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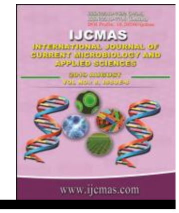

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Management of Root-Knot Nematodes (*Meloidogyne* spp.) Using Different Bio-Agents in Papaya Nursery

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Keywords

Papaya, root-knot nematode, *Pseudomonas putida*, *P. fluorescens*, *Bacillus amyloliquefaciens*, *B. pumilus*, *B. subtilis*, *B. megaterium* and *Purpureocillium lilacinum*

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ABSTRACT

Various methods are used to manage phytonematodes under field conditions. Use of chemicals to manage phytonematodes in various crops is very popular among farmers. But these chemicals are not ecofriendly. Various bio-control agents are found promising against root-knot nematodes. Therefore to study the effect of different bio-agents for the management of *Meloidogyne* spp. in papaya nursery, application of *Pseudomonas putida*, *P. fluorescens*, *Bacillus amyloliquefaciens*, *B. pumilus*, *B. subtilis*, *B. megaterium* and *Purpureocillium lilacinum* each @ 50g/m^2 were tested in nursery having initial nematode population of 293 J_2/200 cm^3 soil. During experiment we have taken different observations *viz.*, number of females, number of egg mass/3 g roots and number of eggs/egg mass and transplantable seedlings of papaya.

Introduction

Papaya (*Carica papaya* Linn.) is a tropical fruit having commercial importance because of its high nutritive and medicinal value. Sudhakar (2014) opined that *C. papaya* is commonly called as paw-paw and it belongs to the family *Caricaceae*. Papaya fruit is a rich source of vitamin A and C. Papain prepared from dried latex of its immature fruits is used in manufacturing of chewing gum, cosmetics, degumming natural silk and to give shrink resistance to wool. It is also used in pharmaceutical industries, textile and garment, cleaning paper and adhesive manufacture, sewage disposal, etc. (Anon., 2016). Papaya cultivation had its origin in South Mexico and Costa Rica. It possesses excellent medicinal properties for treatment of different ailments. Growth, development and yield of papaya are highly influenced by various biotic stresses such as bacteria, fungi, viruses, insects and nematodes (Anon., 2014). Papaya is reported to be a good host of *Meloidogyne* spp. in many tropical and sub-tropical regions. It causes leaf yellowing and low leaf production as well as premature dropping of both leaves and fruits of papaya (Inserra and Cartia, 1977; Roy and Das, 1980 and Khan, 1989). Root-knot nematodes cause swelling and gall formation in the roots, which prevent water and nutrient uptake. Nematode population may be high in papaya without producing symptoms. Heavy nematode infestations can cause wilting, stunting, decreased plant vigor, reduced yields and shortening of the productive life of a papaya tree (Anon., 2008).

Khan *et al.,* (1997) observed beneficial effects of *Paecilomyces lilacinus* and *Trichoderma harzianum* when applied against *M. incognita* and *Fusarium solani* on potted papaya in steam sterilized soil. The combined effect of both the biocontrol agents was more effective than individual biocontrol agents in reducing nematodes and decreasing the incidence of root rot.

Rao (2007) concluded that seed treatment with *Pseudomonas fluorescens* (10^8 cfu/g) and application of *P. fluorescens* and *T. harzianum* (10^6 cfu/g), each at 5 g/kg soil, significantly reduced eggs/egg mass of *M. incognita* in papaya seedlings.

Kumar (2009) carried out an experiment to manage *M. incognita* in papaya with *Pochonia chlamydosporia* and noticed that nematode population was significantly low on 30^{th} day when applied @ 5 kg/ha. He also reported that *M. incognita* caused severe root galling and reduced plant growth and yield.

Pradhan *et al.,* (2012) tested *T. harzianum*, neem oil and carbofuran 5G against root-knot nematode (*M. javanica*) in two papaya varieties Kashempuri and Deshi papaya. Seedlings were inoculated with *M. javanica.* Neem oil (5 ml/ 10g seeds) and *T. harzianum* (1:4) were used as seed treatment. *T. harzianum* significantly increased the root and shoot growth, reduced galls and egg masses and suppressed the development of J_2, J_3, and J_4 and adult females.

