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Research Article

IN VITRO EFFICACY OF BIOCONTROL AGENTS AND SCREENING OF ROOTSTOCKS AGAINST ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* IN MANGO

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Abstract: Use of biocontrol agents and resistant sources proved to be better when compared to chemical means, both in terms of cost and its side effects. Hence *in vitro* biocontrol studies were conducted using four efficient biocontrol agents such as three fungi (*Purpureocillium lilacinum, Pochonia chlamydosporia, Trichoderma harzianum*) and one bacteria (*Bacillus subtilis*). Under laboratory condition, egg hatching and juvenile mortality tests were made to know the efficacy of biocontrol agents in the form of culture filtrate. Among these, *P. lilacinum* and *P. chlamydosporia* were almost equally effective in comparison with other biocontrol agents in controlling the root knot nematode in mango. *P. lilacinum* was very effective in inhibiting the egg hatching of M. incognita (78%) whereas *P. chlamydosporia* was best among the four organisms in juvenile mortality test (90.75%). Screening of mango rootstocks of five different varieties were also made to know their status of resistance against root knot nematode. In case of screening, the rootstock of polyembryonic variety H- 13- 1 was moderately resistant (Root knot index- 3) in comparison with the other four variety rootstocks.

Keywords: *Root knot nematode, Biocontrol agents, Laboratory condition, Rootstocks, Screening.*

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Introduction

About 4100 species of plant parasitic nematodes have been identified worldwide [6]. These nematodes are responsible for about 12% crop loss worldwide and $40.3 million loss in India alone [12]. Amongst these, the most advanced one is root knot nematode [9]. Root knot nematode has a broad host range and survival capacity compared to all other nematodes. Thus, the threat caused by these hidden organisms are serious than the loss caused by insect pests [12]. The use of chemical nematicides and pesticides had spoilt the soil health and many other beneficial organisms [12]. Hence control measures using biocontrol agents or the use of resistant varieties will be safe and good. Though, the use of biocontrol agents like trapping fungi was not practically successful, some of the egg parasitic fungi and toxin producing bacteria have been reported to manage root knot nematode efficiently. Hence for present investigation, four biocontrol agents such as *Purpureocillium lilacinum, Pochonia chlamydosporia, Trichoderma harzianum* and Bacillus subtilis were selected. Their efficacy was preliminarily tested against root knot nematode *in vitro*. Under laboratory conditions, culture filtrate of these organisms was treated against the nematode eggs and juveniles by egg hatching and juvenile mortality tests [11]. Likewise, screening is also an important way in handling nematode problems by the use of resistant cultivars which also reduces the cost of cultivation to farmers. Hence rootstocks of mango varieties such as Alphonso, Banglura, H-13-1, Muvendan and one wild species were screened. The difference in galling index is taken as a factor for assessing the resistance of the varieties [4]. Thus, an attempt was made to find a better solution for managing root knot nematode in mango through the use of a resistant variety and a potential biocontrol agent.

Materials and Methods

Biocontrol studies (*in vitro*)

For biocontrol (*in vitro*) studies, the biocontrol agents were treated against root knot nematode under laboratory conditions.

Two tests such as egg hatching and juvenile mortality were conducted against root knot nematode using biocontrol agents such as *Purpureocillium lilacinum* (TNAU-PI-001), *Pochonia chlamydosporia* (TNAU-Pc-001) taken from Department of Nematology, TNAU, Coimbatore and Pure culture of *Trichoderma harzianum*, *Bacillus subtilis* were taken from the Department of Plant Pathology, TNAU, Coimbatore.

Culture filtrate

Well grown cultures of bioagents in their respective broths were taken. They were filtered using Whatman No.1 Filter paper. This filtrate was centrifuged at 3000 rpm for 3 minutes, and passed through a bacterial filter. The culture filtrate was diluted as 20, 40, 80 and 100% using distilled water to treat them against the egg mass and juveniles of root knot nematode.

Egg Hatching Study

Uniform sized egg masses (containing 150- 200 eggs) from *M. incognita* infested roots were selectively collected. Egg masses were exposed to four biocontrol agents with four different concentrations and four replications. Egg masses were taken in small Petri Dishes of size 5 cm diameter containing 2ml of culture filtrate (One egg mass/2 ml of culture filtrate). Observations were taken at 24, 48 and 72 hours after inoculation by counting the number of juveniles hatched out. The experiment was carried out under room temperature.

