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Influence of *Streptomyces fradiae* against Root knot nematode *Meloidogyne incognita* in Tomato

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Abstract

A protected and field experiment was conducted to evaluate the biocontrol potential of the optimized culture filtrates of Streptomyces fradiae against root-knot nematode Meloidogyne incognita in tomato. The fermentation medium of S. fradiae optimized with pH 6 at 35°C with starch and yeast extract as carbon and nitrogen sources respectively maximized the colonization rate of S. fradiae. The culture filtrate of the optimized medium of S. fradiae resulted in higher degree of inhibition in egg hatching, and mortality of juveniles of M. incognita. The effectiveness of optimized medium of S. fradiae against M .incognita is related to higher production of secondary metabolites subsequent to maximization of colonization. The S. fradiae experimented for the management of M. incognita under protected and field conditions revealed its effectiveness for the suppression of M .incognita population and to reduce the severity of nematode disease in terms of gall index. The suppression in M. incognita population / incidence following the application of S. fradiae resulted in significant improvement in yield attributes including fruit yield of tomato.

Keywords: *Meloidogyne incognita, Streptomyces fradiae,* actinomycetes, Ken Knight, agar medium, potato dextrose agar (PDA), Nutrient agar (NA).

Introduction

Among the plant parasitic nematodes limiting crop production, root-knot nematodes, *Meloidogyne* spp. cause severe damage to a wide variety of crops and leads to significant yield losses which accounts for 78 billions US dollar worldwide annually[1]. It has been estimated that plant parasitic nematodes cause a yield loss of 11 per cent in vegetables in general and 46.2 per cent in tomato due to root knot nematode alone in India[2].

Among biocontrol agents actinomycetes having frequent worldwide occurrence is considered as one of the most promising group[3]. It has been documented that actinomycetes act as antagonists of nematodes by producing nematicidal metabolites[4] and antibiotics[5]. The antibiotics such as avermectins and related compounds are active against plant pathogens including phytoparasitic nematodes [6]. Research on actinomycetes with root knot nematode had yielded varying but often positive results from laboratory, greenhouse and field tests[7] . However the *Streptomyces* spp encountered frequently, little is known about its role in regulating nematode populations[8]. Hence the present study is programmed to evaluate the biocontrol potential of *S. fradiae* against the key nematode pest of *M. incognita* in tomato.

Material and Methods

Preparation of *S. fradiae* inoculums: The isolated indigenous culture of *S. fradiae* was subcultured from the agar slants to Ken Knight agar medium. The medium was then autoclaved at 121^0C and 15 lbs. for 15 min and allowed to cool down to room temperature before being poured into 90 mm Petri dishes. The *S. fradiae* were streaked on the prepared medium and incubated at 28^0C for 7 days under aerobic conditions. The *S. fradiae* inoculum was then prepared by transferring several colonies into sterile SS (Starch Soluable) broth (100 ml) and incubated the medium for 4 days at room temperature.

Optimization of fermentation conditions: A study was conducted as follows to optimize the fermentation conditions/ medium in order to maximize the biocontrol potential of *S. fradiae*.

Effect of temperature and pH: One hundred ml of the SS broth (fermentation broth) was dispended in 250 ml Erlenmeyer flask and steam sterilized. After inoculation flasks were incubated at different temperatures ranges from 20 to 40°C. The other parameters like pH of the substrate kept at their optimum level and fermentation was run for 7-8 days. The growth of actinomycetes measured by the number of cell count at 10^{-7} dilution after incubation and its biocontrol potential against *M. incognita* was assessed. In the present study, suitable replications were maintained for each temperature experimented[9].

Similarly the effect of pH on the fermentation medium of actinomycetes was studied with the pH of 4, 5, 6, 7 and 8 by adding 0.1N sodium hydroxide or 0.1N hydrochloric acid. The

other parameters like temperature of the medium kept at optimum level of 35°C based on the above study and fermentation was run for 7-8 days. After incubation the growth of actinomycetes measured by no.of cell count at 10^{-7} dilution and its biocontrol potential against *M. incognita* was assessed.