Singh *et al.,* (2013) investigated 24 indigenous isolates of *P. lilacinum* (*Paecilomyces lilacinus*) (Thom) Samson against the *M. incognita* collected from different agro-climatic zones of India. Results showed that all the tested isolates were capable to parasitise eggs, inhibit egg hatching and caused juvenile mortality at various levels. Based on the performance under studies, out of eight isolates (NDPL-01, ANDPL-02, SHGPL-03, HYBPL-04, AHDPL-05, PTNPL-06, SNGPL-07 and VNSPL-08), HYBPL-04 caused highest mortality (80%), inhibition of egg hatching (90%) as well as parasitisation of *M. incognita* eggs (75%), in terms of reduction of galls (61%) and reproductive factor (Pf/Pi(RF) = 0.2) achieved through application of HYBPL-04 + FYM compared to control and other tested isolates.

Nematicidal activity of *Bacillus subtilis*, *B. megaterium, B. pumilus* and *P. fluorescens*, *T. harzianum*, *T. viride* and *T. virens* against nematode population of *M. incognita* infecting tropical fruit, studied by Youssef *et al.,* (2017). Results showed that *T. virens* reduced the gall numbers by 74% followed by *B. pumilus* (73%), *T. viride* (73%), *B. subtilis* and *T. harzianum* (71%), *B. megaterium* (71%) and *P. fluorescens* (60%). *T. virens* also caused highest percentage reduction of egg-masses (80%) followed by *B. pumilus*, *T. harzianum* and *T. viride* that caused 74%, 68%, 65% and 61% reduction by *B. megaterium*, *B. subtilis* and *P. fluorescens*, respectively. *T. virens* when applied at one and/or second time was effective in enhancing the growth parameters *viz*., length of shoot, fresh and dry weight of shoot and root weight than *B. pumilus*. Walia and Khan (2018) reported control of root-knot nematode population and their galling density by applying *P. chlamydosporia* @ 5 kg/ha in papaya field. Enrichment of FYM with bio-pesticides @ 2 kg each of *P. lilacinus, P. fluorescens* and *T. harzianum/T. viride* in one ton of FYM and kept it in shade for 15 days at 25-30% moisture for multiplication of beneficial microbes and controlling root-knot nematode population in nursery bed of papaya.

Materials and Methods

Bio-agents were applied with 250 g FYM before sowing in 1.2 x 1.2 m *Meloidogyne* spp. infested bed for the management of root knot nematodes. Seeds of papaya *cv*. Madhubindu were sown at 15 x 10 cm spacing in furrows of bed. Beds without any treatment were kept as untreated check. After 60 days of sowing, the experiment was discontinued by removing the seedlings from the nursery and roots were washed gently under running tap water and observations on fresh seedling weight and root-knot index were recorded. Roots were cut in to 2-3 cm length mixed thoroughly and three gram roots were stained in 0.05 per cent acid fuchsin in lactophenol. Then roots were washed with tap water to remove excess stain and kept overnight in lactophenol, Then the roots were examined for nematode population (Table 1 and 2).

Results and Discussion

Germination count/1.44 m^2

Data presented in table 3 revealed that the effect of various bio-agents on seed germination found non-significant indicated that the bio-agents used in present study have no adverse impact on seed germination.

Fresh seedling weight (g)

Seedlings raised in the beds treated with *P. putida* had maximum fresh seedling weight (1048.33 g) and it was statistically at par with *P. lilacinum* (T_7). *B. amyloliquefaciens* (T_3) was at third rank. Fresh seedling weight was lowest in the control (T_8). However, it was statistically at par with *B. pumilus* (T_4) and *B. megaterium* (T_6) (Table 3).

Transplantable seedlings (per 1.44 m^2)

Significantly higher (79) transplantable seedlings were recorded in treatment of *P. putida* (T_1), followed by *B. amyloliquefaciens* (T_3), *P. lilacinum* (T_7), *B. subtilis* (T_5) and *P. fluorescens* (T_2), statistically all treatments found at par with each other. Lowest number of transplantable seedlings was noticed in the treatment of control (T_8) (Table 3).

Non-transplantable seedlings (per 1.44 m^2)

Lowest number of non-transplantable seedlings (1) was noticed in *P. putida* (T_1) treatment followed by *B. amyloliquefaciens* (T_3), both were at par with each other. Significantly more number of non-transplantable seedlings *i.e.* 5 was found in control. Rest of the treatments registered non-transplantable seedlings ranging from 2 to 3 (Table 3).

Root- knot index (RKI)

Root-knot index was significantly least (1.59) in the treatment of *P. putida* (T_1), but it was at par with *P. lilacinum* (T_7) and *B. amyloliquefaciens* (T_3).Control (T_8) had significantly highest RKI as compared to other treatments. RKI of *B. subtilis* (T_5), *P. fluorescens* (T_2), *B. megaterium* (T_6) and *B. pumilus* (T_4) were in the range of 1.92 to 2.05 and all were statistically at par with each other (Table 4).

