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Effect of various bio control agents and plant extracts on the growth of *Fusarium oxysporum* f. sp. *ubense* (Foc) causing *Fusarium* wilt disease in banana

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Abstract

Banana is considered as an important fruit crop in the livelihood of many people in tropical and subtropical countries. However, the banana crop is threatened by Fusarium wilt incited by the pathogen *Fusarium oxysporum* f. sp. *ubense*, a soil borne fungus. The disease has a destructive effect on the crop and causes up to 80 per cent of yield loss. In the present study, an *in vitro* experiment was conducted for the evaluation of various biocontrol agents and botanicals at lower, recommended and higher doses by poisoned food for control of the pathogen. The biocontrol agents PGPR Mix II, PGPM, *Trichoderma viride* and *Pseudomonas fluorescens* showed 100 per cent inhibition of the pathogen. Among the plant extracts used, highest (39.44 %) per cent inhibition of the pathogen was obtained with extract of *Myristica fragrans* mace followed by *Azadirachta indica* oil (26.22 %) and *Lawsonia inermis* leaves (25.33 %). The lowest per cent of inhibition of the pathogen was noticed in the treatment *Curcuma caesia* (9.33 cm). Biocontrol agents can be effectively used for the management of Fusarium wilt disease in banana.

Keywords: *Fusarium oxysporum* f. sp. *ubense* (Foc), Fusarium wilt, bio control agents, plant extracts, management

1. Introduction

Banana is one of the most important fruit crops in the world. However, its cultivation is constrained by the incidence of various pest and diseases. Among the diseases, Fusarium wilt of banana which is commonly known as Panama wilt disease, caused by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *ubense* (Foc) is the most destructive disease of banana [1]. Fusarium wilt of banana is a typical vascular disease and pathogen enters the plant through water conducting xylem vessels; thus, causes the blockage of water and results in wilted appearance [2]. It results in discolouration of the vascular strands from pale yellow initially to dark brown or black in the advanced stage [3].

Now-a-days, biological control of fusarium wilt diseases using certain microorganisms has been getting more popularity as it is an eco-friendly management strategy compared to the indiscriminate use of chemical fungicides [4, 5].

The medium that can be used for the better growth of *Fusarium oxysporum* was half strength potato dextrose agar consisting of 100 g peeled and diced potatoes, 10 g dextrose and 20 g agar per liter of distilled water [6]. *F. oxysporum* f. sp. *ubense* was isolated from strands of infected banana plants collected from southern parts of India and maintained on PDA [7].

The effect of 110 isolates of *Trichoderma viridae* isolated from rhizosphere of banana and found that thirty-one isolates were effective in the initial screening tests conducted *in vitro* against banana wilt pathogen *F. oxysporum* f. sp. *ubense* [8]. Different strains of *Trichoderma harzianum* were used to evaluate the effect on *F. oxysporum* f. sp. *ubense* *in vitro* using dual culture and volatile organic compounds production test [9]. They reported that all the strains inhibited Foc growth at various level and produced toxic metabolites.

Soil application of *Pseudomonas fluorescens* was the most effective among the various methods *viz.*, corm injection, sucker dipping and capsule application tried for the management of Fusarium wilt of banana in the field experiment [10]. Bio agents such as *T. harzianum*, *T. viride*, *P. fluorescens* and *Bacillus subtilis* along with organic amendments against *F. oxysporum* f. sp. *ubense* causing wilt disease in banana was found to be very effective [11]. *T. harzianum* and *P. fluorescens* were found to be significantly better in the presence of farm yard manure or mustard cake.

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Plant growth promoting rhizobacteria (PGPR) like *P. fluorescens* and *Serratia marcescens* induces systemic resistance in cucumber against Fusarium wilt caused by *F. oxysporum* f. sp. *cucumerianum* [12]. The effect of Arbuscular Mycorrhizal Fungi (AMF) and *Trichoderma harzianum* against *F. oxysporum* f. sp. *cubense* on 'Cavendish' banana plantlets has also studied [13]. The plantlets treated with 70 g of VAM + 50 g of *T. harzianum* reduced *Foc* development in terms of disease symptom and disease incidence.

Leaf extracts and bio-control agents were tested against *F. oxysporum* f. sp. *cubense* [14]. They reported that, leaf extract of *Datura* (10%) inhibited the mycelial growth completely *in vitro*. Under field conditions, combined application of leaf extract along with biocontrol agents like PGPR controlled the wilt incidence significantly. Four types of botanicals form of *Azadirachta indica* leaves, *Moringa oleifera* oil, *Withania somnifera* roots and *Tithonia diversifolia* leaves to control the fungal isolates of *F. oxysporum* f. sp. *elaeidis* causing vascular wilt in oil palm were collected from various regions of Cameroon and found that leaf extracts spray of *A. indica* and *T. diversifolia* could manage the fungal growth compared to the others [15].

