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Research Article

Bio-management of Fusarium wilt disease complex with *Pseudomonas fluorescens* and *Aspergillus niger*

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ABSTRACT: Flue Cured Virginia (FCV) tobacco is a major rain-fed crop grown in light soil regions of Karnataka. Fusarium wilt disease complex caused by *Fusarium oxysporum* f. sp. *nicotianae* in association with Root-knot nematodes, *Meloidogyne incognita* is a major threat to the successful production and also for its sustainability in the region. The tobacco crop affected with above malady exhibit stunted growth, severe wilt symptoms, drying of leaves on one side of the plant and with conspicuous galls on the infected roots resulted in heavy yield and quality loss. Fungicides and nematicides are being used against this wilt disease complex with limited efficacy. Extensive use of pesticides of chemical origin especially in higher doses for disaese control has to be avoided due to higher costs and associated hazards to the environment. Replicated trials were conducted with antagonistic bacterium, *Pseudomonas fluorescens* and antagonistic fungi, *Aspergillus niger* singly and in combinations against fusarium wilt disease complex in FCV tobacco under sick field conditions. Results revealed that application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched with FYM @ 100 g /plant at the time of planting resulted in 61.0% reduction in fusarium wilt disease incidence at 70 DAT compared to untreated check. There was significant reduction in root knot nematode incidence in terms of RKI (Root-Knot Index) to 1.93 and final soil nematode population to 72.5 as compared to RKI of 3.71 and final soil nematode population of 140 in untreated check. Subsequent increase in total cured leaf and bright grade yield was 1311 kg/ha and 926 kg/ha respectively as compared to 1042 kg/ha and 615 kg/ha respectively in untreated check.

KEY WORDS: *Pseudomonas fluorescens*, *Aspergillus niger*, Fusarium wilt complex, *Meloidogyne incognita*, FCV tobacco

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INTRODUCTION

Flue-Cured Virginia (FCV) tobacco is an important rainfed commercial crop grown in Karnataka light soils with lot of export potentiality. The produce is preferred internationally due to its ideal chemistry with below detectable levels of TSNA (Tobacco specific nitrosamines) compounds. Wilt disease caused by *Fusarium oxysporum* f. sp. *nicotianae* is a major threat to FCV tobacco production under field conditions (Shenoi *et al*., 2004). Moreover, it is widely reported that fusarium wilt disease occurs in association with root-knot nematodes causing wilt disease complex. Plant parasitic nematodes often play a major role in disease interactions. Infection by one pathogen usually alters host response to subsequent infection by another pathogen. Mostly, root decay and necrosis of root-knot nematode infected plants are due to the association of nematodes with numerous pathogenic fungi and bacteria (Powel, 1971). Ramakrishnan *et al.* (2008) reported that root knot nematode, *Meloidogyne incognita* predisposes FCV tobacco crop to wilt disease caused by *F. oxysporum* f. sp. *nicotianae* contributing to significantly reduced yields of FCV tobacco in Karnataka. Similar to wilt disease, root knot nematode, *M. incognita* is also a major limiting factor for the successful production of tobacco, both in nursery and main field (Hussaini, 1983; Ramakrishnan *et al.,* 2001). The conspicuous symptoms of the wilt disease complex of FCV tobacco under field conditions are gradual yellowing, wilting and drying of leaves on one side of the plant. Underground symptoms exhibit infected roots turning black and in many cases with conspicuous galls caused by root knot nematodes. For the management of wilt disease complex in FCV tobacco, application of a fungicide or a nematicide alone will be effective only against the target organisms. Moreover, chemical pesticides proved to be not cost effective and many effective chemicals were withdrawn from the market due to their ill-effects and hazards they pose to environment. Hence, bio-management of wilt disease complex with antagonists is an alternative, cost effective and eco-friendly approach. Keeping in view of

export demand of the tobacco crop, efforts were made to evolve safe and eco-friendly management strategies against wilt disease complex in field crop through the use of farm yard manure enriched bio-agents, *Pseudomonas fluorescens* and *Aspergillus niger* in rational combinations.