Effect of carbon and nitrogen sources: Six carbon sources used for optimization of fermentation broth were soluble starch, sucrose, maltose, fructose, galactose and glucose in fermentation broth. One hunderd ml of culture medium amended with different carbon sources were taken in 250 ml Erlenmeyer flask and steam sterilized. The optimum pH of the medium adjusted to 6.0 with diluted NaOH based on the above study. Similarly the effect of different nitrogen source was replaced with other organic nitrogen source such as peptone, soyapeptone, malt extract, yeast extract and beef extract. Both the flask was inoculated with subcultured *S. fradiae* and flasks were incubated at 35°C for 7-8 days. After incubation the growth of actinomycetes measured by no. of cell count at 10^{-7} dilution and its biocontrol potential against *M. incognita* was measured. Three number of replications were maintained for each parameter[10,11] .

Preparation of talc formulation of *S. fradiae*: For field studies the concentrated culture filtrate of *S. fradiae* was prepared as talc formulation based on the method developed [12] and ten gram of carboxy methylcellulose was mixed with 1 kg talc powder and the pH was adjusted to 6.0 by adding calcium carbonate. The mixture was then sterilized using autoclave for 30 min for two consecutive days. The required inoculum of *S. fradiae* was added to 1 kg of the talc mixture and mixed well under sterile conditions to maintain 8 x 10^9 cfu/g. The product was dried under shade to bring the moisture content below 20 per cent. The formulation was packed in polythene bags, sealed and kept at room temperature and used for field experimental purpose.

Influence of *S. fradiae* on *M.incognita* in tomato under protected conditions: Experiments were conducted under glasshouse conditions to evaluate the biocontrol potential of *S. fradiae* against *M. incognita* in tomato. Four weeks old healthy tomato seedlings were transplanted @ 3 seedlings/pot filled with 10 kg of stream sterilized pot mixture prepared with red soil, sand and farm yard manure in 2:1:1 proportion. One week after planting the seedlings were thinned to one per pot and inoculated with freshly hatched J_2 of *M. incognita* (1 J_2/g soil) obtained from the pure culture maintained on tomato followed by the application of the culture filtrates at different concentrations of 0.2 to 1% by making three slanting holes to a depth of 2-3 cm around the seedlings (Plate 6). The pots treated with the nematicide carbofuran 3G at 1g a.i/pot and untreated were served as chemical check and untreated control respectively. All the treatments were replicated thrice in Completely Randomized Design (CRD).

The experiments were concluded at 150 days after transplanting (DAT) and the plants were removed with intact roots system and washed free of soil. Observation on shoot height (cm), root length (cm), shoot weight (g), root weight (g),fruit yield (g), gall index, nematode population in soil and root were made.

Influence of *S. fradiae* on *M.incognita* in tomato under field conditions: Field experiments were conducted at Kalampalayam village in Coimbatore district during 2013 to evaluate the biocontrol potential of *S. fradiae* against *M. incognita* in tomato. The experiments were conducted on tomato var Dhanalaksmi in nematode sick field. The healthy tomato seedlings of four weeks old were transplanted @2 seedlings/hole. One week after planting the seedlings were thinned to one per hole and applied with the *S. fradiae* prepared in talc formulation (8 x 10^9 cfu/g). The plots treated with the nematicide carbofuran 3G at 1kg a.i/ha and untreated were served as chemical check and untreated control respectively. All the treatments were replicated three times with plot size of 20 m^2 and the design adopted was Randomized Block Design (RBD).

At the time of concluding the experiments at 150 DAT ten plants were uprooted from each plot at random and indexed for root gall index. Besides observations were made on plant growth characters including fruit yield, and nematode populations in soil and root.

Results and Discussion

In the process of optimization of fermentation medium with different temperature and pH the highest rate of multiplication of *S. fradiae* (38×10^7cfu/ml) was recorded at 35°C with pH 6 (40 x10^6cfu/ml). Similarly there was significant difference among the different sources of carbon and nitrogen in the multiplication rate of *S. fradiae* which is directly related to the production of secondary metabolites. The highest *S. fradiae* mass multiplication rate of 30×10^7cfu/ml was registered by starch among the different sources of carbon and the yeast extract (58 x10^7cfu/ml) was found to be the optimum source of nitrogen to enhance the multiplication rate of *S. fradiae* (table-1).

