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In vitro Biological Control of *Fusarium oxysporum* f. sp. *Cubense* by using Some Indian Medicinal Plants

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KEYWORDS

Fusarium wilt, Banana, Biological control, Medicinal plants

A B S T R A C T

Fusarium wilt of banana, also called Panama disease is one of the most destructive diseases in banana plant. In the present study, we investigated the *in vitro* biological control of *Fusarium oxysporum* f. sp. *cubense* by using some Indian medicinal plants such as *Calotropis gigantea* L., *Centella asiatica* L., *Ocimum sanctum* L., *Piper betle* L. and *Vitex negundo* L.. The antifungal activity of the distilled water, ethyl acetate, methanol and n-hexane extracts of selected medicinal plants was tested against the *Fusarium oxysporum* f. sp *cubense* by well agar method. Among the five plants tested, *P. betle* L. plant extracts exhibited maximum antifungal activity against the tested plant pathogen *Fusarium oxysporum* f. sp *cubense* followed by *V. negundo, C. gigantea, C. asiatica* and *O. sanctum* plant extracts. The distilled water extracts of all the plants did not show any activity against the tested pathogen.

Introduction

Banana (*Musa* sp.) is among the most important food and fruit crops in many developing countries (Heslop Harrison and Schwarzacher, 2007). *Fusarium* wilt of banana, also called Panama disease is one of the most destructive diseases in banana (Ploetz, 2000). It has been reported in all banana producing countries, including Asia, Central and South America, Africa, and Australia (Ploetz, 2006).

In India, the disease is widespread in almost all banana growing regions, affecting most of the commercial cultivars grown except Red Banana and Nendran (Thangavelu and Mustaffa, 2010).

The pathogen of banana Fusarium wilt was identified as a soil-borne hyphomycete, *Fusarium oxysporum* formae specialis *cubense* (FOC) (Ploetz and Pegg, 1997;

Ploetz, 2006; Fourie *et al*., 2011). This fungus infects banana plants through the roots and invades the vascular tissue (xylem), causing external symptoms like gradual wilting, progressive yellowing of banana leaves, eventual collapse at the petiole, and longitudinal splitting of the outer leaf sheaths in the pseudostem (Yin *et al*., 2011). The distinguishing internal symptoms of the disease is the typical discoloration of vascular tissues varying from light yellow to dark brown, which appears first in the outer or oldest leaf sheath, then extends up to the pseudostem (Ploetz, 2006). Eventually, the disease leads to the death of banana plants.

Scientists around the world have investigated chemical control (Nel *et al*., 2007), biocontrol (Saravanan et al., 2003; Cao et al., 2005; Lian et al., 2009) and molecular methods (Paul *et al*., 2011; Yip *et al*., 2011), in search of methods to control the disease. Some of these studies were found to effectively suppress *Foc* growth in the laboratory and in greenhouses, but were unable to efficiently control *Fusarium* wilt in the field.

Use of biocontrol agents to protect and promote plant growth is generally considered as a potential approach for management of plant diseases (Harish *et al.,* 2009). This method also offers an attractive and environmentally sound alternative for the control of Fusarium wilt of banana (Berg *et al.,* 2001). Medicinal plants have been reported for antifungal activity against some plant pathogens (Asthana *et al*., 1989; Daoud *et al*., 1990; Al-Mughrabi, 2003; Ameziane *et al*. 2007; Bluma *et al*. 2008; Harish *et al*. 2008; Chaijuckam *et al*. 2010). Therefore in the present study we investigated the *in vitro* biological control of *Fusarium oxysporum* f. sp. *cubense* by using some Indian medicinal plants.

Materials and Methods

Collection of Banana Plant

The Fusarium wilt disease affected banana stem (Banana variety - Karpuravalli) was collected from banana cultivated field of Manachanallur, Tiruchirappalli district (Fig. 1).