MATERIALS AND METHODS

The field experiments were conducted for two seasons during 2008-09 in kharif at CTRI Research Station, Hunsur in a field sick with the pathogens, *F. oxysporum* and *M. incognita.* The soil type in experimental site is red sandy loam and the mean initial population of infective juveniles of *M. incognita* was 150 second staged infective juveniles/ 100g soil. The *P. fluorescens* was locally isolated, multiplied by using King's B medium and brought into talc formulation with load of 2.5 x 10^8 cfu g^{-1} and the other fungal antagonist, Kalisena, the commercial formulation of *A. niger* (strain AN 27) obtained from IARI were evaluated either singly and in combinations against *F. oxysporum* complex in FCV tobacco under field conditions. The slurry form of the bio-agent (Kalisena SL) was applied through enriched FYM. The enrichment of FYM with Kalisena SL $20g^{-1}$ was done by heaping the FYM with optimum soil moisture. The heap was turned at regular intervals and allowed for 10 days incubation before application in the field.

The treatment details are as follows, T1-*Pseudomonas fluorescens* (talc formulation) @ 1g/plant at planting, T2 – *Aspergillus niger* enriched FYM @ 100g/plant at planting, T3 – *P. fluorescens* (talc formulation) @ 1g/ plant at planting + *A. niger* enriched FYM @ 100g/plant at planting, T4 – *P. fluorescens* (talc formulation) @ 1g/ plant at planting + *A. niger* enriched FYM @ 100g/plant at 30 DAT, T5 – *P. fluorescens* root dip (5% solution) for six hours before planting, T6 – *P. fluorescens* root dip (5% solution) for six hours before planting + *A. niger* enriched FYM @ 100g/plant at planting and T7 = Untreated check. All the seven treatments were replicated four times in a randomised block design.

P. fluorescens in talc formulation and *A. niger* enriched FYM were applied at the time of planting to each plant hole, as root dip and at 30 DAT as per treatment schedule. All other management and cultural practices were followed as recommended (Shenoi, 1998). Data on FCV tobacco yield parameters (green leaf yield, bright grade yield, medium grade yield, low grade yield and total cured leaf yield), Root Knot Index (RKI) at 0-5 scale at the time of final harvest, initial and final soil nematode population per 100g soil and fusarium wilt disease incidence at 60 and 70 DAT were recorded. Pooled data of the two season trials were statistically analysed and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Pooled results of two seasons data on evaluation of *P. fluorescens* and Kalisena enriched FYM singly and in combinations against fusarium wilt and root-knot nematode disease complex revealed that application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched FYM @ 100 g /plant at the time of planting resulted in 71.7 and 61.4 percent decrease in wilt disease incidence at 60 & 70 DAT respectively, compared to untreated check (Table 1). At 70 DAT, wilt disease incidence in untreated plots was 51.5%, whereas in bio-agents treated plots, disease incidence ranged from 19.9 to 39 per cent. Applications of *P. fluorescens* as seedling root dip (5% solution) for six hours before planting reduced the wilt disease incidence to the extent of 24.3 per cent as compared to untreated check. It is clear from the present investigations, soil applications of bio-agents gave significantly better disease control as compared to seedling root-dip applications. Earlier, Shenoi and Sreenivas (2007) indicated that Kalisena SL was effective against soil borne fungal diseases such as damping-off, blight and black shank also significantly increased the healthy transplants count in FCV tobacco nurseries. Barua and Bora (2008) studied the efficacy of *T. harzianum* and *P. fluorescens* against *M. incognita* and *R. solanacearum* complex in brinjal. Brinjal plants when treated with *T. harzianum* and *P. fluorescens* significantly reduced wilt disease incidence and RKI in brinjal. In case of bacterial population in soil the highest reduction was observed in the treatment with *T. harzianum* and *P. fluorescens*.