Final nematode population

Data (Table 4) recorded on number of females from 3 g root indicated that treatment of *P. putida* (T_1) had significantly least (92) nematode population as compared to other treatments.

Table.1 List of bio-agents tested

Treatments code	Treatments
T_1	*Pseudomonas putida* (2x10^8 cfu/g) @ 50g/m^2
T_2	*Pseudomonas fluorescens* (2x10^8 cfu/g) @ 50g/m^2
T_3	*Bacillus amyloliquefaciens* (2x10^8 cfu/g) @ 50g/m^2
T_4	*Bacillus pumilus* (2x10^8 cfu/g) @ 50g/m^2
T_5	*Bacillus subtilis* (2x10^8 cfu/g) @ 50g/m^2
T_6	*Bacillus megaterium* (2x10^8 cfu/g) @ 50g/m^2
T_7	*Purpureocillium lilacinum* (2x10^8 cfu/g) @ 50g/m^2
T_8	Control (Untreated check)

Table.2 Root-knot Index Scale

Rating scale (0-5), per cent root infection and reaction pertaining to root-knot nematodes		
Root Knot Index (RKI)	**Per cent root infection**	**Reaction**
0	No galling	Highly resistant
0.01-1.0	Up to 20% root galling	Resistant
1.01-2.0	Up to 40% root galling	Moderately resistant
2.01-3.0	Up to 60% root galling	Moderately susceptible
3.01-4.0	Up to 80% root galling	Susceptible
4.01-5.0	More than 80% root galling	Highly susceptible

Table.3 Effect of different bio-agents on plant growth characters of papaya

Treatments	Germination count/1.44 m^2	Fresh seedling weight, g	Transplantable seedlings/1.44 m^2	Non-Transplantable seedlings/1.44 m^2
T_1 (*Pseudomonas putida*)	80	1048.33	79	1
T_2 (*Pseudomonas fluorescens*)	79	670.67	77	2
T_3 (*Bacillus amyloliquefaciens*)	80	869.00	79	1
T_4 (*Bacillus pumilus*)	79	611.00	76	3
T_5 (*Bacillus subtilis*)	79	673.67	77	2
T_6 (*Bacillus megaterium*)	78	650.00	75	3
T_7 (*Purpureocillium lilacinum*)	80	1000.00	78	2
T_8 (Control)	78	495.33	74	5
SEm±	0.90	51.42	1.00	0.24
CD at 5 %	NS	155.97	3.02	0.72
CV %	1.97	11.79	2.24	15.80