Isolation of Plant Pathogen

The pathogen *Fusarium oxysporum* f. sp *cubense* (FOC) was isolated from Fusarium wilt disease affected banana stem (*Musa paradisiaca* L.) by using potato dextrose agar (Himedia, Mumbai) medium and identification was done by macro and micro morphological examinations compared with the standard Manuals of A Manual of Soil Fungi and Fungi in Agricultural Soils (Gillman, 1957; Domsch and Gams, 1972)

Collection of Plant Leaves

The medicinal plants such as *Calotropis gigantea* L., *Centella asiatica* L., *Ocimum sanctum* L., *Piper betle* L. and *Vitex negundo* L. were collected from Tiruchirappalli district and identified (Fig. 2)

Preparation of Extracts

Plant extracts were prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1996). A known weight of 5 g of leaf powdered sample was macerated separately with 50 mL of sterile distilled water for 10 min; the macerate was filtered through double layered muslin cloth and then centrifuged at 4000 rpm for 30 min.

A known weight of 5 g of powdered sample was extracted separately with the 50 mL selected solvents *viz.* ethyl acetate, methanol

and n-hexane. The leaf powder was filled in the thimble and extracted with the solvents by using a soxhlet extractor for 24 h. The obtained extracts were concentrated by using a rotary flash evaporator. The extracts were well preserved in airtight containers for further analysis.

Antifungal Activity

The antifungal activity of the selected medicinal plants was tested against the *Fusarium oxysporum* f. sp *cubense* by well agar method (Perez *et al*., 1990). In the freshly prepared and sterilized potato dextrose agar medium supplemented with streptomycin (10 mg/ml) for prevent bacterial contamination was poured into each petriplate and allowed to solidify. The test fungal culture was evenly spread over the media by using sterile cotton swab. Then wells (5 mm) were made in the medium by using sterile cork borer, 200 µl of the distilled water, ethyl acetate, methanol and n-hexane extracts of selected medicinal plants were transferred into separate wells. Then these plates were incubated at 28 °C for 48-72 h. After incubation period the results were observed and measured the diameter of inhibition zone around each well.

Effect of Commercial Fungicide on *Fusarium oxysporum* f. sp *cubense* (Positive Control)

The commercial fungicides *viz*., carbendazim, dithane M-45 and thiophanate-methyl (mg/ml) was tested against *Fusarium oxysporum* f. sp *cubense* by well agar method.

Effect of Solvents on *Fusarium oxysporum* f. sp *cubense* (Negative Control)

The effect of solvents such as distilled water, ethyl acetate, methanol and n-hexane on *Fusarium oxysporum* f. sp *cubense* was studied by well agar method.

Results and Discussion

Isolation of *Fusarium oxysporum* f. sp *cubense* from Infected Banana Stem

The plant pathogen *Fusarium oxysporum* f. sp *cubense* (FOC) causal agent of wilt disease in banana plant was isolated from infected stem of banana plant using PDA medium (Plate 1).

Scientific Classification of *Fusarium oxysporum* f. sp *cubense*

Form class	- Deuteromycetes
Order	- Moniliales
Family	- Tuberculariaceae
Genus	- *Fusarium*
Species	- *oxysporum* f. sp *cubense*

Macroscopic Observation of *Fusarium oxysporum* f. sp *cubense*

In potato dextrose agar (PDA) medium, the aerial mycelium first appeared as white in colour and then changed to dark purple colour (Plate 1).

Microscopic Observation

Fusarium oxysporum f. sp *cubense* produces three types of asexual spores, microconidia, macroconidia and chlamydospores. Microconidia were two celled, and it was the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia were three to five celled, gradually pointed and curved toward the ends. These spores were commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick walled spores, produced either terminally or intercalary on older

mycelium or in macroconidia. These spores were either one or two celled (Fig. 3).

Antifungal Activity

The results of antifungal efficacy of distilled water, ethyl acetate, methanol and n-hexane extract of selected medicinal plants such as *Calotropis gigantea* L., *Centella asiatica* L., *Ocimum sanctum* L., *Piper betle* L. and *Vitex negundo* L. were summarized in table 1.

Among the five plants tested, *P. betle* L. plant extracts exhibited maximum antifungal activity against the tested plant pathogen *Fusarium oxysporum* f. sp *cubense* followed by *V. negundo, C. gigantea* and *C. asiatica* extracts. *O. sanctum* plant extracts were exhibited least antifungal activity against the tested plant pathogen. The distilled water extracts of all the plants did not show any activity against the tested pathogen (Plate 2).

