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DEVELOPMENT OF INTEGRATED BIOCONTROL STRATEGY FOR THE MANAGEMENT OF STEM ROT DISEASE (*FUSARIUM OXYSPORUM* F.SP. *VANILLAE*) OF VANILLA

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ABSTRACT

Stem rot disease of vanilla caused by *Fusarium oxysporum* f.sp. *vanillae* is a major constraint in its cultivation in Kanyakumari District. The *in vitro* studies on the mycelial growth of the pathogen expressed the superior efficacy of the native isolate of *Pseudomonas fluorescens* (Pf_{NI}) that recorded an inhibition zone of 25.00mm and was followed by *T. harzianum*. Under field conditions soil application of $Pf_{(NI)}$ followed by carbendazim spray (0.2%) after 30 days of *Pseudomonas* application recorded the lowest disease incidence of 3.77, 4.03 and 3.67 per cent in three years and exerted 79.95, 80.50, 80.48 per cent reduction in disease over the control plot respectively. Among the bioagents, the soil application of $Pf_{(NI)}$ followed *T. harzianum* after 30 days and another dose of $Pf_{(NI)}$ after 30 days recorded a significant disease reduction of 69.84, 68.24 and 70.21 per cent in three consecutive years.

Vanilla (*Vanilla planifolia*) cultivated for its aromatic beans occupies a significant position in the spice economy of the country. In Kanyakumari district the crop is infected by number of fungal pathogens that poses an impediment to achieve the higher yield target. Stem rot disease caused by the fungus, *Fusarium oxysporum* f.sp. *vanillae* is the major disease causing severe reduction in plant stand and yield. Symptoms appear in the form of water soaked lesions extending to both sides of the stem giving a brown coloured appearance. Later, enlarged patches develop on the stem which, results in rotting of the stem tissues and severe loss in yield. Hence the present study was carried out to develop an effective management strategy to combat this serious disease.

***In vitro* efficacy of biocontrol agents :** The selected bioagents listed in Table 1 were tested for their efficacy in arresting the mycelial growth of the pathogen by following dual culture technique.

Dual culture technique : A nine mm actively growing culture disc of the fungus was placed onto the sterilized Petri dish containing solidified PDA medium approximately at a distance of 1.5 cm away from the edge of the plate. In the same way a nine mm culture disc of the purified antagonistic fungus was placed onto the medium at the opposite side of the plate at 1.5 cm away from the edge of the plate. For the bacterial antagonists, the fungal pathogen alone was inoculated and incubated at room temperature (28± 2° C) for three days. Then the actively growing 48 h old cultures of the antagonistic bacteria were separately streaked onto the medium at the opposite side of the plate at 1.5 cm away from the edge of the plate. PDA medium inoculated with the pathogen alone served as control. The treatments were replicated thrice in completely randomized block design. The radial growth of the pathogen was measured after 7 days of incubation. The results were expressed as per cent inhibition of the mycelial growth (I) of the pathogen over the control.

$$I = 100 (C-T)/C$$

I - per cent inhibition of the mycelial growth,
C - growth in control, T - growth in treatment

Field efficacy of bioagents against *F. oxysporum* f.sp. *vanillae* : The effective bioagents under *in vitro* condition were further tested under field condition for successive three years (Table 2). The treatments were replicated thrice in randomized block design.

Table 1. *In vitro* efficacy of antagonists against growth of stem rot pathogen

Treatment	Inhibition zone (mm)
Chaetomium globosum	2.33[d]
Gliocladium virens	11.00[c]
Trichoderma viride	15.67[b]
T. harzianum	17.33[b]
Pseudomonas fluorescens (Pf_1)	14.67[b]
Fluorescent Pseudomonas ($Pf_{(NI)}$)	25.00[a]
Bacillus subtilis	8.67[c]
Control (Sterile water)	0.00[e]
CD (P=0.05)	0.41

The results revealed the superior efficacy of the native isolate of *P. fluorescens* (Pf_{NI}) that recorded the inhibition zone of 25.00mm and 17.33mm in *T. harzianum* (Table 1). Antimicrobial chemicals produced by the bioagents attributed for the growth inhibition of the pathogen.