Therefore similarly the culture filtrate of fermented medium optimized with suitable source of starch as carbon and yeast extract as nitrogen; temperature (35°C) and pH (6) had significant inhibitory effect on *M. incognita* (figure-1 and 2). The direct relationship between the biomass and secondary metabolites production by *S. fradiae* is explained as probable reason for the same as reported by earlier workers[13].

There was significant reduction in *M. incognita* population followed by the soil application of *S. fradiae*. The reduction in nematode population ranging from7.78 to 65.35 per cent (table-2) was found to be directly proportional to the concentration of *S. fradiae*. The above trend was observed in respect of reduction in *M. incognita* population in root followed by the application of *S. fradiae* through soil. Similarly there was significant reduction

in *M. incognita* female population from 11.31 to 79.40 with increase in the concentration of *S. fradiae* from 0.2 to 1 per cent and female population in root was significantly reduced in the population of *M. incognita* females with egg mass due to the treatmental effect of *S. fradiae*. The gall index recorded in different treatments differed significantly among each other. The lowest gall index of 1 with 80 per cent reduction over untreated control was registered by the *S. fradiae* at 1 per cent. The significant effect of *S. fradiae* in the suppression of *M. incognita* population/ incidence resulted in increase in the plant growth parameters (table-3) of tomato viz., shoot length, shoot weight, root lengh and root weight and fruit yield also significantly increased.

Table-1
Effect of different parameters in the optimization of fermentation medium of *S. fradiae*

Parameters		**Cell count**		**SEd**	**CD (P=0.05)**
		Cfu/ml	**Log value**		
Carbon sources	Starch	30×10^7	8.505^a	0.24	0.52
	Sucrose	17×10^6	7.255^c		
	Maltose	83×10^5	6.929^c		
	Glucose	70×10^5	6.833^c		
	Fructose	35×10^6	7.556^b		
	Galactose	93×10^6	7.914^b		
Nitrogen sources	Peptone	25×10^7	8.398^a	0.25	0.55
	Soya peptone	28×10^6	7.447^b		
	Beef extract	30×10^6	7.477^b		
	Malt extract	25×10^7	8.398^a		
	Tryptone	65×10^6	7.813^b		
	Yeast extract	58×10^7	8.763^a		
Temperature(°C)	20	22×10^4	5.301^d	0.09	0.20
	25	24×10^5	6.398^c		
	30	29×10^6	7.462^b		
	35	38×10^7	8.556^a		
	40	28×10^6	7.447^b		
pH	4	27×10^3	4.462^e	0.09	0.21
	5	26×10^4	5.398^d		
	6	40×10^6	7.591^a		
	7	44×10^5	6.681^b		
	8	20×10^5	6.301^c		