In the case of root knot nematode incidence, combined application of *P. fluorescens* @ 1g/plant with *A. niger* enriched FYM @ 100 g /plant at the time of planting recorded significantly reduced RKI to 1.93 and final soil nematode population was 72.5 infective juveniles / 100g soil as compared to RKI of 3.71 and final soil nematode population of 140 infective juveniles/100 g soil respectively, in untreated check. But, application of *P. fluorescens* (talc formulation) alone @ 1g/plant at planting and *A. niger* enriched FYM alone @ 100g/plant at planting reduced the RKI and final soil nematode population to the tune of 36.4% and 40.76 and 46.0 per cent respectively, compared to untreated check. Reduction in root knot nematode incidence in terms of RKI and final soil nematode population in bio-agents treated plots ranged from 23.2 to 48.04 per cent and 15.7 to 48.2 per cent respectively, compared to untreated check. Sobita Devi

Table 1: Effects of bioagents on Fusarium wilt disease incidence and root knot nematode population in FCV tobacco field crop

Sl. No.	Treatments	Wilt at 60 DAT		Wilt at 70DAT		Root-knot Index (0-5 Scale) at 90 DAT100		Nematode population / g. soil	
		A	B	A	B	A	B	A	B
1	*Pseudomonas fluorescens* @1g/plant at planting	19.4 (11.04)	47.7	29.3 (24.0)	43.1 43.1	2.36 2.36	36.4 36.4	83.0 83.0	40.7 40.7
2	*Aspergillus niger* enriched FYM@ 100g/ plant at planting	16.9 (8.43)	54.4	(34.35) (34.35)	30.3 30.3	2.36 2.36	36.4 36.4	75.5 75.5	46.0 46.0
3	*P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@ 100g/plant at planting	10.5 (3.32)	71.7 71.7	19.9 (11.55)	61.4 61.4	1.93 1.93	48.0 48.0	72.5 72.5	48.2 48.2
4	*P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at 30DAT	17.5 (9.01)	52.8 52.8	35.9 (34.45)	30.3 30.3	2.14 2.14	42.0 42.0	90.5 90.5	35.3 35.3
5	*P. fluorescens* root dip(@ 5% solution) for 6hrs. before planting	18.8 (10.34)	49.3 49.3	39.0 (39.55)	4.3 24.3	2.85 2.85	23.2 23.2	118.0 118.0	15.7 15.7
6	*P. fluorescens* root dip (@ 5% solution) for 6hrs. before planting + *A. niger* enriched FYM@ 100g/plant at planting	15.9 (7.55)	57.1 57.1	32.1 (28.27)	37.7 37.7	2.55 2.55	31.3 31.3	120.0 120.0	14.2 14.2
7	Untreated check	37.1	–	51.5	–	3.71	–	140.0	–
		(36.47)	–	(61.28)	–	3.71	–	140.0	–
	S.Em	1.33		1.72		0.08		0.72	
	CD at ($p = 0.05$)	3.70		4.78		0.13		2.31	
	CV%	37.67		27.43		7.98		9.20	
	Seasons mean 2005-06	10.99		31.55		2.48		102.3	
	2006-07	27.88		38.0		2.63		97.5	
	S.Em	1.38		1.80		0.04		1.91	
	CD at ($p = 0.05$)	4.79		6.24		0.13		6.30	
	CV%	19.41		14.01		8.64		10.50	
	S x T interaction S.Em	1.89		2.44		0.11		2.46	
	CD at ($p = 0.05$)	5.23		NS		NS		NS	

A = Incidence (%); B – Percent Incidence over untreated check Figures in parenthesis are arc sine transformed values

and Pandey (2001) also had studied the field application of *P. fluorescens* in chick pea crop against *M. incognita* and *Fusarium oxysporum* f.sp. *ciceri* on chickpea and observed significant reduction in root knot disease incidence in field in terms of reduced gall formation and soil nematode population.

