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Bioefficacy of different bioagents against root-knot nematode, *Meloidogyne incognita* infesting bottle gourd under laboratory conditions

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ABSTRACT

The bioefficacy of different bioagents *viz.*, *Paecilomyces lilacinus* ($2x10^6$ cfu/g), *Pseudomonas fluorescens* ($1x10^9$ cfu/g), *Trichoderma viride* ($2x10^6$ cfu/g), Phule *Trichoderma* plus ($2x10^6$ cfu/g) and *Pochonia chlamydosporium* ($2x10^6$ cfu/g) against second stage juveniles of root-knot nematode infesting bottle gourd crop was studied under laboratory conditions. In this bioassay *Paecilomyces lilacinus* fungus caused higher rate of juvenile mortality at 1.00 per cent concentration was found to be promising in laboratory bioassay studies and recorded 80.00 per cent mortality of second stage juveniles of root-knot nematode. This was followed by 76.67 per cent mortality of second stage juveniles of root-knot nematode by *Pseudomonas fluorescens* at 1.00 per cent concentration after 72 hrs treatment of respective bioagents. The fungi Phule *Trichoderma* plus, *Trichoderma viride* recorded 70.00, 66.67 per cent juvenile mortality, while as in case of fungi *Pochonia chlamydosporium* 53.33 per cent juvenile mortality was observed after 72 hrs treatment of respective bioagents at 1.00 per cent concentrations.

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INTRODUCTION

Plant-parasitic nematodes, particularly root-knot nematodes, are widely distributed and cause significant yield losses in a wide range of crops (Sasser and Freckman, 1986). *Meloidogyne incognita* is a limiting factor affecting production of vegetables including Bottle gourd in India. The loss to Indian agriculture is estimated at about Rs.210 crore annually (Jain *et al.*, 2007). Since use of chemicals is prohibitive as well as hazardous, attention has now been directed towards use of biopesticides, *Paecilomyces lilacinus, Pseudomonas fluorescens, Trichoderma viride, Phule Trichoderma plus* and *Pochonia chlamydosporium* has a good potential as biocontrol agent to manage root-knot nematode, *M.incognita*. Nematode management through

biocontrol agents is gaining importance in the new millennium as other measures have become less attractive to growers/farmers where economics demand specialization and intensification. In the last decade nematode management through biocontrol agents was in the forefront of research and development. Management of plant parasitic nematodes through ecofriendly means is the need of this era. In view of the increasing awareness about environment and demand of organic farming, The present study was initiated to investigate the boiefficacy of different bioagents against root-knot nematode, *M. incognita* on Bottle gourd under laboratory condition.

MATERIAL AND METHODS

Source of material :

The bioefficacy of formulations of different bioagents *viz*., *Paecilomyces lilacinus* ($2x10^6$ cfu/g), *Trichoderma viride* ($2x10^6$ cfu/g), Phule *Trichoderma* plus ($2x10^6$ cfu/g), *Pseudomonas fluorescens* ($1x10^9$ cfu/g) and *Pochonia chlamydosporium* ($2x10^6$ cfu/g) were studied under laboratory conditions by bioassay method .

Host culture :

The soil population of root knot nematode, *Meloidogyne incognita* was needed, the soil from the root zone of brinjal (cv. MBH-110) which was grown continuously in earthen pots as well as in the field for culture was taken and processed by Cobb's Sieving and Decanting Method (Cobb, 1918).

Four concentrations of each formulation *i.e.* 0.25, 0.50, 0.75, 1.00 per cent were prepared from stock solution by adding distilled water and stored in cool dry conditions tested to observe the possibility of defecting small changes in virulence and the spore count obtained in resulting suspension.Freshly hatched juveniles of *M. incognita* were obtained by incubating egg masses collected from the infected brinjal roots of the pure nematode culture in distilled water at 25^0C. The test was conducted in sterile Petri plates filled with 5 ml of each concentration of fungal and bacterial culture and 1 ml of nematode suspension containing 100 nematodes/ml.

