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BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *VANILLAE*, THE CASUAL AGENT OF STEM ROT OF VANILLA IN VITRO

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ABSTRACT
The aim of this study was to evaluate the ability biocontrol agents in suppressing the growth of *Fusarium oxysporum* f. sp. *vanillae* causing stem rot in Vanilla *in* vitro by employing dual culture technique. *Trichoderma harzianum, Pseudomonas fluorescens and Bacillus subtilis inhibited the growth of pathogen.* In nature microbial interactions involve competition, hyper parasitism or antibiosis and these phenomena play an important role in striking ecological balance and keeping several plant pathogens in check. In recent times biological control of plant pathogenic fungi has received a considerable attention, as it has several advantages such as possibility of multiple pathogen suppression, low cost and promotion of soil fertility.

KEY WORDS*:* Vanilla, biological control, F. oxysporum, antibiosis.

INTRODUCTION

In nature microbial interactions involve competition, hyper parasitism or antibiosis and these phenomena play an important role in striking ecological balance and keeping several plant pathogens in check. In recent times biological control of plant pathogenic fungi has received a considerable attention, as it has several advantages such as possibility of multiple pathogen suppression, low cost and promotion of soil fertility.

Among the few pathogens causing diseases in vanilla, *Fusarium oxysporum* f.sp. *vanillae* is the most important pathogen becoming a serious constraint in the successful cultivation of vanilla. Field control of these diseases was possible to a limited extent, with the help of bio-control agents and fungicides alone. These methods had not given satisfactory results, so there is an urgent need to formulate integrated disease management module. These includes introduction of antagonistic microorganisms into field especially *Trichoderma* spp. (Papavizas, 1984) and bacterial antagonists (Campbell and Faull, 1979); application of organic amendments to soil to stimulate resident antagonist and chemical control methods that help enormously in enhancing crop growth and disease management. (Joseph Thomas and Susheela Bhai, 2000).

In vanilla, stem rot is a serious problem throughout the year. Now a days majority of vanilla growers never want to use chemicals for the disease management even if disease is at its peak because chemical treated beans fetch low price. Biological control offers potential alternatives to combat many soil-borne pathogens (Spadaro and Gullino, 2005). Several reports have previously demonstrated the successful use of biological control agents against Fusarium diseases of various crops. Several biocontrol bacteria, including Pseudomonas spp., Serratia marcescens, Bacillus sp., Streptomyces sp. have been used to control Fusarium wilt diseases (Scher and Baker, 1982). Moreover, use of Trichoderma spp. on banana (Thangavelu et al., 2003) have also been reported .Keeping this in view, an attempt was made to find out efficacy of a fungal (*Trichoderma harzianum*) and two bacterial antagonists (*Pseudomonas fluorescens* and *Bacillus subtilis*) on mycelial growth of *F. oxysporum* f. sp. *vanillae* causing stem rot of Vanilla under lab conditions.

MATERIALS AND METHODS

Isolation of the pathogen from stem rot infected plants

Direct tissue isolation technique was followed for the isolation of pathogen from Vanilla vines showing typical stem rot symptoms. The samples were collected from different places and were utilized for isolation. The most common method for isolating the pathogen from infected stem involves disinfecting the outer surface by wiping with 10 per cent house hold bleach, followed by cutting of infected stem into several small sections of 0.5 to 1cm length, then these sections are washed in three changes of sterile water and are finally placed on potato dextrose agar medium usually 2 to 3 sections per dish and were incubated at $28^0 \pm 1^0$C temperature.

Purification of *Fusarium oxysporum* f.sp. *vanillae* isolates

Spore suspensions of *F. oxysoporum* f.sp. *vanillae* isolates were made in sterile distilled water in test tubes and two milliliter of dilute spore suspension was added to two per cent water agar in sterilized Petri plates. The Petri plates were rotated well for uniform spread of the spores on the medium. After 24 hours, the plates were viewed under low power objective of the microscope by keeping the plate in inverted position to locate well isolated and germinated single spores. Such spores were marked with marker pen by viewing through the microscope. Finally the single spore was picked up along with agar medium, and transferred on to the PDA slants under aseptic conditions and and these slants were utilized for subsequent studies.

Maintenance of pure cultures

The slants containing *F. oxysoporum* f.sp. *vanillae* isolates were stored in a deep freezer at 5^0C for further

investigations and were sub cultured at regular intervals during the course of investigation.

Evaluation of biocontrol agents against *Fusarium oxysporum* f. sp. *vanillae* Isolates *in vitro*

The bioagents *viz*., *Pseudomonas. fluorescens* and *B. subtilis* were obtained from Department of Agricultural microbiology, UAS, Bangalore and *T.harzianum* was isolated from the rhizosphere of wilted plants using TSM medium to study their bio-efficacy against F. *oxysporum* f.sp. *vanillae in vitro* as well as during poly house and field studies.