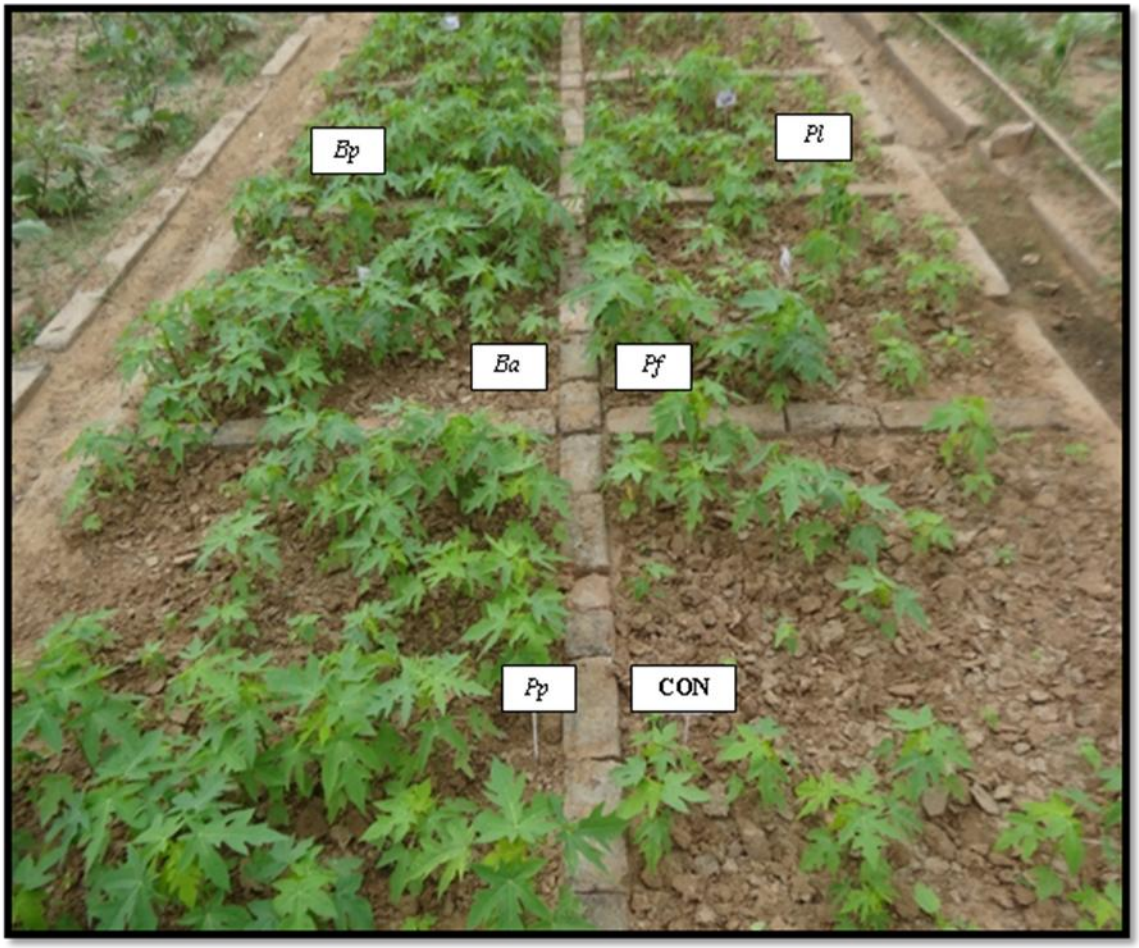
NS = Non-significant

Table.4 Effect of different bio-agents on multiplication of *Meloidogyne* spp. on papaya

Treatments	**RKI (0-5)* (√x+0.5)**	**Nematode population**				
		No. of females/ 3g root (Log X+1)	**No. of egg mass/ root (Log X+1)**	**No. of eggs/ egg mass (Log X+1)**	**No. of juveniles/ 200 cm^3 soil (Log X+1)**	**Total (Log X+1)**
T_1 (*Pseudomonas putida*)	1.45 (1.59)	1.97 (92)	1.86 (71)	2.34 (218)	2.75 (563)	2.98 (954)
T_2 (*Pseudomonas fluorescens*)	1.94 (3.26)	2.39 (243)	1.92 (82)	2.45 (281)	3.57 (3723)	3.64 (4364)
T_3 (*Bacillus amyloliquefaciens*)	1.58 (2.00)	2.10 (126)	1.81 (64)	2.30 (199)	2.81 (643)	3.02 (1046)
T_4 (*Bacillus pumilus*)	2.05 (3.69)	2.44 (276)	1.92 (82)	2.46 (287)	3.57 (3740)	3.64 (4364)
T_5 (*Bacillus subtilis*)	1.92 (3.19)	2.35 (225)	1.92 (80)	2.45 (281)	3.57 (3723)	3.63 (4265)
T_6 (*Bacillus megaterium*)	2.03 (3.62)	2.39 (247)	1.94 (86)	2.58 (379)	3.61 (4101)	3.68 (4785)
T_7 (*Purpureocillium lilacinum*)	1.57 (1.96)	2.18 (150)	1.88 (75)	2.40 (250)	2.97 (926)	3.15 (1412)
T_8 (Control)	2.28 (4.70)	2.59 (386)	1.97 (92)	2.59 (388)	3.68 (4818)	3.75 (5622)
SEm±	0.05	0.03	0.02	0.04	0.03	0.02
CD at 5 %	0.16	0.07	0.07	0.11	0.08	0.06
CV %	4.98	2.47	1.99	2.52	1.45	0.97

*0 = Free; 5 = Maximum disease intensity.
Figures in parentheses are retransformed values of transformation value

Effect of different bio-agents on papaya in nursery condition



Treatment of *B. amyloliquefaciens* (T_3) found next best treatment followed by *P. lilacinum* (T_7). Control treatment had maximum population and similar trend was observed for number of egg mass/root and number of eggs/egg mass. Significantly least (563) number of nematode population from soil was recorded in the treatment of *P. putida* (T_1) followed by *B. amyloliquefaciens* (T_3), it was at par with each other. Untreated control had maximum nematode population and it was at par with *B. megaterium* (T_6) (Table 4).

Total nematode population

In case of total nematode population, it was significantly least (954) in the treatment of *P. putida* (T_1) and found statistically at par with *B. amyloliquefaciens* (T_3), followed by *P. lilacinum* (T_7) and it was significantly differed with next best treatment. It is obvious that control had maximum (5622) nematode population (Table 4).

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