The effective bioagents $Pf_{(NI)}$ and *T. harzianum* were tested for their efficacy under field condition along with the soil amendments *viz.*, neem cake, vermicompost and

Table 2. Integrated biocontrol methods against the stem rot disease of Vanilla

Treatment	Disease Incidence (%)						Green Beans Yield (Kg/ha)	Cost : benefit ratio
	2004		2005		2006			
	D.I. (%)	% DC	D.I. (%)	% DC	D.I. (%)	% DC		
Soil application of *Trichoderma harzianum* three times	10.76[h]	42.77	10.73[f]	42.93	11.00[g]	41.49	1486	1: 3.07
Soil application of *T. harzianum* two times + vermicompost	8.83[fg]	53.03	8.66[de]	53.94	8.53[e]	54.63	1452	1: 2.95
Soil application of *T. harzianum* two times + neem cake	9.50[g]	49.47	9.71[ef]	48.35	9.53[f]	49.31	1450	1:2.93
Soil application of Pf^{-}_{NI} three times	7.47[d]	60.27	7.70[d]	59.04	7.40[d]	60.64	1500	1:3.09
Soil application of Pf^{-}_{NI} two times + vermicompost	5.83[c]	68.99	6.33[c]	66.33	5.73[c]	69.52	1541	1:3.13
Soil application of Pf^{-}_{NI} two times + neem cake	7.57[de]	59.73	7.77[d]	58.67	7.86[de]	58.19	1478	1: 2.98
Soil application of Pf^{-}_{NI} - *T. harzianum*- Pf^{-}_{NI}	5.67[c]	69.84	5.97[c]	68.24	5.60[c]	70.21	1550	1: 3.20
Soil application of *T. harzianum*- Pf^{-}_{NI} - *T. harzianum*	8.37[ef]	55.48	8.30[d]	55.85	8.53[e]	54.63	1522	1: 3.14
Soil application of *T. harzianum*- Carbendazim (0.2%)	4.60[b]	75.53	4.93[b]	73.78	4.70[b]	75.00	1334	1: 2.76
Soil application of Pf^{-}_{NI} - Carbendazim (0.2%)	3.77[a]	79.95	4.03[a]	80.50	3.67[a]	80.48	1400	1: 2.89
Control	18.8[i]	-	20.67[g]	-	18.63[h]	-	1000	1: 2.13
CD (P=0.05)	0.94	-	1.12	-	0.76	-	-	-

(Note - Treatments were applied at 30 days interval)

carbendazim. The treatments were tested consecutively in the field for three years (2004 – 2006) in randomized block design by maintaining three replications (Table 2). Soil application of $Pf_{(NI)}$ followed by carbendazim spray (0.2%) after 30 days of *Pseudomonas* application recorded the lowest disease incidence of 3.77, 4.03 and 3.67 per cent in three years and exerted 79.95, 80.50, 80.48 per cent reduction in disease over the control plot respectively. Among the bioagents, the soil application of $Pf_{(NI)}$ followed by *T. harzianum* after 30 days and another dose of *Pseudomonas* after 30 days recorded significant disease reduction of 69.84, 68.24 and 70.21 per cent with highest CBR of 1:3.20 in three consecutive years. Similar findings were reported by Sivamani and Gnanamanickam (1988) wherein *P. fluorescens* reduced the wilting and internal colonization due to *F. oxysporum* f.sp. *cubense* in banana.

These findings are in support of Furuya *et al*. (1992) that the production of several antibiotics *viz*., pyrrolnitrin, pyoluteorin, phenazine and 2,4-di acetyl phloroglucinol by *P. fluorescens* for their inhibitory activity against the pathogen. Phloroglucinol producing strains of *P. fluorescens* have been shown to be effective against root pathogens *viz*., *Pythium ultimum*, *Thielaviopsis basicola*, *Gaumanomyces graminis* var.*tritici* in wheat as evidenced by Maurhofer *et al*. (1995). Besides this, phloroglucinol induced defense mechanism against the fungal infection. The metabolites phenazine and pyrrolnitrin also had antifungal effect. Saravanan *et al*., (2004) also reported that the several mechanisms *viz*., production of siderophores, rhizhosphere colonizing ability, production of antibiotics and induced systemic resistance have been identified in the disease suppression by various strains of *P. fluorescens* against fusarial infection.

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