The details of experiment are given below:

Treatment No.	Concentration (%)
T_1	0.25
T_2	0.50
T_3	0.75
T_4	1.00
T_5	Untreated control

Observations :

The effect of fungal and bacterial culture filtrates on the root-knot nematode activity was determined after 24, 48, 72 hours. Toxicity was estimated according to the percentage of paralysed nematodes. Nematodes that were rigid and elongated with head and tail sometimes slightly bent were considered as immobilized and if they did not react when probed with fine needle were considered as paralyzed. Percentage of dead nematodes or nematode mortality was determined after revival test for which nematodes were washed three times with sterile water using centrifugation for 3 min at 1000 rpm and incubated in distilled water for 24 hr, nematodes still inactive were considered as dead. There were three replicates for each treatment.

Statistical analysis

Cumulative nematode mortality were analysed and subjected to ANOVA.

RESULTS AND DISCUSSION

Five different concentration of of different bioagents *viz.*, 0.25, 0.50, 0.75, 1.00 per cent were tested for determining the bioefficacy on the second stage juveniles of root knot nematode *M. incognita* of are presented (Table 1).

The data recorded at 24 hrs revealed that the treatment with 1.00 per cent concentration was superior over other treatments and recorded 20.00 per cent 2^{nd} stage juvenile mortality. Significant differences did not exist among the rest of treatments.

At 48 hrs the treatment with 1.00 per cent concentration was effective and recorded 43.33 per cent 2^{nd} stage juvenile mortality and it was found to be significantly superior over all other treatments. The treatment with concentration of 0.75, 0.50, 0.25 per cent recorded 26.67, 23.33, 13.33 per cent 2^{nd} stage juvenile mortality, respectively.

While, at 72 hrs the treatment with 1.00 per cent concentration was effective and recorded 80.00 per cent

2nd stage juvenile mortality and it was found to be significantly superior over all other treatments. The treatment with concentration of 0.75, 0.50, 0.25 per cent recorded 50.00, 36.67, 23.33 per cent 2nd stage juvenile mortality, respectively.

Bioefficacy of *Pseudomonas fluorescens* against second stage juveniles of *M.incognita* under laboratory conditions :

The second bioagent, *Pseudomonas fluorescens* which was tested with 0.25, 0.50, 0.75, 1.00 per cent concentration and 2nd stage juvenile mortality data at 24, 48 and 72 hrs are presented in Table 2.

It could be seen from the Table 2 that at 24 hrs, 2nd stage juvenile mortality in 1.00 per cent concentration was 16.67 per cent which was significantly superior to untreated control. The treatments with concentration of 0.75, 0.50, 0.25 per cent recorded 10.00, 6.67, 3.33 per cent 2nd stage juvenile mortality, respectively.

The observations recorded at 48 hrs revealed that the treatment with 1.00 per cent concentration recorded 33.33 per cent 2nd stage juvenile mortality which was significantly superior over the rest of the treatments. The treatment with concentrations of 0.75, 0.50, 0.25 per cent recorded 16.67, 13.33, 10.00 per cent second stage juvenile mortality, respectively.

At 72 hrs, treatment with 1.00 per cent concentration recorded highest (76.67%) 2nd stage juvenile morality. The mortality of 2nd stage juvenile ranged maximum (76.67) in treatment with 1.00 per cent concentration to a minimum (20.00 %) in treatment with 0.25 per cent concentration. Thus, the treatment with concentration of 1.00 per cent proved to be consistently superior to other treatments at all the intervals of observations.

Bioefficacy of Phule *Trichoderma* plus against second stage juveniles of *M. incognita* under laboratory conditions :

The bioagent, Phule *Trichoderma* plus was tested for the determining the biological activity on the 2nd stage juveniles of *M. incognita*. It was tested at 0.25, 0.50, 0.75, 1.00 per cent concentration and 2nd stage juvenile mortality data at 24, 48 and 72 hrs are presented in Table 3.

The data recorded at 24 hrs revealed that the treatment with 1.00 per cent concentration recorded 16.67 per cent 2nd stage juvenile mortality and found to be superior over all other treatment.

