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Biocontrol Potential of Optimized Culture Filtrates of *Streptomyces fradiae* Against Root Knot Nematode *Meloidogyne incognita* in Tomato

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A study was conducted to evaluate the biocontrol potential of the optimized culture filtrates of *Streptomyces fradiae* against root-knot nematode *Meloidogyne incognita* in tomato under field conditions. The fermentation medium of *S. fradiae* optimized with pH 6 at 35$_0$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* caused in higher degree of inhibition in egg hatching, and mortality of juveniles of *M. incognita*. The effectiveness caused medium of *S. fradiae* against *M .incognita* was related to higher production of secondary metabolites subsequent to maximization of colonization. The *S. fradiae* experimented for the management of *M. incognita* under field conditions revealed its effectiveness for the suppression of nematode population and reduced the severity of nematode disease in terms of gall index. The suppression in *M. incognita* population / incidence following application of *S. fradiae* resulted in significant improvement in yield attributes including fruit yield of tomato.

Key words: *Meloidogyne incognita, Streptomyces fradiae*, actinomycetes, bio control, tomato.

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 (Barker, 1998). 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 (Mohsin, 1987).

Among biocontrol agents, actinomycetes having frequent worldwide occurrence is considered as one of the most promising group (Hay and Skipp,1993). It has been documented that actinomycetes act as antagonists of nematodes by producing nematicidal metabolites (Dicklow *et al*., 1993) and antibiotics (Challis and Hopwood, 2003). Antibiotics such as avermectins and related compounds are active against plant pathogens including phytoparasitic nematodes. (Jansson and Rabatin, 1998). Research on actinomycetes with root knot nematode yielded varying but often positive results from laboratory, greenhouse and field tests (Krechel *et al.,* 2002). However, little is known about the role of *Streptomyces* spp regulating nematode populations (Man- Hong Sun *et al*., 2006). Hence the present study was programmed to evaluate the biocontrol potential of *S. fradiae* against nematode pest of *M. incognita* in tomato.

Materials and Methods

Preparation of S. fradiae inoculum

The isolated indigenous culture of *S. fradiae* was subcultured by streaking the same from the agar slants to Ken Knight agar medium. All the compounds were added to the diluents and allowed to dissolve completely. The medium was then autoclaved at 121 °C 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 °C for 7 days under aerobic conditions. The *S. fradiae* inoculum was then prepared by transferring several colonies into sterile SS(Starch Soluble) broth (100 ml) and incubated the medium for 4 days at room temperature.

Optimization of fermentation conditions

A study was conducted 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 dispensed in 250 ml Erlenmeyer flask and steam sterilized. After inoculation, flasks were incubated at different temperatures ranging from 20 to 40$_0$C. The other parameters like pH of the substrate was kept at their optimum level and fermentation was run for 7-8 days. After incubation, the growth of

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actinomycetes measured by the number of cell count at 10^{-7} dilution and its biocontrol potential against *M. incognita* was assessed. Suitable replications were maintained for each temperature experimented in the present study (Ellaiah,*et al* 2004).

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^{o}C based on the above study and fermentation was run for 7-8 days. After incubation the growth of actinomycetes measured by the number 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 was taken in 250 ml Erlenmeyer flask and steam sterilized. The optimum pH of the medium adjusted was.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 flasks were inoculated with subcultured *S. fradiae* and incubated at 35 ^{o}C for 7-8 days. After incubation the growth of actinomycetes was measured by number of cell count at 10^{-7} dilution and its biocontrol potential against *M. incognita* was measured. Three replications were maintained for each parameter (Agenes *et al.,* 2005 and Yang and Yuan, 1990).

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 by Vidhyasekaran and Muthamilan (1995). 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 in an 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 experiments.

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. 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^{7}cfu/ml) was recorded at 35^{o}C with pH 6 (40×10^{6}cfu/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^{7}cfu/ml was registered by starch among the different sources of

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

Parameter		Cell count		CD
		Cfu/ml	Log value	SEd (P=0.05)
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(^{o}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}	

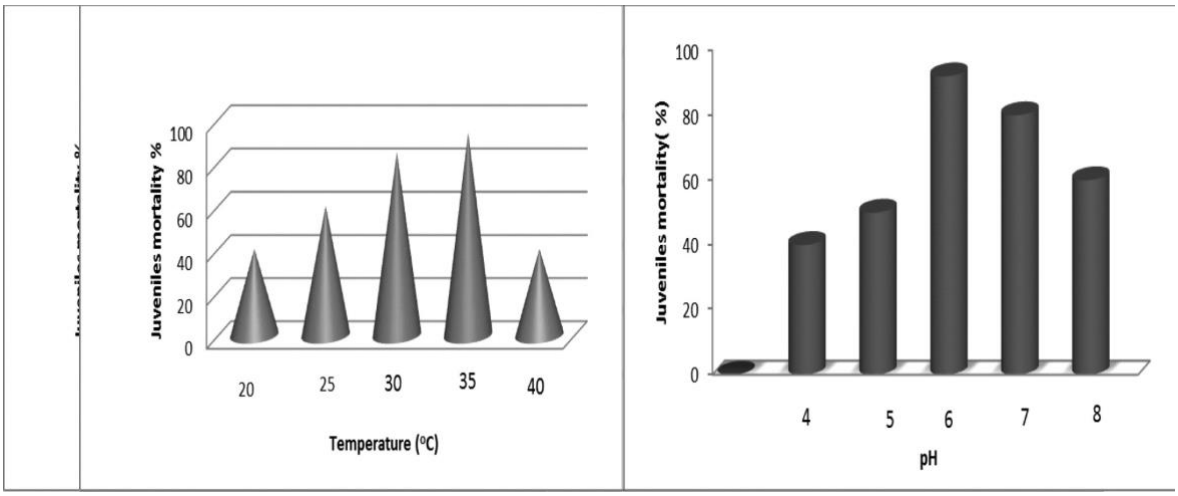

Fig. 1. Effect of temperature and pH used for the optimization of fermentation medium on juveniles of *M. incognita*

