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Effect of different organic amendments on the parasitic behavior of *Pasteuria penetrans* for the management of *Meloidogyne javanica* infecting tomato

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Abstract

An experiment was conducted to assess the effect of organic amendments (neem cake, mustard cake, castor cake, and farmyard manure) on parasitic behavior of *Pasteuria penetrans* for the management of root knot-nematode, *Meloidogyne javanica* infecting tomato under screen house conditions. After one week, seedlings were inoculated with 1000 J₂ of *M. javanica* encumbered with *P. penetrans* (at least 5-7 endospores of *P. penetrans* per juvenile). Organic amendments were applied at the rate of 10 g/pot, either individually or in combination with *P. penetrans*. Untreated (having nematode) and uninoculated (healthy) plants served as control. Each treatment was replicated three times. The results revealed that there was synergistic effect of bacteria and organic amendments in the management of *M. javanica*. The growth parameters of tomato were significantly increased by the combined application of *P. penetrans* @ $1x10^4$ spores/g of soil and mustard cake @ 10 g/kg soil. Minimum number of galls, eggs per root system and final nematode population in 200 cc soil was recorded, where combined application of *P. penetrans* with mustard cake were applied. Overall assessment of the results of the present study suggested that amongst the four organic amendments tested, combined application on *P. penetrans* with mustard cake seemed to be more promising approach in the management of *M. javanica* in tomato nursery as it caused greater nematode suppression and improved plant growth parameters.

Keywords: Organic amendments, tomato, mustard cake, Pasteuria penetrans, Meloidogyne javanica

Introduction

Tomato (Solanum lycopersicum) an annual herbaceous plant of solanaceae family, is one of the most versatile crops in the world. The crop is adversely affected by a number of pest and disease including plant parasitic nematode (PPNs) in which root-knot nematodes (RKNs) is most important. In India, an estimated yield loss of 27.2 per cent was recorded in tomato infested with root-knot nematode, *Meloidogyne* spp. (Jain, Mathur and Singh)^[1]. The management of this nematode is more difficult as compared to other pests due to its hidden mode of life, and attack on the underground plant parts (Stirling)^[2]. The global focus on sustainability in the agricultural environment is increasing in order to produce healthy, safe and good-quality food. Currently, the trend is to use control measures alternative to chemical pesticides. Therefore, development of ecologically safer biological methods of nematode management is absolutely essential. Use of organic soil amendments is a traditional cultural practice to improve soil fertility and structure. The exploitation of antagonistic activities of microorganisms may be an effective bio-control agent that could be developed to manage PPNs. The efficacy of organic additives depends on their chemical composition and type of microorganisms that develop during degradation (Rodriguez-Kabana)^[3]. The most sustainable approach to RKNs control is to integrate several tools and strategies, including reasonable use of organic amendments, bio-control agent, resistant cultivars and nematicides. Nevertheless, not much information is available on the effectiveness of P. penetrans as bio-control agent against *M. javanica* in individually and in combination with organic amendments. In view of this, the current study was undertaken to determine the efficacy of P. penetrans with and without the amendment of oil cakes of mustard, castor, neem and farmyard manure in controlling root-knot nematode, M. javanica infecting tomato under screen house conditions.

Material and Methods

The experiment was carried out under screen house, Department of Nematology, CCS Haryana Agricultural University, Hisar. Organic amendments (neem cake, castor cake, mustard cake and farmyard manure) were applied separately at the rate of 10 g per pot containing 1 kg steam sterilized sandy soil. The pots were watered after treatment to ensure proper decomposition of amendments. After one week waiting period; sowing of tomato var. Hisar arun was done. After one week, seedlings were inoculated with 1000 J₂ of M. javanica encumbered with at least 5-7 endospores of P. penetrans per juvenile. Organic amendments were applied either individually or in combination with P. penetrans. Untreated (having nematode) and uninoculated (healthy) plants served as control. Each treatment was replicated three times. After 45 days of inoculation, plants were uprooted and roots carefully washed under running tap water. Observations were recorded on plant growth parameters viz. plant length, fresh and dry weight of both roots and shoots and nematodes reproduction parameters i.e. number of galls, egg masses per root system by using magnifying glass and final nematode population per 200cc soil by Modified Baermann's Funnel Technique (Christie and Perry)^[4].