Juvenile mortality Test

Juveniles (J2) were collected from the egg masses kept for hatching. 100 J2/Petri Dish of 5 cm diameter were exposed with 2ml of culture filtrate with the same treatments, concentrations and replications made for the previous study. Observations were taken by counting the number of dead juveniles after making survival test at 24, 48 and 72 hours after inoculation. The experiment was carried out under room temperature.

Table-1 *Screening of Mango rootstocks against rootknot nematode*

Variety	No. of eggmass / plant	No. of eggs / eggmass	No. of Galls / plant	RF	Rootknot index	Reaction
Alphonso	24.36	198.95	38.05	2.05	4	Susceptible
Banglura	29.45	227.80	34.50	1.55	4	Susceptible
Muvendan	21.05	235.35	32.35	1.92	4	Susceptible
H- 13-1	5.05	89.30	10.85	1.02	3	Moderately Resistant
Wild species	3.25	78.75	20.35	1.05	3	Moderately Resistant

[Note: RF (Reproduction factor) = Final nematode population/ Initial population]

Table-2 *Efficacy of biocontrol agents over the eggs of M. incognita (Egg hatching Test)*

SN	Conc. (%)	No. of eggs hatched after exposure period											
		P. lilacinum			*P. chlamydosporia*			*B. subtilis*			*T. harzianum*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
1	20%	68.75 (8.2)	67.25 (8.2)	71.75 (8.4)	46.25 (6.6)	53.50 (7.5)	62.25 (7.9)	77.00 (8.8)	82.25 (9.0)	94.25 (9.6)	79.00 (8.8)	83.50 (9.0)	88.25 (9.3)
2	40%	52.75 (7.2)	61.25 (7.7)	68.00 (8.1)	47.75 (6.8)	58.00 (7.5)	65.75 (6.8)	47.75 (8.5)	57.00 (7.5)	62.25 (7.8)	73.00 (8.5)	77.25 (8.7)	84.25 (9.1)
3	80%	44.50 (6.6)	49.25 (6.9)	56.50 (7.4)	48.25 (6.9)	53.25 (7.2)	55.50 (7.4)	57.25 (7.4)	60.00 (7.3)	62.50 (7.5)	56.00 (7.4)	58.25 (7.5)	65.00 (8.0)
4	100%	39.00 (6.2)	34.75 (5.8)	44.50 (6.3)	32.00 (5.6)	33.50 (5.7)	41.00 (6.3)	37.25 (6.8)	40.50 (6.3)	34.00 (6.7)	48.00 (6.8)	52.50 (7.2)	56.75 (7.4)
5	Control	89.00 (8.8)	170.00 (12.4)	200.00 (13.6)	79.00 (9.3)	149.00 (11.8)	152.00 (12.6)	68.00 (8.7)	101.00 (9.4)	143.00 (10.9)	84.00 (8.7)	94.00 (10.6)	116.00 (11.4)
	SEd±	0.52	0.21	0.35	0.48	0.44	0.45	0.25	0.56	0.30	0.25	0.30	0.29
	CD(P=0.01)	1.50	0.88	1.10	0.43	1.31	1.32	0.73	0.79	0.90	0.74	0.88	0.86

Figures in the parentheses are square root transformed values

Table-3 *Efficacy of biocontrol agents over juvenile mortality of M. incognita (Juvenile mortality Test)*

SN	Conc. (%)	No. of dead juveniles after exposure period											
		P. lilacinum			*P. chlamydosporia*			*B. subtilis*			*T. harzianum*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
1	20%	34.00 (5.7)	42.25 (6.4)	50.75 (7.0)	25.75 (4.9)	35.00 (5.8)	40.25 (6.3)	20.25 (4.4)	23.75 (4.8)	31.00 (5.5)	21.00 (4.5)	26.25 (5.0)	29.50 (5.3)
2	40%	42.75 (6.4)	51.25 (7.1)	56.50 (7.4)	28.50 (5.2)	40.50 (6.3)	50.75 (7.0)	26.50 (5.0)	31.50 (5.5)	34.50 (5.8)	40.50 (6.3)	48.00 (6.9)	13.75 (7.5)
3	80%	62.75 (7.8)	72.00 (8.4)	78.25 (8.7)	61.00 (7.8)	66.75 (8.1)	75.75 (8.6)	60.25 (7.7)	66.50 (8.1)	72.75 (8.4)	60.50 (7.7)	66.00 (8.0)	72.00 (8.5)
4	100%	77.25 (8.7)	82.25 (9.0)	83.75 (9.1)	79.00 (8.8)	87.75 (9.3)	90.75 (9.4)	75.00 (8.6)	80.00 (8.8)	83.50 (9.0)	66.00 (8.0)	69.50 (8.2)	73.25 (8.5)
5	Control	2.00 (1.3)	6.20 (2.2)	10.45 (3.1)	2.80 (1.3)	4.50 (1.9)	7.50 (2.6)	1.50 (1.2)	2.50 (1.3)	10.00 (2.7)	2.50 (1.2)	5.00 (1.9)	11.00 (3.5)
	SEd±	0.29	0.34	0.41	0.53	0.29	0.20	0.38	0.21	0.24	0.14	0.14	0.19
	CD(P=0.01)	0.87	1.10	1.20	1.50	0.88	0.59	1.03	0.62	0.73	0.41	0.42	0.56