The above mentioned fungal and bacterial bioagents were evaluated for their antagonistic effect against *F. oxysporum* f.sp. *vanillae* by dual culture method (Dennis and Webster, 1971) on PDA medium *in-vitro*.

Around 15-20ml of PDA medium was poured into sterile Petri plates and allowed for solidification. Seven days old 5 mm disc of *F. oxysporum* f.sp. *vanillae* was cut near the periphery of the colony with the help of sterile cork borer and placed on one side of the PDA plate. Similarly, *T. harzianum* was placed on other side of PDA plate and both the pathogen and bioagent were kept at a distance of 2cm at an angle of 180^{o}. Plates without antagonists were maintained as control. All inoculated plates were incubated at $27^{o} \pm 1^{0}$C for seven days and each treatment was replicated five times and the data was statistically analyzed.

In another set of experiment *B. subtilis* and *P. fluorescens* were tried as antagonistic organisms as described earlier and the per cent inhibition of the colony over control was calculated by using the formula given by Vincent (1927).

TABLE 1: Evaluation of biocontrol agents on *Fusarium oxysporum* f.sp. *vanillae* isolates *in vitro*.

Sl. No.	Bio agents	Isolates	Mean Radial growth (mm)	Percent Inhibition (PI)	Mean (PI)
1	*Trichoderma harzianum*	Fov-1	10.70	82.83	
		Fov-2	12.10	80.58	
		Fov-3	05.92	90.50	83.63
		Fov-4	10.13	83.74	
		Fov-5	12.86	74.11	
		Fov-6	06.21	90.03	
2	*Pseudomonas*	Fov-1	14.25	77.13	
	fluorescens	Fov-2	18.66	70.05	
		Fov-3	15.32	75.41	76.80
		Fov-4	12.78	79.49	
		Fov-5	12.44	80.03	
		Fov-6	13.28	78.69	
3	*Bacillus subtilis*	Fov-1	16.21	73.98	
		Fov-2	17.11	72.54	
		Fov-3	17.45	71.99	72.70
		Fov-4	16.24	73.94	
		Fov-5	19.12	69.31	
		Fov-6	15.92	74.45	
	Control	-	62.32	-	

RESULTS AND DISCUSSION

In vitro evaluation of the antagonist's *viz., Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *vanillae* isolates showed strong antagonistic activity in dual plate culture method (Table 1 and Plate 1). Among the antagonists tested, *T. harzianum* effectively reduced the mean radial growth of all the isolates of *F. oxyspoum* f. sp. *vanillae*. *T. harzianum* showed highest inhibition of 90.50 per cent against isolate Fov-3 followed by Fov-6 (90.03%) and Fov-4 (83.74%). Least per cent inhibition of (74.11%) was observed with isolate Fov-5 with a mean per cent inhibition of 83.63 per cent. Bacterial bio-control agent's *viz., P. fluorescens* and *b. subtilis* both reduced the growth of all the isolates drastically compared to control. *P. fluorescens* showed 80.03 per cent inhibition of isolate Fov-5 followed by Fov-6 (78.69%) and Fov-4 (79.49%). However, least per cent inhibition was recorded with isolate Fov-2 (70.05%) with a mean per cent inhibition of 76.80 per cent. *B. subtilis* showed highest per cent inhibition of 74.45 per cent against isolate Fov-6 followed by Fov-1 (73.98%). However, the least per cent inhibition was recorded with isolate Fov-5 by 69.31 per cent with a mean per cent inhibition of 72.70 per cent. Present investigation studies on the usage of biocontrol agents for the management of *F. oxysporum f.sp.vanillae* revealed that, all the three biocontrol agents viz., *Trichoderma harzianum, Psuedomonas fluorescens* and *Bacillus subtilis* were significantly reduced the growth of *F. oxysporum* f. sp. *vanillae in vitro,* pot as well as under field condition.

However, among the biocontrol agents *Trichoderma harzianum* found to be most efficient in reducing the

disease incidence.These results are in confirmation with the reports of several workers who reported the inhibitory effect of volatile compounds produced by *Trichoderma* spp. on several soil borne pathogens (Dennis and Webster, 1971; Bell et.al. 1982; Chet et.al. 1979; Hutchinson and Cowan, 1972 and Upadhyay and Mukhopadhyay, 1993). The antagonistic effect of *T. hamatum* and *Gliocladium virens* against *F. oxysporum* f. sp. *dianthi* was reported by Anandraj *et.al.* (1995). whereas antagonistic nature of *P. fluorescens* against *F.oxysporum* f. sp. *cubense* was reported by Scher and Baker, (1982) Kenny and Couch, (1981) Shivamani and Gnanamanicum, (1988).

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