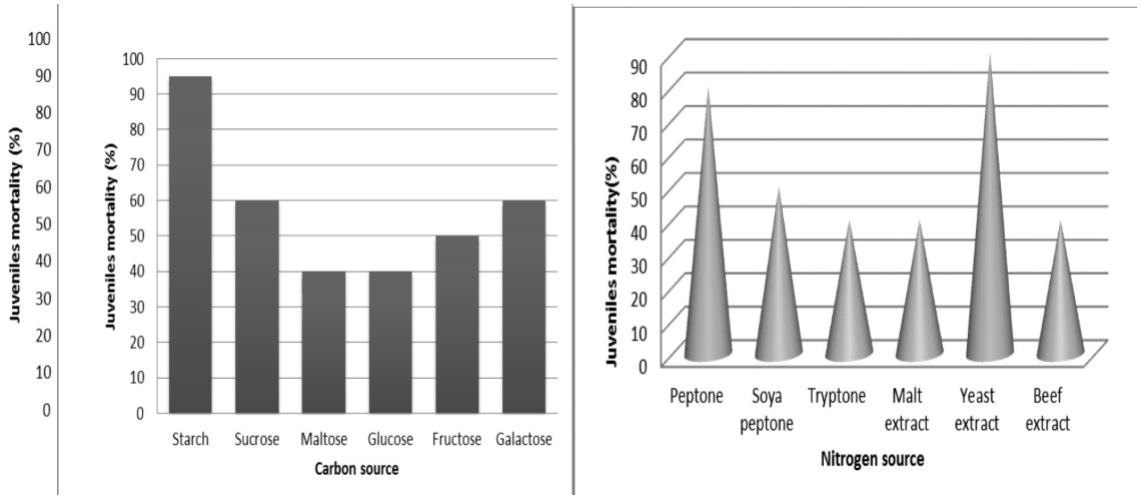

Fig. 2. Effect of carbon and nitrogen sources used for the optimization of fermentation medium on juveniles of *M. incognita*

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).

The culture filtrate of fermented medium optimized with suitable source of starch as carbon and yeast extract as nitrogen; temperature (35$_0$C) and pH (6) had significant inhibitory effect on *M. incognita* (Fig 1 and 2). The direct relationship between biomass and secondary metabolites production by *S. fradiae* might be responsible for the same as reported by earlier workers (Pornthip Ruanpanun *et al*.,2011)

Soil application of *S. fradiae* in talc formulation (8 x 10_9 cfu/ml) prior to planting of tomato resulted in significant reduction in *M. incognita* population (Table 2). 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 increased with increase in the dosage of *S. fradiae* from 1 to 5 kg/ha. The results of the present study were also supported by many authors who attempted for the management of *M. incognita*,and *R. reniformis* in crops(Jayakumar *et al*., 2005; Pornthip Ruanpanun *et al*., 2011; Faske and Starr., 2006).

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

Table 2. Influence of *S. fradiae* on *M. incognita* in tomato under field conditions.

Treatment	Nematode population in		Females / 5g root	Fe males 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)
Carbo furan 3G 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	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 parentheses are % decrease over control.
Values followed by same letter in a row are not significantly different from others by Least Square Mean Test (P≤0.05).

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

Treatment	Shoot		Root		Fruit yield / ha (tons)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	
S. fradiae 1 kg/ha	51.67^{bc} (17.43)	49.67^{cd} (28.44)	20.00^{de} (9.11)	19.43^{e} (17.04)	16.85^{e} (7.66)
S. fradiae 2kg /ha	54.33^{bc} (23.47)	51.00^{c} (31.89)	23.00^{cd} (25.47)	20.33^{cd} (22.46)	18.45^{d} (17.89)
S. fradiae 3kg/ ha	55.00^{b} (25.00)	52.67^{c} (36.20)	25.33^{bc} (38.18)	22.97^{bc} (38.37)	19.53^{c} (24.79)
S. fradiae 4kg/ha	61.33^{a} (39.38)	58.33^{b} (50.86)	27.33^{ab} (49.09)	23.00^{ab} (38.55)	20.16^{b} (28.81)
S. fradiae 5 kg/ha	67.67^{a} (53.79)	61.67^{a} (59.47)	29.52^{a} (61.04)	27.67^{a} (66.68)	20.45^{a} (30.67)
Carbo furan 3G 1kg a.i/ha	49.67$_{c}$ (12.88)	47.33^{d} (22.41)	21.67^{de} (18.22)	20.63^{cd} (24.27)	18.50^{b} (18.21)
Un treated 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 parentheses are % increase over control.
Values followed by same letter in a row are not significantly different from others by Least Square Mean Test (P≤0.05).

recorded in the treatment of *S. fradiae* @ 5kg/ha (Table 3). Subhashini and Padmaja (2011) proved that many species of actinomycetes effectively colonize plant roots, protect plant roots from plant pathogens and improve biometric characters of plants.

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 was well documented by Aldesuqui *et al*. (1998). 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 (Glick 1995).

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

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