Figures in the parentheses are square root transformed values

Screening

Transplanting

Mango rootstocks of five varieties such as Alphonso, Benglura, H-13-1, Muvendhan and one wild species with four replications in each variety were screened. Alphonso and Banglura rootstocks were concentrated because these two varieties are commercially important (for raising seedlings). Pot mixture were filled in polythene bags and autoclaved. The sterilised soil was used for potting the rootstocks of all five varieties and replications were labelled clearly. The pots were arranged in completely randomised design under glasshouse conditions [Fig-1].

Fig-1 Varietal screening for five different mango variety rootstocks (Glasshouse condition).

Inoculation of nematodes

Egg masses of *M. incognita* were separated from the infested roots and placed in a 100 ml beaker containing water. They were periodically aerated until the juveniles hatched out from all the egg masses. These second stage juveniles were counted and 2 juveniles / g of soil were inoculated into the rhizosphere region of mango rootstocks. The plants left uninoculated with nematodes were taken as control.

Fig-2 Varietal screening of mango rootstocks against *M. incognita* (a:Control; b:Wild variety; c:Alphonso; d:Banglura; e:Muvendan; f:H-13-1)

Analysis
45 days after inoculation, the plants were pulled out to assess the root knot index [Fig-2]. Root knot index was found to know the resistance status of the rootstocks by comparing with that of the control plants [4]. 1: no gall – highly resistant; 2: 1-10 galls- resistant; 3: 11-30 galls- moderately resistant; 4: 31-100 galls- susceptible; 5: ›100- highly susceptible [4].

Results and Discussion
In the experiment conducted using four biocontrol organisms, two were found to be effective. *P. lilacinum* was very effective in inhibiting the hatching (78%) of *M. incognita* eggs [Table-2] whereas *P. chlamydosporia* was best in juvenile mortality (90.75%) tests [Table-3]. Hence to conclude, both the organisms (*P. lilacinum* and *P. chlamydosporia*) were more or less equally effective in controlling the root knot nematode in mango, followed by *Bacillus subtilis* and *Trichoderma harzianum*. The organisms chosen here for biocontrol studies were all found to be efficient in different aspects. They are all commercially used bioagents in controlling the pest and disease problems in plants. Here they were compared to evaluate their efficiency in controlling root knot nematode infesting mango. According to Cayrol *et al*. (1989), *P. lilacinum* was best in producing toxic metabolites against nematodes. This was now found to be in line with the work done in this research. Moreover, *P. lilacinum* was found to produce highest number of metabolites extracted through High Pressure Liquid Chromatography that are toxic against nematodes [12]. Bailey *et al*. (2008) reported that *P. chlamydosporia* has an important role as a biocontrol agent. *Bacillus subtilis* which was reported to produce iturin and surfactin genes that is toxic against the nematodes involved in biocontrol of root knot nematodes [7]. Massoud *et al*. (2000) worked with *Trichoderma virens* (strain GI-3), which resulted in 49% reduction of hatching and mortality of juveniles. The promising biocontrol agents *viz., P. lilacinum* and *P. chlamydosporia* could be further tested under glass house followed by field condition for the management of root knot nematode in mango. According to Root knot index calculated, the varieties such as Alphonso, Banglura and Muvendan were found to be susceptible [Table-1]. Wild species and polyembryonic variety H-13- 1 were moderately resistant. The root knot index was three in case of H-13-1 and Wild species and four for all the other three variety rootstocks such as Alphonso, Banglura and Muvendan [Table-1]. The highest reproduction factor and number of galls per plant was recorded from the variety Alphonso as 2.05 and 38.05 respectively. Though the number of egg mass/plant was high (29.45) in variety Banglura, number of eggs/egg mass was maximum (235.35) in Muvendan [Table-1].

Application of research: Studies related to biomanagement of root knot nematode in mango.

Research category: Nematology

Abbreviations: h: hours, J2: Infective second stage juvenile, rpm: revolutions per minute, RF: Reproduction Factor.

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