Results and Discussion

A. Plant Growth Parameters

The results (Table 1) revealed that plant height of tomato increased significantly (P < 0.05) with the application of P.

penetrans, organic amendments and their combined application as compared to that of untreated check. Among all treatments, maximum and significantly (P < 0.05) higher plant height (22.3 cm) was observed in treatment where P. penetrans at the rate of 1×10^4 spores was applied in combination with mustard cake at the rate of 10 g/kg soil. Treatments, where P. penetrans was integrated with neem cake and FYM, were statistically at par (P < 0.05) with each other. Minimum height (9.6 cm) of tomato plant was recorded in untreated check (Table 1). There was 132.29% increase in plant height over untreated check was recorded with the application of *P. penetrans* integrated with mustard cake. Maximum and significantly (P < 0.05) higher fresh shoot weight (22.3 g) and dry shoot weight (2.6 g) was observed in treatment where *P. penetrans* was applied at the rate of 1×10^4 spores/g soil in combination with mustard cake at the rate of 10 g/kg soil. The minimum fresh shoot weight (5.1 g) and dry shoot weight (0.7 g) was observed in untreated check (Table 1). Maximum and significantly (P < 0.05) higher fresh root weight (9.47 g) and dry root weight (1.97 g) was observed in treatment where *P. penetrans* at the rate of 1×10^4 spores were applied in combination with mustard cake at the rate of 10 g/kg soil (Table 1). In other words, the treatment mustard cake @ 10 g/kg soil + P. penetrans @ 1x10⁴ spores/g soil showed significant increased fresh root weight (669.91%), dry root weight (885%), fresh shoot weight (337.25%), and dry shoot weight (271.42%) over untreated check.

 Table 1: Effect of different organic amendments in combination with Pasteuria penetrans on growth parameters of tomato infested with rootknot nematode, Meloidogyne javanica

	Seedling Height	Fresh root	Dry root	Fresh shoot	Dry shoot
Treatments	(cm)	weight (g)	weight (g)	weight (g)	weight (g)
Neem cake @ 10g/kg soil	14.9	3.47	0.68	123 (141 17)	1.4
	(55.20)	(182.11)	(240)	12.3 (141.17)	(100)
Mustard cake @ 10 g/kg soil	17.3	5.23	1.06	15.8 (200.80)	1.6
	(80.20)	(325.20)	(430)	15.8 (207.80)	(128.57)
Castor cake @ 10 g/kg soil	15.6	4.73	0.99	13 2 (158 82)	1.4
	(62.50)	(284.55)	(395)	15.2 (150.02)	(100)
Formword Monure (EVM) @ 10 g/kg soil	13.3	2.77	0.56	11.2 (119.60)	1.1
	(38.54)	(125.20)	(180)	11.2 (119.00)	(57.14)
Neem cake @ 10 g/kg soil + P. penetrans @ $1x10^4$ spores/g soil	18.4	6.37	1.33	17 1 (235 29)	2.1
	(91.66)	(417.88)	(565)	17.1 (235.27)	(200)
Mustard cake @ 10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	22.3	9.47	1.97	22 3 (337 25)	2.6
	(132.29)	(669.91)	(885)	22.3 (331.23)	(271.42)
Castor cake @ 10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	20.1	7.40	1.55	187 (266 66)	2.4
	(109.37)	(501.62)	(675)	18.7 (200.00)	(242.85)
FYM @10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	18.3	5.93	1.25	15 4 (201.06)	2.0
	(90.62)	(382.11)	(525)	13.4 (201.90)	(185.71)
<i>P. penetrans</i> @ $1x10^4$ spores/g soil alone	12.5	1.73	0.35	8.7	0.8
	(30.20)	(40.65)	(75)	(70.58)	(14.28)
Carbofuran @ 1kg a.i./ha	12.6	1.57	0.30	8.6	0.9
	(31.25)	(27.64)	(50)	(68.62)	(28.57)
Uninoculated control	13.3	2.23	0.46	9.2	1.1
	(38.54)	(81.30)	(130)	(80.39)	(57.14)
Untreated check	9.6	1.23	0.20	5.1	0.7
CD (P=0.05%)	0.87	0.26	0.13	0.77	0.23
SE(m)	0.299	0.091	0.009	0.264	0.075