2. Materials and methods

2.1 Pathogen, bio control agents and plant extracts

Vascular strands of banana cultivar Rasthali/Poovan infected with *Fusarium oxysporum* f. sp. *cubense* (Foc) was collected from Banana Research Station, Kannara and used for the further studies. Bio control agents and plant extracts obtained from various research station of Kerala Agricultural University (KAU) were tested in this study. Bio control agents used were plant growth promoting rhizobacteria (PGPR) mix II, plant growth promoting microorganisms (PGPM), *Pseudomonas fluorescens* and *Trichoderma viride*, whereas botanicals used were extracts of *Azadirachta indica* (leaves), *Myristica fragrans* (mace), *Lawsonia inermis* (leaves), *Areca catechu* (seed kernel), *Kaempferia galanga* (rhizome), *Curcuma caesia* (rhizome), *Calotropis gigantea* (flower), *Bacopa monnieri* (aerial part), *Curcuma angustifolia* (rhizome) and *Justicia gendarussa* (leaves).

2.2 Preparation of media and fungal culture

Pathogen isolated from infected, brown vascular strand was grown on half strength potato dextrose agar (PDA) media in 90 mm Petri dishes. Medium with same composition incorporated with bio control agents and botanicals was used for the *in vitro* management of pathogen.

2.3 Formulation of bio control agents and plant extracts

Talc formulation of bio control agents containing a minimum colony count of 2×10^6 /g was used for the study. The fungicides were evaluated at three different concentrations viz., 1%, 2% and 3%. Plant extracts collected from Aromatic and medicinal plants research station, Odakkali, KAU were dissolved in equal volume of dimethyl sulfoxide (DMSO) and employed for the evaluation at 0.05%, 0.1% and 0.15% concentrations.

2.4 Impact of formulations on inhibition of mycelial growth *in vitro*

In vitro suppression of bio control agents and botanicals was done by poisoned food technique [16]. Desired concentration of the formulation was amended with melted and cooled medium. The media incorporated with fungicides were poured into Petri plates and inoculated with 7mm mycelial discs of 4 days old Foc culture under aseptic condition. The inoculated

plates were incubated at a temperature of 25°C along with control. The percentage inhibition was calculated using the formula given below [17].

$$I = \frac{C-T}{C} \times 100$$

Where, I – Percentage inhibition

C - Growth of Foc in unamended medium

T – Growth of Foc in amended medium

2.7 Statistical analysis

Lab experiment was analyzed using completely randomized design (CRD) whereas field experiment was conducted with randomized block design (RBD).

3. Results and discussion

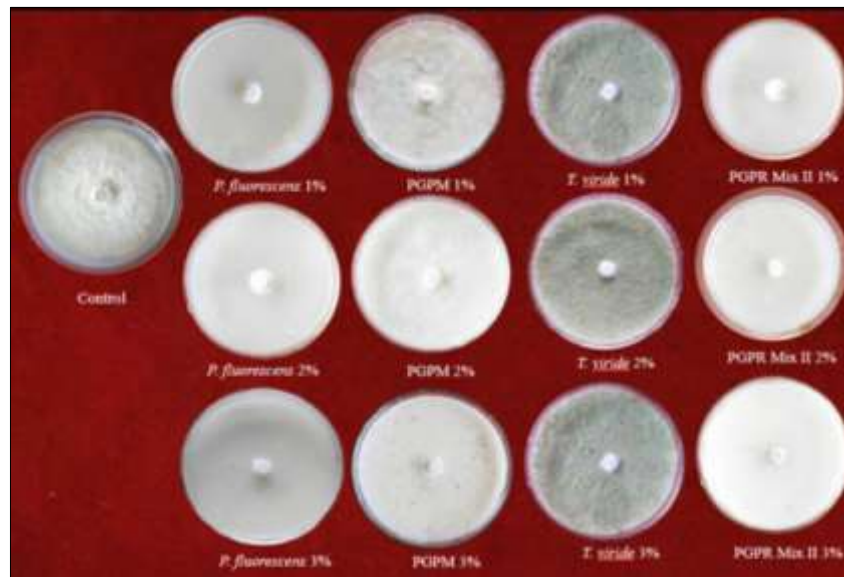
The banana production is seriously constrained by several pests and diseases. Among the diseases, the Fusarium wilt is the most destructive one which is incited by the pathogen, *Fusarium oxysporum* f. sp. *cubense*. It is a classic example of vascular wilt disease in plants. The pathogen is soil-borne and produces three types of spores namely microconidia, macroconidia and chlamydospores. It can survive for several years through chlamydospores. The pathogen causes up to 20 to 80 per cent yield loss. The pathogen has four recognised races from 1 to 4 based on the host specificity. Foc race 1 attacks cultivars Gros Michel (AAA), Silk (AAB), Rasthali (AAB) and Pome (AAB); Foc race 2 causes disease in the varieties such as Bluggoe and other cultivars (ABB genome). Whereas, race 3 was previously described as Foc which is pathogenic to *Heliconia* spp [18,19]. an ornamental flowering plant, but is no longer considered to belong to race structure of Foc [20]. Race 4 attacks Cavendish and all banana cultivars susceptible to races 1 and 2. Fusarium wilt of banana causes significant economic loss in agriculture scenario of tropical and subtropical countries in the world. It is a major threat to banana cultivation with 80 to 90 per cent disease severity [21]. In India, the pathogen causes up to 80 per cent disease incidence and yield loss of up to 40 per cent in northern states. Whereas, in southern states yield loss up to 90 per cent have been reported [22].