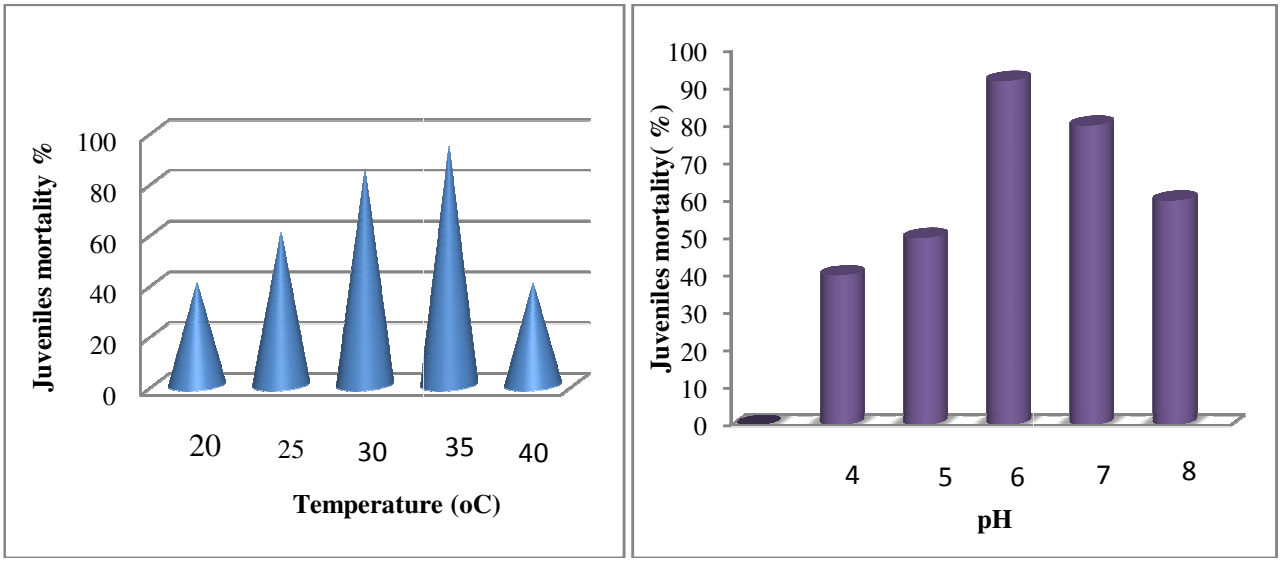

Figure-1
Effect of temperature and pH used for the optimization of fermentation medium on juveniles of *M. incognita*

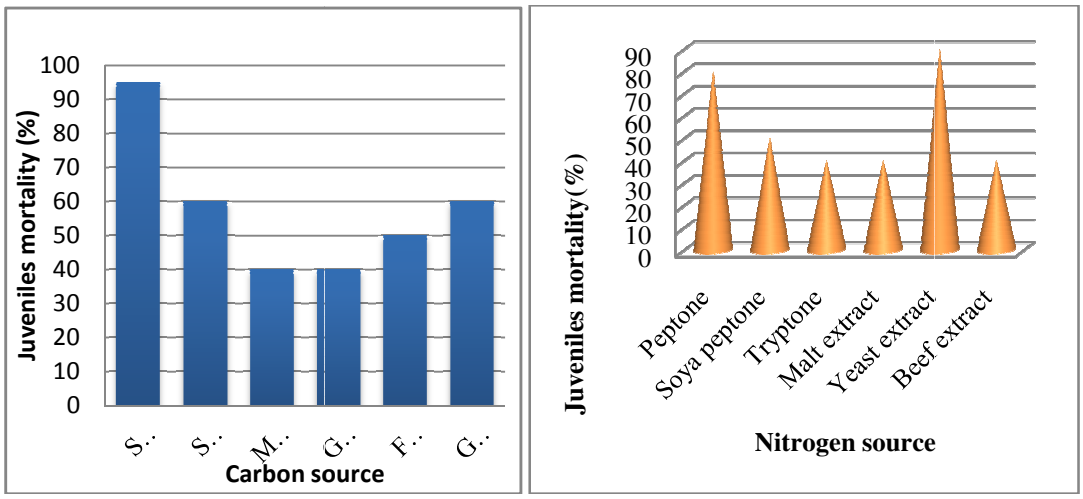

Figure-2
Effect of carbon and nitrogen sources used for the optimization of fermentation medium on juveniles of *M. incognita*

Table-2
Influnce of *S. fradiae* on *M.incognita* under glasshouse conditions

Treatments	Nematode population		Females/5g root	Females with egg mass/5g root	Eggs/egg mass	Gall index
	Soil (250cc)	Root (5g)				
S.fradiae at 0.2%	285.06^{f} (7.78)	189.10^{f} (7.87)	91.43^{f} (11.31)	43.06^{f} (8.38)	234.16^{f} (6.99)	4.00^{f} (20.00)
S.fradiae at 0.4%	239.16^{e} (22.63)	143.16^{e} (30.25)	72.96^{e} (29.23)	36.16^{e} (23.06)	209.13^{e} (16.92)	3.34^{e} (33.20)
S.fradiae at 0.6%	187.10^{d} (39.47)	108.06^{d} (47.35)	64.70^{d} (37.60)	31.76^{d} (32.42)	189.10^{d} (24.88)	2.33^{d} (53.40)
S.fradiae at 0.8%	140.00^{c} (54.71)	83.06^{c} (59.53)	45.30^{c} (56.06)	22.56^{c} (52.00)	156.06^{c} (38.01)	1.67^{c} (66.60)
S.fradiae at 1.0%	107.10^{a} (65.35)	46.16^{a} (77.51)	21.23^{a} (79.40)	8.70^{a} (81.48)	113.10^{a} (55.07)	1.00^{a} (80.00)
Carbofuran 3G @1kg a.i/ha	120.16^{b} (61.12)	66.13^{b} (67.78)	31.50^{b} (69.44)	16.10^{b} (65.74)	130.10^{b} (48.32)	1.33^{b} (73.40)
Untreated Control	309.13^{g}	205.26^{g}	103.10^{g}	47.00^{g}	251.76^{g}	5.00^{g}
CD(P=0.05)	1.78	1.82	3.89	1.58	1.83	0.36