Data are average value of three replications

Initial inoculum level: 1 larvae/g soil

Fig. in parentheses are per cent increase over untreated check

B. Nematode parameters

Data presented in Table 2 revealed that the treatment where *P*. *penetrans* at the rate of 1×10^4 spores was applied in combination with mustard cake at the rate of 10 g/kg soil

significantly (P < 0.05) reduced the number of galls per root system (51.30%), number of eggs per root system (88.06%) and final nematode population per 200cc soil (88.06%) as compared to control. The interaction between organic amendments and *P. penetrans*, was synergistic as addition of amendments reduced the reproduction potential of root-knot nematode. Maximum and significant (P < 0.05) decrease in nematode population was observed in treatment where *P. penetrans* and mustard cake were applied followed by application of castor cake along with *P. penetrans*. Highest nematode population (771.5) was recorded in untreated check. Chemical treatment of carbofuran also showed a significant (P < 0.05) decline in final nematode population as compared to the untreated check. All the organic amendments had synergistic effect with *P. penetrans*. There was significantly (P < 0.05) better parasitization when the bacterial parasite was integrated with organic amendments. Maximum and significantly (P < 0.05) higher number of infected females (55.0) was observed in combined treatment of bacteria and mustard cake. The minimum number of infected female (17.6) was recorded where *P. penetrans* at the rate of 1×10^4 spores/g soil was applied in the soil (Table 3).

Table 2: Effect of Pasteuria penetrans along with different organic amendments on galling and reproduction potential of root-knot nematode
(<i>M. javanica</i>) infecting tomato

Treatments	No. of galls per root system	No. of eggs per root system	No. of J ₂ per 200 cc soil
Neem cake @ 10 g/kg soil	35.0 (17.25)	1855.3 (66.24) (43.10)*	227.7 (70.48) (15.12)*
Mustard cake @ 10 g/kg soil	30.0 (29.07)	970.3 (82.34) (31.20)*	196 (74.59) (14.04)*
Castor cake @ 10 g/kg soil	31.6 (25.29)	1183.0 (78.47) (34.4)*	389.9 (49.46) (19.77)*
Farm Yard Manure (FYM) @ 10 g/kg soil	37.3 (11.82)	3242.7 (41) (56.90)*	475.9 (38.31) (21.84)*
Neem cake @ 10 g/kg soil + <i>P. penetrans</i> @ 1x10 ⁴ spores/g soil	25.6 (39.47)	1233.0 (77.56) (35.1)*	136.7 (82.28) (11.74)*
Mustard cake @ 10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	20.6 (51.30)	656.0 (88.06) (25.60)*	92.1 (88.06) (9.65)*
Castor cake @ 10 g/kg soil + <i>P. penetrans</i> @ 1x10 ⁴ spores/g soil	23.0 (45.62)	758.7 (86.19) (27.60)*	128.2 (83.38) (11.37)*
FYM @ 10 g/kg soil + <i>P. penetrans</i> @ 1x10 ⁴ spores/g soil	28.3 (33.09)	2897.0 (47.29) (53.80)*	172.5 (77.64) (13.17)*
Carbofuran @ 1kg a.i./ha	32.3 (23.64)	1918.3 (65.09) (43.80)*	265.6 (65.57) (16.30)*
Untreated check	42.3	5496.3 (74.10)*	771.5 (27.79)*
CD (P=0.05%)	2.78	1.79	0.61

Data are average value of three replications

Initial inoculum level: 1 larvae/g soil

Fig. in parentheses are per cent decrease over untreated check

Fig. in parentheses with * are \sqrt{n} transformed values

 Table 3: Effect of Pasteuria penetrans along with different organic amendments on parasitisation of P. penetrans on root-knot nematode, M.

