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Field evaluation of promising fungicides and bioagents against Fusarium wilt and root knot complex disease in FCV tobacco crop

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Fusarium wilt and root knot are two important diseases of tobacco in Hunsur tract of Karnataka, India. Both diseases also form disease complex and are known to cause severe damage to the crop. The present investigation was carried out under *in vitro* and *in vivo* conditions to find out the possibility of using ecofriendly measures to manage the disease complex. Fungal and bacterial bioagents *viz*., *Trichoderma viride* , *Trichoderma harzianum*, *Bacillus subtilis, Pseudomonas fluorescens* were evaluated along with nine chemical fungicides such as Carbendazim, Copper hydroxide, Propiconazole, Difenoconazole, Thiophanate methyl, Mancozeb, Tridemorph, Metalaxyl and Triadimefon. The bioagents *viz*., *T. viride, P. fluorescens* and chemical fungicides like Carbendazim, Thiophanate methyl, Mancozeb, Difenoconazole and Propiconazole were capable of inhibiting the mycelial growth of wilt pathogen *in vitro* conditions. The effective chemical fungicides and biocontrol agents were evaluated during 2009 to 2011 under *in vivo* conditions. The pooled analysis from two years of comparative data between chemicals and bioagents indicated that among chemicals Carbendazim and Propiconazole were found to be very effective in controlling the wilt to an extent of 61.47 and 60.29% respectively. *T. viride* and *P. fluorescens* in talc formulations controlled the wilt disease to an extent of 58.46% and 60.15 % respectively. Neem cake formulation of *T. viride* was found to be more promising than talc formulation and affected 60.9% control over check. Among chemicals Carbendazim and Propiconazole treatments resulted in increased yield of total cured leaf to an extent of 24.53 and 31.77% respectively. Similarly the bright grade leaf yield also increased by 18.59 and 34.98% respectively. Among bioagents *T. viride*-neem cake formulation, *T. viride* and *P. fluorescens* talc formulation treatments resulted in increase in yield of total cured leaf to an extent of 34.26 %, 29.97% and 26.88% respectively. This is the first report on the neem cake formulation of *T. viride* successfully controlling tobacco wilt pathogen. Similarly the bright grade leaf yield also increased by 40.56%, 26.85% and 36.51% respectively over untreated check. Propiconazole was found to be the most promising fungicide and *T. viride* in neem cake formulation, the most reliable bioagent. The management strategy gave an Incremental Cost Benefit Ratio (ICBR) of 1: 7.35 and 1: 7.38 in chemical and bioagent respectively. The root-knot complex incidence

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was evaluated by observing root-knot symptom in the wilt affected plants. Sodium tetra thiocarbamate and talc formulation of *P. fluorescens* effected 49% and 52% control of root-knot incidence over check respectively.

Key words: Chemical fungicides, biocontrol agents, *Trichoderma viride*, *Pseudomonas fluorescens*, neem cake and talc formulations

Introduction

Flue-Cured Virginia (FCV) tobacco is a major rain fed commercial crop cultivated in the transitional belt between the eastern slopes of Western Ghats and the plains in Karnataka under monsoon conditions. Among several diseases, wilt caused by *Fusarium oxysporum* f. sp. *nicotianae* and wilt-root knot complex caused by *Fusarium oxysporum* f. sp. *nicotianae* and *Meloidogyne incognita* are the most devastating diseases resulting in loss in yield and quality of cured tobacco (Shenoi and Sreenivas, 2007). In view of economic importance of FCV tobacco it is imperative that a coherent and comprehensive control measures be meticulously undertaken. This can be achieved by devising the management strategies either through chemical or biocontrol agents. Biocontrol strategy has become the alternative method in recent years due to the indiscriminate and frequent use of chemical agents and its ill effects to soil and environment (Anand and Goutham, 2006; Bohra *et al.,* 2006; Mukherjee and Tripathi, 2000; Mukhtar, 2007; Seema and Devaki, 2012). Several antagonistic organisms have been used successfully as biocontrol agents for controlling soil borne pathogens (Bhaskaran *et al*., 1988; Chen *et al.,* 1995; Deacon, 1991). Soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma*, *Bacillus subtilis, Pseudomonas fluorescence* (Anitha and Dass, 2011; Chet, 1990; Gary *et al.,* 2003; Weller, 1988; David, 2007). Currently, cost-effective fungicide is also not available that gives guaranteed control against wilt-root knot complex disease.