Table 1 : Bioefficacy of *Paecilomyces lilacinus* against second stage juveniles of *M.incognita* under laboratory conditions

Treatments No.	Concentration (%)	Cumulative 2nd stage juvenile mortality (%) *		
		24 hr	48 hr	72 hr
T_1	0.25	6.67 (12.29)	13.33 (21.14)	23.33 (28.78)
T_2	0.50	6.67 (12.29)	23.33 (28.78)	36.67 (37.22)
T_3	0.75	13.33 (21.14)	26.67 (31.00)	50.00 (45.00)
T_4	1.00	20.00 (26.07)	43.33 (41.15)	80.00 (63.93)
T_5	UC	0.00 (0.00)	0.00 (0.00)	6.67 (12.29)
	S.E.±	0.910	0.408	1.895
	C.D. (P=0.05)	2.585	1.16	5.384

(*Figures in parentheses are arcsin transformation)

Table 2: Bioefficacy of *Pseudomonas fluorescens* against second stage juveniles of *M.incognita* under laboratory conditions

Treatment No.	Concentration (%)	Cumulative 2nd stage juvenile mortality (%) *		
		24 hr	48 hr	72 hr
T_1	0.25	3.33 (6.14)	10.00 (18.43)	20.00 (26.07)
T_2	0.50	6.67 (12.29)	13.33 (21.14)	26.67 (31.00)
T_3	0.75	10.00 (18.43)	16.67 (23.86)	36.67 (37.22)
T_4	1.00	16.67 (23.86)	33.33 (35.01)	76.67 (61.22)
T_5	UC	0.00 (0.00)	3.33 (6.14)	6.67 (12.29)
	S.E.±	1.22	1.106	1.721
	C.D. (P=0.05)	3.464	3.141	4.890

* Figures in parentheses are arcsin transformed values

At 48 hrs, 26.67 per cent second stage juvenile mortality was recorded in treatment with 1.00 per cent concentration and it was superior over other treatments. However, it was on par with treatment with 0.75 per cent concentration where, 23.33 per cent 2nd stage juvenile mortality of *M. incognita* was recorded.

The maximum juvenile mortality (70.00%) was recorded in treatment with 1.00 per cent concentration at 72 hrs, which was superior over the rest of treatments. The treatment with 0.75 per cent concentration recorded 43.33 per cent 2nd stage juvenile mortality. The least 16.67 per cent 2nd stage juvenile mortality was observed in treatment with 0.25 per cent concentration.

Thus, the treatment with 1.00 per cent concentration proved to be consistently superior to other treatment at all the intervals of observations.

Bioefficacy of *Trichoderma viride* against second stage juveniles of *M.incognita* under laboratory conditions :

The bioagent, *T.viride* was tested for determining the biological activity against 2nd stage juvenile of *M. incognita*. The bioagent, *Trichoderma viride* was found to be effective to cause mortality in 2nd stage juvenile of *M. incognita*, when applied at 0.25, 0.50, 0.75, 1.00 per cent concentration. The tested fungal concentration and

Table 3: Bioefficacy of Phule *Trichoderma* plus against second stage juveniles of *M.incognita* under laboratory conditions

Treatment No.	Concentration (%)	Cumulative 2nd stage juvenile mortality (%) *		
		24 hr	48 hr	72 hr
T_1	0.25	3.33 (6.14)	6.67 (12.29)	16.67 (23.86)
T_2	0.50	3.33 (6.14)	13.33 (21.14)	20.00 (26.07)
T_3	0.75	10.0 (18.43)	23.33 (28.08)	43.33 (41.15)
T_4	1.00	16.67 (23.86)	26.67 (31.00)	70.00 (57.00)
T_5	UC	0.00 (0.00)	3.33 (6.14)	3.33 (6.14)
	S.E.±	1.465	1.274	1.704
	C.D. (P=0.05)	4.160	3.620	4.84

*Figures in parenthesis are arcsin transformed value

Table 4 : Bioefficacy of *Trichoderma viride* against second stage juveniles of *M.incognita* under laboratory conditions

Treatment No.	Concentration (%)	Cumulative 2nd stage juvenile mortality (%) *		
		24 hr	48 hr	72 hr
T_1	0.25	3.33 (6.14)	6.67 (12.29)	16.67 (23.86)
T_2	0.50	6.67 (12.29)	13.33 (21.14)	20.00 (26.07)
T_3	0.75	10.00 (18.43)	23.33 (28.08)	33.33 (35.01)
T_4	1.00	10.00 (18.43)	26.67 (31.00)	66.67 (54.78)
T_5	UC	0.00 (0.00)	3.33 (6.14)	3.33 (6.14)
	S.E.±	1.801	1.618	1.684
	C.D. (P=0.05)	5.114	4.596	4.783