Soil application of *S. fradiae* in talc formulation (8 x 10^{9} cfu/ml) prior to planting of tomato resulted with significant reduction in *M. incognita* population (table-3). The effect of *S. fradiae* in the per cent reduction of *M. incognita* population ranging from 49.38 to 79.21 is positively correlated with increase in the dosage of *S. fradiae* from 1 to 5 kg/ha. Hence the highest reduction of 79.21 per cent in nematode population was registered by the treatment of *S. fradiae* @ 5 kg/ha and it was on par with the dosage *S. fradiae* at 4 kg /ha and chemical check of carbofuran 3G @ 1kg a.i/ha (77.76 %). Similar trend was followed in respect of suppression in nematode population in root, number of females, females with egg masses and eggs per eggmass. The per cent reduction in gall index of 20 to 80 per cent was found to be increased with increase in the dosage of *S. fradiae* from 1 to 5 kg/ha. The results of the present study also supported by many authors attempted for the management of *M. incognita*, *R. reniformis* in different crops[13-15].

The suppression in root knot population/ incidence in tomato followed by the application of *S. fradiae* resulted in improvement in plant growth characters significantly. In this regard the highest shoot length and weight of 67.67 cm and 61.67 g was recorded in the treatment of *S. fradiae* at 5 kg/ha. Similarly the highest per cent increase (61.04) in root length was recorded in the treatment of *S. fradiae* @ 5kg/ha (table-4) and it proved that many species of actinomycetes effectively colonize plant roots, protect plant roots from plant pathogens and improve biometric characters of plants[16].

There was significant improvement in fruit yield of tomato in plots treated with *S. fradiae*. The history of *Streptomyces* and its capacity to enhance plat growth is well documented[17]. The possibility of *Streptomyces* as PGPR to enhance the growth of plants includes nitrogen fixation, siderophore synthesis,

phytohormone synthesis and solubilization of minerals to make them available for plant uptake and use[18].

Conclusion

It is concluded that *S. fradiae* in talc formulation @ 5 kg/ha is effective for the management of *M. incognita* and to enhance the fruit yield of tomato.

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Table-3
Influence of *S. fradiae* on plant growth of tomato under glasshouse conditions

Treatments	Shoot		Root		Fruit yield /plant (g)
	Length(cm)	Weight(g)	Length(cm)	Weight(g)	
S.fradiae at 0.2%	48.33^{e} (11.48)	44.22^{e} (6.34)	12.70^{e} (16.83)	7.80^{d} (6.41)	172.30^{e} (8.91)
S.fradiae at 0.4%	50.20^{d} (15.80)	46.54^{d} (11.92)	13.53^{d} (24.47)	8.48^{c} (15.68)	180.00^{d} (13.78)
S.fradiae at 0.6%	53.26^{c} (22.86)	48.51^{c} (16.66)	15.35^{c} (41.21)	9.10^{b} (24.14)	218.30^{c} (37.98)
S.fradiae at 0.8%	57.55^{b} (32.75)	51.33^{b} (23.44)	17.56^{b} (61.54)	10.11^{a} (37.92)	247.00^{b} (56.13)
S.fradiae at 1.0%	61.34^{a} (41.52)	54.17^{a} (30.27)	19.42^{a} (64.58)	11.99^{a} (63.57)	263.60^{a} (66.62)
Carbofuran3G @1kg a.i/ha	48.37^{e} (11.58)	47.40^{c} (13.99)	12.40^{e} (14.07)	8.00^{d} (9.14)	232.30^{b} (46.83)
Untreated Control	45.16^{f}	46.10^{e}	10.87^{f}	7.33^{e}	158.20^{f}
CD(P=0.05)	1.73	1.82	1.41	0.93	12.55