Thus, considering the seriousness of the loss and ineffectiveness of the available chemicals to control the disease, the present investigation was undertaken to evaluate some promising fungicides and formulations (neem cake and talc) of bioagents against wilt-root knot complex in FVC tobacco crop.

Materials and methods

Maintenance and evaluation of biocontrol agents in vitro

T. viride was obtained from Division of Plant Pathology, Ghandhi Krishi Vijnan Kendra (GKVK) Bangalore. *P. fluorescens* (13525) were obtained from ATCC. In the present study *T. viride* and *P. fluorescens* were selected for field evaluation. The pure cultures were maintained in *Trichoderma* selective medium (Elad and Chet, 1983) and *Pseudomonas* selective medium (King *et al.,* 1954) at 4°C. Dual culture technique by inoculating the pathogen and each bioagent in a single petri plate was adopted to evaluate the efficacy of these bioagents.

Development of formulations of the antagonists for field application Trichodermaviride talc formulation

Mycelial disc (0.5cm) of actively growing *T. viride* was transferred to 100 ml of molasses yeast medium in 250 ml conical flasks and incubated at room temperature for 14 days. Five grams of Carboxy Methyl Cellulose (CMC) was added to 1 kg of talc and mixed well. The mycelial mat was homogenized and blended with talc powder at 1:2 ratio. The materials were shade dried and packed in polypropylene bags, heat sealed and kept at room temperature (Ramakrishnan *et al.* 1994; Thiruvudainambi *et al.*2010).

Pseudomonas fluorescens talc formulation

A loop full of *P. fluorescens* was transferred into King's B broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature. After 48 h of incubation the broth containing 9 x 10^8cfu/ml was used for the preparation of talc based formulation. 400 ml of bacterial suspension, 1 kg of the talc powder, calcium carbonate 15 g (to adjust pH to neutral) and CMC 10 gm (as adhesive) were mixed under sterile condition. The materials were dried and packed in polypropylene bags, heat sealed and kept at room temperature (Vidhyasekaran and Muthamilan, 1999).

Trichodermavirideneem cake formulation

The mycelial mat of *T. viride* was homogenized and mixed 10 g with 100 g decomposed neem cake. The preparations were dried and packed in polypropylene bags, heat sealed and kept at room temperature until use (Rini and Sulochana, 2007).

In vivo screening of fungicides, fungal and bacterial antagonists

The study was conducted at Central Tobacco Research Institute, Research station, Hunsur during 2009 to 2011 in wilt and root knot pathogen infested field. The experiments were performed in Randomized Completely Block Design (RCBD) with 9 treatments with replications using the tobacco cultivar Kanchan. The plot size was 48 m^2, gross size 20 m^2 and net size 68 m^2. The chemical agents were evaluated at 0.05 to 0.2% as plant hole + drench around the plant at 60 and 70 days after transplant (DAT). The chemical mix were applied at 200 ml/plant, bioagents were applied at 1 g/plant and both the formulations were compared with currently recommended schedule involving carbendazim 0.2% as plant hole + drench around the plant. Tobacco crop was raised following all recommended agronomic practices (Shenoi and Sreenivas, 2007). Disease spread at regular intervals and leaf yield parameters were studied.

Disease symptom investigation

Wilt disease symptom were recorded on 60 DAT and 70 DAT. On 70 DAT, 20% of the wilted plants were uprooted randomly in all the experimental fields and these plants were evaluated for root-knot incidence.

Leaf picking and grading

Leaves were picked from the field grown in treated and control plants at 90 DAT and the fresh green leaves were weighed and later cured as per the standard procedure (Shenoi and Sreenivas, 2007) and were graded as bright, medium and low based on the decreasing order of quality.

Results and discussions

In vitro studies

The antagonists tested *in vitro* conditions revealed the suppression of the pathogen to some extent. *T. viride* and *P. fluorescens* were found to be very effective in controlling the wilt pathogen (Sumana *et al.,* 2012) and these two bioagents are selected for *in vivo* screening.

In vivo screening of fungicides, fungal and bacterial antagonists

All chemicals and bioagents tested were found to be significant in controlling the wilt disease. The data of 2009 and 2011 were pooled and the

pooled analysis of data (Table 1) suggested that among the chemical formulation, propiconazole was found to be most effective at lower doses of 0.05% when given as plant hole application during transplantation. It recorded 60.29% control over check and leaf yield was recorded to be 12693, 909, 398, 173 and 1479 kg/ha of green leaves, bright grade, medium grade, low grade and total cured leaf respectively. It resulted with 1:7.35 of an Incremental Cost Benefit Ratio (ICBR) (Table 2). Treatments involving plant hole application of Propiconazole 0.05 %+drench around the plant 0.05% at 60 and 70 DAT were identified as the best schedules for the control of *Fusarium oxysporum* f. sp *nicotianae*.

