and hence cannot be cultured in an artificial media. The bacterial inoculum was multiplied by introducing spore suspension along with J_2 of *M. incognita* in root-knot susceptible tomato. Initially spores of *P. penetrans* was obtained by crushing infested adult females of *M. incognita* from the pure culture already maintained in the Department of Nematology, AAU, Jorhat. Two months after nematode inoculation, plants were uprooted washed free of soil, sun dried and then grounded to fine powder. This root powder was used as a source of bacterial inoculums.

The root powder was then mixed with water to make a suspension and inoculated in the pots with root-knot susceptible tomato to maintain the root-knot population on which the bacteria will grow and multiply. The Delhi isolate, in form of root powder was obtained from Division of Nematology, IARI New Delhi.

The quantitative estimation of endospores in water suspension was made by using a haemocytometer. Ten mg root powder of both the isolates were taken in two measuring cylinder and 1ml of water was added to each of it. Spore suspension so formed was thoroughly bubbled and aliquot of 0.1ml was drawn and numbers of spore were counted on a haemocytometer. Average of three counts was multiplied with total volume of suspension to get the actual number of spores present in 1ml suspensions.

Thirty five sterilized petriplates were filled with10gm of sterilized sand-soil mixture. In each petriplate 1ml of spore suspension of three concentrations (1 x 10^6, 1.5 x 10^6, 2.0 x 10^6) of Assam and Delhi isolates respectively was added on the sand- soil mixture. Subsequently, 5000 active juveniles of *M. incognita* were added and then incubated at 27^{0}C for 24 hrs. In untreated control only nematode was added. Nematodes were extracted from sand through 350 mesh sieve and 20 J_2 from each petriplate was picked at random and examined for number of spores attached, and number of nematode with bacterial spores under compound microscope at 40x .

Preparations of pots:

Forty earthen pots of 1.5 kg soil capacity were cleaned and sundried. Few broken pieces of bricks were placed at the pots and filled with sterilized soil. Proper labeling of each pot was done with T-shaped labels. In treated control only nematode was added where as in untreated control no no nematode was used.

Inoculation of encumbered J_2 in pots:

Active J_2 of *M. incognita* with more than 5 endospores attached on the cuticle @ 2000 J_2/ pot was inoculated to the seedlings of tomato. Both the isolates of *P. penetrans* was inoculated at three different concentrations viz., 1.0 x 10^6, 1.5 x 10^6 and 2.0 x 10^6 spores per ml of water. Experiment was replicated 5 times. Inoculation of both the nematode and bacterial spores were done with a syringe in the root zone of the seedlings and then covered with soil, followed by light watering. Plants were kept in the Green house and observations were taken two months after inoculation.

Result and Discussion

The number of spore encumbered J_2 were more (79, 80 and 82) in the treatments with Assam isolate as compared to the treatments with Delhi isolate (47, 58 and 62) in the respective treatments (Table 1). However, highest numbers of encumbered J_2 was found in the highest levels of spore inoculum i.e., 82 in Assam isolate and 62 in Delhi isolate. The number of spores per J_2 was more (68, 70 and 72) in Assam isolate as compared to the Delhi isolate (54, 56 and 60) in the respective treatments (Table 1). *Pasteuria* infected J_2 were found to be less active and less mobile with a tendency to aggregate forming clumps. Brown and Smart (1985) also observed that bacterial spores of *Pasteuria* attached rapidly to J_2 of *Melodogyne javanica*.

Studies on the efficacy of bacterial isolates on the management of root-knot nematode showed that both the isolates were effective in reducing nematode population in tomato roots thereby improving the plant growth parameters. Similar results were also obtained by Dube and Smart (1987) that *P. penetrans* can significantly reduce population of root-knot nematode.

Plant growth parameters like plant height, fresh and dry shoot weight was found to be increased in accordance with the increase in bacterial inoculum levels. Experimental data revealed that maximum plant height of 39.80 cm was recorded in the untreated control followed by 39.60 cm in the treatment with highest levels of bacterial inoculum of Assam isolate and 37.20 cm in Delhi isolate (Table 2).

Likewise, number of galls, egg masses and nematode population also declined with corresponding increase in inoculum levels of bacterial parasite (Plate 4. a, b). Significantly higher numbers of galls and egg masses were recorded in treated control (40.00 and 38.80) followed by (39.60 and 35.60) in the lowest concentration of bacterial inoculum of delhi isolate (Table 3). Similarly, minimum numbers of eggs per egg mass was recorded in the treatment with highest levels of bacterial inoculum in both the isolates (Table 3). Chen *et al*. (1996) also reported that populations of *Pasteuria* on peanuts can effectively reduce root and pod galls of *M. arenaria* by 60 and 95 per cent respectively.

The stained galled roots when teased out were filled with adult *Pasteuria* infected females of root-knot nematodes. *Pasteuria* spores burst out from these females when it was subjected to slightest injury (Plate 2. b, c).

Conclusion

The comparative account of both the isolates of the bacterial bioagent signifies the importance of the native isolate of bio-agents in the place of origin. Being evolved and adopted to the hot and humid environmental conditions of Assam, the Assam isolate is found to be best fitted for Assam condition. The Delhi isolate is also no doubt performing well in all the tests, but showing slightly lesser activity as compared to Assam isolate. Being adapted to dry hot and cold temperature conditions of Delhi, this isolate may exhibit lower efficacy in Assam environmental conditions. At times the effectiveness of the bacterial isolate is also determined by the host nematode and the plant host, that too may have a bearing on the place of origin. The adaptability and performance of an organism is best at its own niche. While considering the selection of a biocontrol agent for managing a particular pest, emphasis should be on native or local organisms/ isolates. Further research is needed for studies relating to acclimatization of bio-agents like *Pasteuria* in a different environmental condition in different agroclimatic zones of our country.