Table.1 In vitro Biological Control of *Fusarium oxysporum* f. sp *cubense* by Some Indian Medicinal Plants

S. No.	Name of the plant	Zone of inhibition (Dia meter in mm)			
		Distilled water extract	Ethyl acetate extract	Methanol extract	n-Hexane extract
1.	*Calotropis gigantea*	-	9.5 ± 1.10	11.4 ± 0.70	8.7 ± 0.78
2.	*Centella asiatica*	-	-	9.7 ± 0.79	8.6 ± 0.56
3.	*Ocimum sanctum*	-	-	8.8 ± 0.83	-
4.	*Piper betle*	-	12.7 ± 0.91	20.5 ± 0.87	11.5 ± 0.92
5.	*Vitex negundo*	-	10.4 ± 1.14	12.5 ± 0.70	9.5 ± 0.93

Results expressed as Mean ± Standard Deviation (n-3); (-) indicates no activity

Fig.1 Collection of Wilt Disease Affected Banana Stem

Fig.2 Selected Indian Medicinal Plants

***Calotropis gigantea* L.**

***Centella asiatica* L.**

***Ocimum sanctum* L.**

***Piper betle* L.**

***Vitex negundo* L.**

Fig.3 Microscopic View of *Fusarium oxysporum* f. sp *cubense*

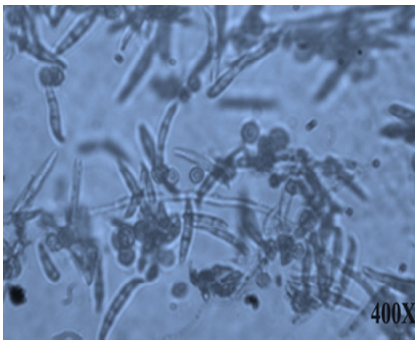


Fig.4 Effect of Commercial Fungicide on *Fusarium oxysporum* f. sp *cubense*

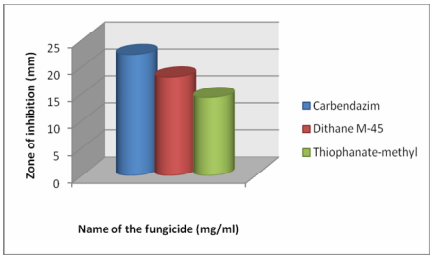
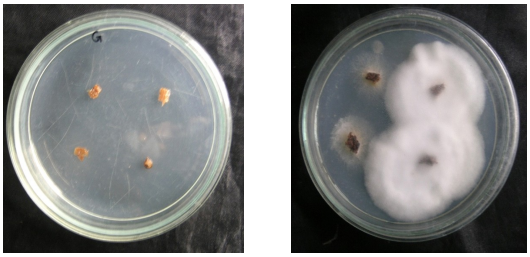


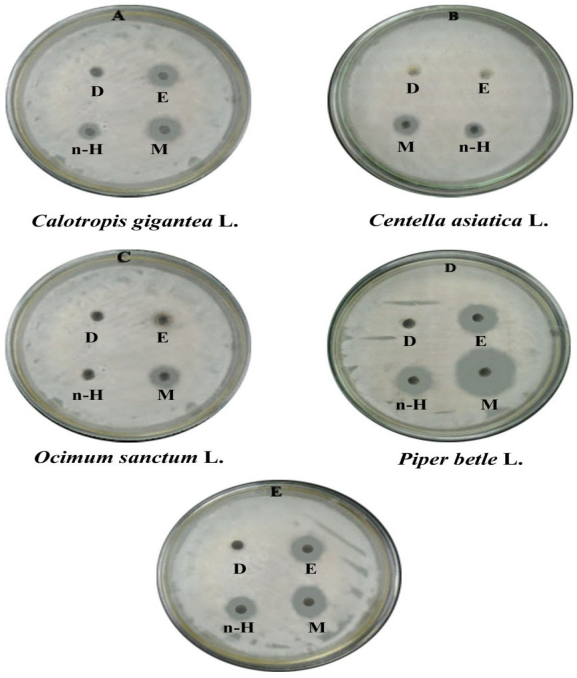
Plate.1 Isolation of *Fusarium oxysporum* f. sp *cubense*



Initial day 3rd day

Infected banana stem on PDA plate

Plate.2 In Vitro Biological Control of *Fusarium oxysporum* f. sp *cubense* by Some Indian Medicinal Plants



Calotropis gigantea L. *Centella asiatica* L.