Table 1. Effect of chemicals and bioagents on the incidence of wilt and root-knot complex (pooled data of 2010 & 2011)

Treatments	Wilt and root-knot incidence					Leaf yield (kg/ha)					
	60 DAT*	% wilt control over check	70 DAT	% wilt control over check	% Root-knot control over check	Greens (%)	Bright grade (%)	Medium grade (%)	Low grade (%)	Total cured (%)	Incremental Cost Benefit Ratio (ICBR)
Carbendazim (0.2%)	22.31 (28.17)	57.89	22.44 (28.27)	61.47	13.8	12580 [28.76]	726 [18.59]	409 [38.63]	202 [17.32]	1337 [24.53]	1: 3.92
Copper hydroxide (0.2%)	22.92 (28.59)	56.74	27.90 (31.87)	52.09	5.6	12041 [25.57]	903 [34.55]	334 [24.85]	209 [20.09]	1446 [30.22]	1: 5.0
Propiconazole (0.05%)	20.16 (26.67)	61.94	23.13 (28.73)	60.29	10.7	12693 [29.40]	909 [34.98]	398 [36.93]	173 [3.46]	1479 [31.77]	1: 7.35
Thiophanate methyl (0.2%)	24.14 (29.42)	54.44	25.62 (30.40)	56.00	17.1	10229 [12.39]	706 [16.28]	321 [21.80]	178 [6.17]	1205 [16.26]	1: 0.88
Sodium tetra Thiocarbamate (0.2%)	34.17 (35.76)	35.500	35.57 (36.60)	38.93	49.0	8908 [-0.59]	750 [21.20]	376 [33.24]	226 [26.10]	1352 [25.36]	1: 4.01
T. viridae (talc formulation) (1gm/plant)	23.93 (29.27)	54.83	24.19 (29.45)	58.46	36.4	9723 [7.83]	808 [26.85]	407 [38.32]	226 [26.10]	1441 [29.97]	1: 7.08
T. viridae (neem cake formulation) (1gm/plant)	22.39 (28.23)	57.74	22.77 (28.49)	60.90	45.2	10413 [13.94]	927 [40.56]	410 [38.78]	199 [16.08]	1535 [34.26]	1: 7.38
P. fluorescens (talc formulation) (1gm/plant)	22.84 (28.54)	56.89	23.21 (28.79)	60.15	52.0	10501 [14.66]	931 [36.51]	321 [21.80]	128 [-30.46]	1380 [26.88]	1: 7.16
Check	52.98 (46.69)	NS*	58.24 (49.72)	NS*	---	8961 [0.0]	591 [0.0]	251 [0.0]	167 [0.0]	1009 [0.0]	---
SEM	0.05	---	0.15	---	---	5.78	4.68	1.40	2.00	3.51	---
CD (0.05)	0.14	---	0.43	---	---	19.97	12.97	3.87	5.53	9.72	---

*Figures in parenthesis are arc sine transformed values; [ ] represents % leaf yield; NS = Not significant
*DAT-Days after transplant

Table 2. Cost Economics of Fusarium wilt management through chemical and biocontrol agents in FCV tobacco crop (Kg /ha)