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Table 1: Effect of *Pasteuria* isolates and spore concentration on number of encumbered juveniles and spore encumbrance per juvenile

Treatments	Spore encumbered juveniles	No. of spores per juvenile
$T_1(A_M)$ = 1.0 x 10^6	79^{a}	68^{abc}
$T_2(A_M)$ = 1.5 x 10^6	80^{a}	70^{ab}
$T_3(A_M)$ = 2.0 x 10^6	82^{a}	72^{a}
$T_4(D_L)$ = 1.0 x 10^6	47^{e}	54^{e}
$T_5(D_L)$ = 1.5 x 10^6	58^{c}	56^{de}
$T_6(D_L)$ = 2.0 x 10^6	62^{b}	60^{d}
T_7 (Control)	0	0
S.Ed	**4.54**	**2.74**
CD(0.05)	**9.31**	**5.78**

Data are mean of five replications

Table 2. Effect of bacterial isolates and spore concentration on root-knot population affecting plant growth parameters

Treatments (spores per ml of water)	Plant Height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)
$T_1(A_M)$ = 1.0 x 10^6	37.60^{c}	11.62^{ef}	15.36^{bcd}	3.35^{bcde}	4.67^{cd}
$T_2(A_M)$ = 1.5 x 10^6	38.60^{b}	12.60^{de}	14.40^{def}	3.51^{bcd}	4.34^{def}
$T_3(A_M)$ = 2.0 x 10^6	39.60^{a}	13.93^{bc}	13.25^{f}	3.95^{ab}	3.92^{f}
$T_4(D_L)$ = 1.0 x 10^6	35.60^{d}	15.14^{ab}	16.33^{b}	2.82^{def}	4.73^{c}
$T_5(D_L)$ = 1.5 x 10^6	36.80^{c}	14.66^{abc}	15.66^{bc}	3.41^{bcde}	4.42^{cd}
$T_6(D_L)$ = 2.0 x 10^6	37.20^{c}	13.41^{cd}	13.79^{def}	3.70^{abc}	4.25^{cde}
T_7 (Treated control)	34.20^{e}	11.55^{f}	17.54^{a}	2.52^{e}	6.19^{a}
T_8 (Untreated control)	39.80^{a}	15.82^{a}	14.82^{de}	4.34^{a}	5.38^{b}
S.Ed	**0.37**	**0.61**	**0.55**	**0.39**	**0.31**
CD(0.05)	**0.76**	**1.25**	**1.13**	**0.81**	**0.64**

Data are mean of five replications

Table 3. Effect of bacterial isolates and spore concentration on root-knot population in affecting disease parameters

Treatments	Avg. No. of galls/plant	Avg. No. of egg mass/plant	No. of eggs per egg mass
$T_1(A_M)$ = 1.0 x 10^6	35.80^{abcd}	34.00^{abc}	113^{bcd}
$T_2(A_M)$ = 1.5 x 10^6	34.80^{bcd}	32.00^{bc}	112^{bcd}
$T_3(A_M)$ = 2.0 x 10^6	33.80^{cd}	30.00^{bc}	110^{bcd}
$T_4(D_L)$ = 1.0 x 10^6	39.60^{ab}	35.60^{abc}	116^{ab}
$T_5(D_L)$ = 1.5 x 10^6	37.00^{abc}	34.60^{abc}	115^{abc}
$T_6(D_L)$ = 2.0 x 10^6	36.00^{abc}	33.20^{abc}	114^{bcd}
T_7 (Treated control)	40.00^{a}	38.80^{a}	121^{a}
T_8 (Untreated control)	0	0	0
S.Ed	**2.65**	**2.87**	**3.79**
CD(0.05)	**5.43**	**5.86**	**7.74**

Data are mean of five replications

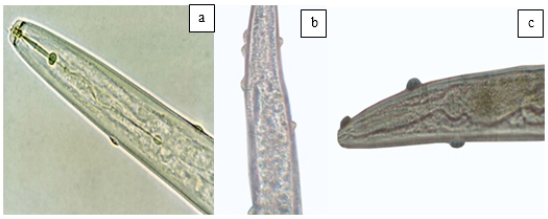

Plate 1 a, b, c. Spore encumbered root knot juvenile (J_2)

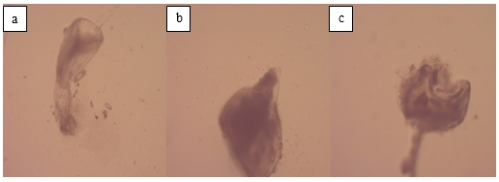

Plate 2. a, b, c. Pre-mature deformed females releasing Pasteuria spores

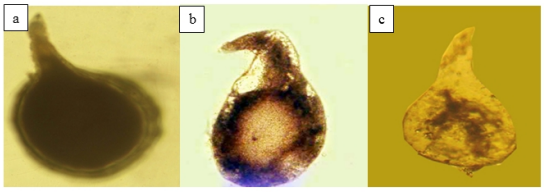

Plate 3. a. A healthy adult Female of M. incognita, b, c. adult root knot female infected with *P. penetrans*

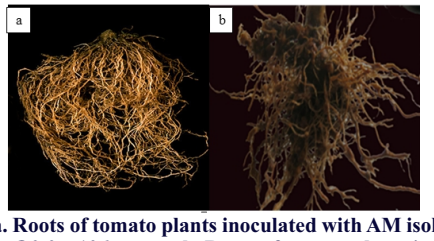

Plate 4. a. Roots of tomato plants inoculated with AM isolate of *P. penetrans* @2.0 x 106 spores b. Roots of tomato plants inoculated with DL isolate of *P. penetrans* @ 2.0 x 106 spores

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