Ocimum sanctum L. *Piper betle* L.

Vitex negundo L.

D - Distilled water extract, E - Ethyl acetate extract,
M- Methanol extract, n-H - n-Hexane extract

Effect of Commercial Fungicides and Solvents on *Fusarium oxysporum* f. sp *cubense*

The commercial fungicides *viz*., carbendazim, dithane M-45 and thiophanate-methyl (mg/ml) were tested against *Fusarium oxysporum* f. sp *cubense* studied to compare to the potentials of plant extracts.

The results were presented in fig. 4. The most effective fungicides were found to be carbendazim (22.3 mm) followed by dithane M-45 (18.1 mm) and thiophanate-methyl (14.3 mm). The effects of solvents on *Fusarium oxysporum* revealed that no activity was observed. In the present investigation, *Fusarium oxysporum* f. sp. *cubense was isolated from* Fusarium wilt disease affected banana stem. The antifungal

activity of *Calotropis gigantea* L., *Centella asiatica* L., *Ocimum sanctum* L., *Piper betle* L. and *Vitex negundo* L. plant extracts was studied against the plant pathogen *F. oxysporum* f. sp. *cubense.* Among the five plants tested, *P. betle* L. plant extracts exhibited maximum antifungal activity against the tested *F. oxysporum* f. sp. *cubense. Our results are harmony with the findings of* Satish *et al*. (2009) and Singha *et al*. (2011) who reported that *P. betle* L. plant extracts were found to be effective in controlling of *Fusarium* sp.

Likewise, Huang *et al.* (2012) reported the inhibitory effects of *Allium tuberosum* on *F. oxysporum* f. sp. *cubense.* In PDA medium the crude extract of *A. tuberosum* at 0.5, 1.0, and 2.0 ml per petridish significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *cubense* by 12.1 %, 43.5 %, and 100 %, respectively. Phay *et al*. (1999) reported a novel antifungal compound, fistulosim, isolated from *A. fistulosum* L. was showed high activity against *Fusarium* oxysporum. Hadi *et al.* (2013) investigated antifungal properties of some plant species and *Mentha piperita* extracts exhibited remarkable antifungal activity against *F. oxysporum*. Recently, Neela *et al*. (2014) reported the significant antifungal activity of ethanol and acetone extract of leaves of *Piper betel, Carica papaya, Andrographis paniculata* and *Lawsonia inermis* against *F. oxysporum* the causal agent of Fusarium wilt in tomato.

In the same way, antifungal activity of medicinal plants against various plant pathogens has been reported by several researchers. For example, Ho *et al*. (2007) reported the crude extracts of *A. fistulosum*, *A. sativum* and *A. tuberosum* were inhibited the germination incidence of *Alternaria brassicicola* by 100%. Caraway and peppermint crude extracts showed the complete growth inhibition of *Sclerotium rolfsii* (El-Mougy and Alhabeb, 2009). Leaf crude extracts of *Abrus precatorius* and *Aegle marmelos* were strongly inhibited the growth of *Colletotrichum capsici* and *Alternaria alternata* (Anand *et al*., 2009). Al-Rahmah *et al*. (2013) reported the fungicidal activity of five methanolic plant extracts from *Lantana camara, Salvadora persica, Thymus vulgaris, Zingiber officinale* and *Ziziphus spina-christi* against tomato phytopathogenic fungi, *Fusarium oxysporum, Pythium aphanidermatum* and *Rhizoctonia solani*, the causative agents of tomato damping-off diseases *T. vulgaris* extract was the most effective in suppressing the mycelial growth of phytopathogenic fungi followed by *Z. officinale* and *S. persica.*

Conclusion

In conclusion, the present investigation, *in vitro* screening of plant extracts has given encouraging results, indicating their potential use in the management of Fusarium wilt in banana caused by *Fusarium oxysporum* f. sp *cubense*. Further field trial and photochemical analysis of the active compounds of those plants would give a strong antifungal activity comparable to synthetic fungicides.

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