* Figures in parenthesis are arcsin transformed values

Table 5: Bioefficacy of *Pochonia chlamydosporium* against second stage juveniles of *M.incognita* under laboratory conditions

Treatment No.	Concentration (%)	Cumulative 2nd stage juvenile mortality (%) *		
		24 hr	48 hr	72 hr
T_1	0.25	0.00 (0.00)	3.33 (12.29)	10.00 (18.43)
T_2	0.50	3.33 (12.29)	10.00 (18.43)	16.67 (23.86)
T_3	0.75	6.67 (12.29)	16.67 (23.86)	30.00 (33.00)
T_4	1.00	10.00 (18.43)	23.33 (28.78)	53.33 (46.92)
T_5	UC	0.00 (0.00)	0.00 (0.00)	6.67 (12.29)
	S.E.±	1.959	1.737	1.713
	C.D. (P=0.05)	5.564	4.935	4.865

*Figures in parenthesis are arcsin transformed values

juvenile mortality data due to *T. viride* at respective hrs interval presented in Table 4.

The data recorded at 24 hrs revealed that the treatment with 1.00 per cent concentration recorded 10.00 per cent second stage juvenile mortality and found to be superior over all other treatments.

At 48 hrs, 26.67 per cent second stage juvenile mortality was recorded in treatment with 1.00 per cent concentration and it was superior over other treatments. However, it was on par with 0.75 per cent concentration treatment where, 23.33 per cent second stage juvenile mortality of of *M. incognita* was recorded.

The maximum juvenile mortality (66.67%) was recorded in treatment with 1.00 per cent concentration at 72 hrs, which was superior over the rest of treatments. The treatment with 0.75 per cent concentration recorded 33.33 per cent second stage juvenile mortality. The least 16.67 per cent 2nd stage juvenile mortality observed in treatment with 0.25 per cent concentration.

Thus, the treatment with 1.00 per cent concentration proved to be consistently superior to other treatment at all the intervals of observations.

Bioefficacy of *Pochonia chlamydosporium* against second stage juveniles of *M.incognita* under laboratory conditions :

The bioagent, *P. chlamydosporium* was tested for determining the biological activity against 2nd stage juvenile stage of *M.incognita*. The bioagent, *P. chlamydosporium* was found to be effective to cause mortality in 2nd stage juvenile of *M. incognita*, when applied at 0.25, 0.50, 0.75, 1.00 per cent concentration. The tested fungal concentration and 2nd stage juvenile mortality data due to *Pochonia chlamydosporium* at respective hrs interval presented in Table 5.

The data recorded at 24 hrs revealed that the treatment with 1.00 per cent concentration recorded 10.00 per cent 2nd stage juvenile mortality and found to be superior over all other treatment.

At 48 hrs, 23.33 per cent 2nd stage juvenile mortality was recorded in treatment with 1.00 per cent concentration and it was superior over other treatments, while treatments with 0.75 per cent concentration treatment where 16.67 per cent 2nd stage juvenile mortality of *M. incognita* was recorded.

The maximum second stage juvenile mortality (53.33%) was recorded in treatment with 1.00 per cent concentration at 72 hrs, which was superior over rest of the treatments. The treatment with 0.75 per cent concentration recorded 30.00 per cent mortality of 2nd stage juveniles. The least, 16.67 per cent second stage juvenile mortality was observed in treatment with 0.25 per cent concentration.

Thus, the treatment with 1.00 per cent concentration proved to be consistently superior to other treatments at all the intervals of observation.

The results of present investigations of effectiveness of *P.lilacinus* are in agreement with those reported by Siddiqui *et al*. (2000) who studied the efficacy of *Pseudomonas aeruginosa* alone or in combination with *Paecilomyces lilacinus* against root knot nematode and root infecting fungi under laboratory and field conditions. The effectiveness of *Bacillus subtilis, P. fluorescence, T. harzianum* and *T. viride* at 10 per cent concentration was reported by El-Nagdi and Abd-El-khair (2006) for management of *M. incognita* under *in vitro* conditions. The inhibitory effects on egg hatching of root knot nematode due to application of *P. lilacinus* and *T.viride* is also reported by Goswami and Singh (2004) under laboratory conditions.

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