The bioagents used namely PGPR Mix II, PGPM, *Trichoderma viride* and *Pseudomonas fluorescens* gave 100 per cent inhibition of the pathogen with no mycelial growth under *in vitro* condition. *T. viride* can be effectively used for the management of Foc [8].

Among the botanicals used for the experiment, the highest (39.44 %) per cent inhibition of the pathogen was observed in treatments applied with extract of *Myristica fragrans* mace followed by an extract of *Azadirachta indica* oil (26.22 %) and *Lawsonia inermis* leaves (25.33 %). The lowest per cent of inhibition of the pathogen was noticed in the treatment *Curcuma caesia* (9.33 cm). The highest mean mycelial growth was recorded in the treatment *Curcuma caesia* (8.16 cm) followed by *Curcuma angustifolia* (7.98 cm) and *Kaempferia galanga* (7.68 cm). Whereas the lowest mean mycelial growth was observed in the treatment with *Myristica fragrans* (5.45 cm) followed by *Azadirachta indica* (oil) (6.64 cm) and *Lawsonia inermis* leaves (6.72 cm). Similar results were reported by others in an experiment against *F. oxysporum* f. sp. *elaeidis* causing vascular wilt in oil palm [15]. Leaf extracts and bio-control agents were found to be effective against Foc in banana [14]. The results are shown in Table 1 and Figures 1 and 2.

Table 1: Effect of fungicides, bicontrol agents and botanicals on the growth of the pathogen Foc

Sl. No.	Treatments	Mycelial growth (Diameter in cm)				Percentage inhibition (%)
		Lower dose	Recommended dose	Higher dose	Mean	
12	PGPR Mix II	0.00 (0.00) ^f	0.00 (0.00) ^j	0.00 (0.00) ^k	0.00	100
13	PGPM	0.00 (0.00) ^f	0.00 (0.00) ^j	0.00 (0.00) ^k	0.00	100
14	<i>Trichoderma viride</i>	0.00 (0.00) ^f	0.00 (0.00) ^j	0.00 (0.00) ^k	0.00	100
15	<i>Pseudomonas fluorescens</i>	0.00 (0.00) ^f	0.00 (0.00) ^j	0.00 (0.00) ^k	0.00	100
16	<i>Azadirachta indica</i> (oil)	7.20 (0.914) ^b	6.56 (0.879) ^d	6.16 (0.853) ^e	6.64	26.22
17	<i>Myristica fragrans</i> (mace)	6.50 (0.869) ^{bc}	5.12 (0.790) ^e	4.73 (0.758) ^e	5.45	39.44
18	<i>Lawsonia inermis</i> (leaves)	7.20 (0.914) ^b	6.77 (0.890) ^c	6.23 (0.859) ^e	6.72	25.33
19	<i>Areca catechu</i> (seed kernel)	7.27 (0.917) ^b	7.10 (0.908) ^b	6.87 (0.896) ^c	7.08	21.33
20	<i>Kaempferia galanga</i> (rhizome)	8.70 (0.987) ^a	8.03 (0.956) ^a	6.60 (0.881) ^d	7.68	14.66
21	<i>Curcuma caesia</i> (rhizome)	8.67 (0.985) ^a	8.13 (0.961) ^a	7.67 (0.938) ^a	8.16	9.33
22	<i>Curcuma angustifolia</i> (rhizome)	8.53 (0.979) ^a	8.07 (0.957) ^a	7.33 (0.921) ^b	7.98	11.33
	CD (0.05)	0.049	0.009	0.012		

**Fig 1:** Effect of biocontrol agents on the growth of Foc under *in vitro* condition**Fig 2:** Effect of botanicals on the growth of Foc under *in vitro* condition

4. Conclusion

An *in vitro* experiment was conducted for the evaluation of biocontrol agents and botanicals for control of the pathogen Foc in banana. Among the bio control agents PGPR Mix II, PGPM, *Trichoderma viride* and *Pseudomonas fluorescens* and among the botanicals, comparatively the extract of *Myristica fragrans* mace and an extract of *Azadirachta indica* oil were found to be effective for the management of Foc.

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