Table-4
Influence of *S. fradiae* on *M. incognita* in tomato under field conditions

Treatments	Nematode population		Females/ 5g root	Females with egg mass/5g root	Eggs/ egg mass	Gall index
	Soil (250cc)	Root (5g)				
S. fradiae @ 1 kg /ha	151.00^{d} (49.38)	65.33^{d} (57.66)	39.33^{c} (73.89)	18.33^{c} (72.36)	181.00^{e} (38.90)	4.00^{f} (20.00)
S. fradiae @ 2kg /ha	107.00^{c} (64.13)	57.00^{c} (63.06)	26.67^{bc} (82.29)	15.67^{bc} (76.37)	161.67^{d} (45.44)	3.26^{e} (34.80)
S. fradiae @ 3kg /ha	88.00^{b} (70.50)	51.00^{b} (66.95)	21.00^{ab} (86.06)	14.67^{bc} (77.88)	146.00^{c} 50.73)	2.67^{d} (46.60)
S. fradiae @ 4kg /ha	76.67^{ab} (74.30)	42.67^{ab} (72.35)	17.67^{ab} (87.82)	10.33^{ab} (84.42)	138.00^{c} (53.43)	1.8^{c} (64.00)
S. fradiae @ 5kg /ha	62.00^{a} (79.21)	33.00^{a} (76.67)	11.33^{a} (92.48)	7.00^{a} (89.77)	113.33^{a} (61.75)	1.00^{a} (80.00)
Carbofuran3G @1kg a.i/ha	66.33^{a} (77.76)	36.00^{a} (76.67)	12.33^{a} (91.81)	7.33^{a} (88.94)	124.00^{b} (58.15)	1.33^{b} (73.40)
Untreated control	298.33^{e}	154.36^{e}	150.67^{d}	66.33^{d}	296.33^{f}	5.00^{g}
CD (P=0.05)	14.75	8.50	12.72	5.16	9.86	0.34

Figures in parenthesis are % decrease over control. Values followed by same letter in a row are not significantly different from others as determined by Least Square Mean Test ($P \leq 0.05$).

Table-5
Influence of *S. fradiae* on plant growth of tomato under field conditions

Treatments	Shoot		Root		Fruit yield / ha (tons)
	Length(cm)	Weight(g)	Length(cm)	Weight(g)	
S. fradiae @1 kg /ha	51.67^{bc}	49.67^{cd}	20.00^{de}	19.43^{e}	16.85^{e}
	(17.43)	(28.44)	(9.11)	(17.04)	(7.66)
S. fradiae @2kg /ha	54.33^{bc}	51.00^{c}	23.00^{cd}	20.33^{cd}	18.45^{d}
	(23.47)	(31.89)	(25.47)	(22.46)	(17.89)
S. fradiae @3 kg /ha	55.00^{b}	52.67^{c}	25.33^{bc}	22.97^{bc}	19.53^{c}
	(25.00)	(36.20)	(38.18)	(38.37)	(24.79)
S. fradiae @4kg /ha	61.33^{a}	58.33^{b}	27.33^{ab}	23.00^{ab}	20.16^{b}
	(39.38)	(50.86)	(49.09)	(38.55)	(28.81)
S. fradiae @5 kg /ha	67.67^{a}	61.67^{a}	29.52^{a}	27.67^{a}	20.45^{a}
	(53.79)	(59.47)	(61.04)	(66.68)	(30.67)
Carbofuran3G @1kg a.i/ha	49.67^{c}	47.33^{d}	21.67^{de}	20.63^{cd}	18.50^{b}
	(12.88)	(22.41)	(18.22)	(24.27)	(18.21)
Untreated control	44.00^{d}	38.67^{e}	18.33^{e}	16.60^{f}	15.65^{f}
CD(P=0.05)	4.79	3.50	3.65	2.74	14.55

Figures in parenthesis are % increase over control. Values followed by same letter in a row are not significantly different from others as determined by Least Square Mean Test ($P \leq 0.05$).

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