Beside reduction in Fusarium wilt disease and root knot nematode incidence, application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched FYM @ 100 g/plant at the time of planting significantly improved FCV tobacco yield parameters under field conditions (Table 2). There was 20.5% increase in total cured leaf yield and 33.6% increase in bright grade out turn compared to untreated check. Application of *P. fluorescens* alone and *A. niger* enriched FYM alone recorded the cured leaf yield of 1215 and 1213 kg/ha respectively. Whereas, combined application of *P. fluorescens* and *A. niger* enriched FYM recorded significantly improved yield of 1311 kg/ha as compared to 1042 kg/ha in untreated check plots. Improvement in total cured leaf yields in plots treated with bio-agents ranged from 13.2 to 20.5% compared to untreated check. Similar increase in plant growth and yield parameters due to application of *P. fluorescens* @ 2.5 kg/ha against root knot nematode in various crop plants were reported

Table 2: Effects of bioagents on yield parameters of FCV tobacco (Kg ha^{-1})

Sl. No.	Treatments		Yield Parameters (kg/ha)						
			Green leaf	Low grade	Medium grade	Bright grade	% increase over check	Total cured leaf	% increase over check
1	*Pseudomonas fluorescens* @1g/plant at planting		13235	130	295	791	22.3	1215	14.2
2	*Aspergillus niger* enriched FYM @100g/plant at planting		13587	156	276	801	23.2	1213	14.1
3	*P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at planting		13299	142	244	926	33.6	1311	20.5
4	*P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at 30DAT		13681	138	281	782	21.4	1200	13.2
5	*P. fluorescens* root dip @5% solution for 6hrs. before planting		12728	144	186	698	11.9	1028	–
6	*P. fluorescens* rot dip (@ 5% solution for 6hrs. before planting + *A. niger* enriched FYM@ 100g/plant at planting		12712	186	239	782	21.4	1207	13.7
7	Untreated check		12436	229	202	615	–	1042	–
		S.Em	330	11.47	11.82	14.0	–	18.28	–
		CD ($p = 0.05$)	1040	31.80	32.75	38.81	–	63.26	–
		CV%	12	21.3	15.72	5.43	–	8.22	–
	Seasons mean	2005-2006	13789	164	246	828	–	1238	–
		2006-2007	14404	157	246	713	–	1115	–
		S.Em	300	6.46	7.30	7.91	–	18.28	–
		CD ($p = 0.05$)	1040	NS	NS	27.36	–	63.26	–
		CV%	7	20.22	13.58	5.14	–	4.76	–
	S x T interaction	S.Em	466	16.23	16.71	19.80	–	28.03	–
		CD ($p = 0.05$)	NS	NS	NS	NS	–	NS	–

Table 3: Cost economics of Bio management of Fusarium wilt disease complex per ha

Particulars	Bio management by *Pseudomonas fluorescens* @1g/plant at planting + *Aspergillus niger* enriched FYM@100g / plant at planting	Check (Un treated)
Cost of Cultivation (Rs. /-)	75000	75000
Crop protection measure for wilt disease complex with application cost	6500	–
Yield (kg/ha)	1311	1042
Bright Grade (kg/ha)	926 (106628.9)*	615 (70817.25)*
Medium Grade (kg/ha)	244 (19654.2)	202 (16271.1)
Low Grade (kg/ha)	142 (7206.5)	229 (11621.75)
Gross returns (Rs. /-)	133489.6	98710.1
Net Profit (Rs. /-)	51989.6	23710.1
Additional income over check	28279.5	–
ICBR	1:4.4	–

Cost of FYM = Rs. 800 / Tone
Cost of Bio agents = Rs. 5200/-
Cost of Bio agent application = Rs. 500/-
Market price of tobacco on average (Rs./-) (Bright = 115.15, Medium 80.55 and Low = 50.75)
* Figures in parenthesis are amount realised at the market

earlier (Santhi and Sivakumar, 1995; Kavitha *et al*., 2007). Mani *et al*. (1998) also reported significant inhibition of potato cyst nematode in potato crop in Nilgiris with *P. fluorescens* application under field conditions. Ramakrishnan *et al*. (2009) had evaluated *P. fluorescens* under FCV tobacco nursery conditions against root knot nematodes and obtained reduced incidence of root knot index and final soil nematode population and also subsequent increase in root knot free and healthy transplants count of FCV tobacco seedlings. It is concluded from the study that the schedule involving *P. fluorescens* @1g/plant + *A. niger* enriched FYM@100g/plant at planting resulted in cost effective management of Fusarium wilt – root-knot disease complex with a better ICBR of 1:4.4 (Table 3).

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