Particulars	Carbendazim	Kocide	Tilt	Thiophanate methyl	Suzone	T. viride (talc)	T. viride (Neem)	P. fluorescens (talc)	Check
Cost of Cultivation (₹)	48,750/-	48,750/-	48,750/-	48,750/-	48,750/-	48,750/-	48,750/-	48,750/-	48,750/-
Cost of crop protection for wilt management (₹)	8,540/-	8,600/-	6,540/-	18,140/-	8,540/-	6,100/-	6,900/-	6,100/-	---
Yield (Kg/ha)	1337.16	1446.33	1479.34	1204.5	1351.5	1440.66	1535.34	1379.50	1009.00
Bright grade (Kg/ha)	725.50 (57,314.5)*	902.83 (71,323.57)*	909.00 (71811.0)*	706.17 (55,787.43)*	750.00 (59250.0)*	808.00 (63,832.0)*	926.67 (73,206.93)*	930.83 (73,535.57)*	591.17 (46,702.43)*
Medium grade (Kg/ha)	409.33 (25,378.46)*	334.17 (20,718.54)*	397.67 (24,655.54)*	320.50 (19871.0)*	376.00 (23312.0)*	406.83 (25,223.46)*	410.00 (25,420.0)*	320.50 (19871.0)*	250.83 (15,551.46)*
Low grade (Kg/ha)	202.33 (8,093.2)*	209.33 (8,373.2)*	172.67 (6,906.8)*	177.83 (7,113.2)*	225.50 (9020.0)*	225.83 (9,033.2)*	198.67 (7,946.8)*	128.17 (5,126.8)*	167.00 (6,680.0)*
Gross returns (₹)	1,337.16 (90786.16)*	1,446.33 (100415.31)*	1479.34 (103373.34)*	1204.5 (82771.63)*	1351.5 (91582.0)*	1440.66 (98088.66)*	1535.34 (106573.73)*	1379.5 (98533.37)*	1009.0 (68933.89)*
Net profit (₹)	33496.16	43065.31	48083.34	15881.63	34292.0	43238.66	50923.73	43683.37	20183.89
Incremental Cost Benefit Ratio (ICBR)	1:3.92	1:5.00	1:7.35	1:0.88	1:4.01	1:7.08	1:7.38	1:7.16	---

Cost of chemical and biocontrol agents per Kg: Carbendazim = ₹ 460/-; Kocide = ₹ 480/-; Tilt = ₹ 1260/-; Thiophanate methyl = ₹ 1040/-; Suzone = ₹ 460/-; *Trichodermaviride* (talc) = ₹ 110/-; *Trichodermaviride* (neem) = ₹ 130/-; *Pseudomonas fluorescens* = ₹ 110/-

Market price of tobacco on average ₹ (Bright grade = 79/- ; Medium grade = 62/- ; Low grade = 40/-)
Volume of chemical at plant hole/drench around the plant = 200ml/plant; biocontrol agents = 1gm/plant
*Figures in parenthesis are amount released at the market

Neem cake formulation of the bioagent performed which compared to talc formulation. Plant hole application of *T. viride* neem cake formulation 1g/plant at 60 and 70 DAT was found to be more superior which exhibited 60.9 % control over check. The leaf yield was found to be 10413, 928, 410, 199 and 1535 of green leaves, bright grade, medium grade, low grade and total cured leaf respectively. It recorded an Incremental Cost Benefit Ratio (ICBR) of 1:7.38. Results demonstrated that the application of bioagents increased the leaf yield with least percentage of disease incidence which in accordance with Al-Jedabi (2009). *T. viride* in neem cake formulation was identified as promising for the control of wilt disease. The results were consistent with the findings of Thiruvudainambi *et al.* (2010) Vimala *et al.* (2009) who showed the efficacy of *T. viride* neem cake formulation on *Sclerotium rolfsii*, *Macrophomina phaseolina* respectively. This is the first report on the neem cake formulation of *T. viride* successfully controlling tobacco wilt pathogen. Earlier researchers like Thiruvudainambi (2010) have been used neem cake and applied directly to the field along with the talc formulations of the bioagent. However, well decomposed neem cake is used to make the formulation and this type of applications to control wilt disease is the first report. Our studies conducted

earlier also showed well decomposed neem cake has no inhibitory effect on the bioagent.

Chemicals and bioagents were also evaluated for their efficiency in controlling the root knot, which can be exploited further to formulate the wilt-root knot disease resistant strategies for different soil borne diseases of plants. Among the promising chemicals and bioagents, Sodium tetra thiocarbamate (0.2%) recorded 49.0% and *P. fluorescens* recorded 52% root-knot control over check. Efficacy of *P. fluorescens* on *M. incognita* has been established earlier by Hanna *et al.* (1999) in tomato, Jonathan *et al.* (2000) in betel vine, Becker *et al.,* (1988) in clover plants, Rajendran *et al.* (2001) in horticultural crops like citrus, potato, chilli and Khan *et al.* (2001) in chick pea. *P. fluorescens* was found to be effective in controlling wilt pathogen by 60.15% and root-knot by 52.0%. Thus *P. fluorescens* can be considered as an effective bioagent for managing wilt root-knot complex.

It is concluded that the application of *T. viride* neem cake formulation (22 $x10^7$ cfu/g) and *P. fluorescens* talc formulation (9 x 10^8 cfu/ml) at 1 g per plant at 60 and 70 DAT were the best schedules for effectively managing Fusarium wilt and wilt-root knot complex disease incidence in an ecofriendly way.

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