 iavanica

Treatments	No. of root-knot female infected with <i>P. penetrans</i>
Neem cake @ 10 g/kg soil + P. penetrans@ $1x 10^4$ spores/g soil	36.3
Mustard cake @ 10 g/kg soil + P. penetrans@ 1x104 spores/g soil	55.0
Castor cake @ 10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	48.6
FYM @ 10 g/kg soil + P. penetrans @ 1x10 ⁴ spores/g soil	24.0
Control (P. penetrans @ 1x10 ⁴ spores/g soil)	17.6
CD (P=0.05%)	4.88

Efforts were made to integrate the *P. penetrans* with organic amendments for the management of root-knot nematode. The application of organic amendment alone also gave good results in improving the growth parameters of plant and in suppression of root-knot nematode multiplication but when integrated with organic amendments particularly mustard

cake, proved best, among all the organic amendments and their combination. The efficacy of *P. penetrans* in suppression of root-knot nematode has been observed by various workers. The nutritional and nematode management properties of various oil seed cakes are well recognized (Schmutterer)^[5]. *P. penetrans* is not capable of producing the rapid 'knock-down'

effect that is associated with a nematicide; however, in the longer term it is capable of limiting nematode egg production. (Gowen and Channer) ^[6] showed decreased numbers of egg masses in a two crop-cycle pot experiment where initially, soil had been amended with 1.1 and 5.5×10^4 *P. penetrans* spores per cubic centimeter. Organic oil amendments stimulate the activities of microorganism that are antagonistic to plant parasitic nematodes.

The decomposition of organic matter results in accumulation of specific compounds in the soil that may be nematicidal. Various mechanisms are involved in nematicidal action of organic matter (Stirling, 1991)^[2]. Some materials release compound toxic to nematodes, like phenol, tannin, azadirachtin, ricinin (Rich)^[7] or derived from the decomposition process in the soil, like ammonia, nitrates, hydrogen sulphide (Rodriguez-kabana)^[3]. Amendments may also provide a favorable substrate for the sustenance of soil microfauna and microflora (Linford, Yap and Oliveira)^[8] which can include direct predators (micro-arthropods) or parasites (fungi, bacteria) of nematodes, or which suppress soil nematode population indirectly through the production of enzyme (Galper, Cohn, Spiegel and Chet)^[9] or toxic metabolites, such as antibiotic of bacterial origin. Moreover, the addition of organic materials usually improve soil structure and consequently the capacity of the soil to hold water and exchange ions that, together with the nutrient released by the organic matter, positively affect the plant growth. P. penetrans when integrated with other practices of nematode management gave synergistic effect on nematode management. (Prasad) ^[10] reported that the green house tomatoes inoculated with M. incognita had fewer galls on roots when grown in soil infected with P. penetrans. (Maheshwari, Mani and Rao)^[11] concluded in their study that soil application of nematicides and P. penetrans together generally has synergistic effect on nematode control. Commonly used pesticides don't have any noticeable adverse effects. (Javed, El-Hassan, Gowen, Pembroke and Inam-ul-Haq) ^[12] while studying the combined effect of *P. penetrans* and organic amendments on the management of root-knot nematode in tomato in pot experiment recorded fewer root galling and the greatest growth of tomato plants treated with a combined application of P. penetrans and neem cake. (Trudgill, Bala, Blok, Daudi,, Davies, Gowen and Voyoukallou)^[13] while assessing the potential of combining P. penetrans and neem (leaves and cake) formulations as a management system for root-knot nematodes and growth of tomato, recorded a synergistic effect of P. penetrans and neem products on juvenile behavior, resulting in a reduction in nematode invasion, for this reason it may well complement in treatments with P. penetrans. (Chaudhary and Kaul) ^[14]evaluated the efficacy of *P. penetrans* under the influence of organic amendments of four oil seed cakes namely neem, castor, mustard and citrullus on suppression of populations of Meloidogyne incognita in chilli in which combination of castor and P. penetrans showed greater reduction in galling index and final population over the M. incognita control than other treatments.

Conclusion

There was synergistic effect of bacteria and organic amendments in the management of root-knot nematode, *M. javanica*. The combined application of bacterial parasite and organic amendment reduced nematode reproduction in tomato nursery. Minimum number of galls, eggs per root system and

final nematode population were significantly reduced, where combined application of *P. penetrans* with mustard cake was applied. Bacterial parasite with all the organic amendments reduced nematode multiplication over untreated control.

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