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In vitro biological control of *Fusarium oxysporum* f. sp. *cubense* (Foc), casual agent of wilt disease in *Musa paradisiaca* L.

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Abstract

Fusarium wilt of banana, also called Panama disease, is one of the most important and destructive diseases of banana plant (*Musa paradisiaca* L.) in the world, which has been reported from all banana producing countries. Control of *Fusarium oxysporum* f. sp. *Cubense,* the casual agent of wilt disease, by using the plant extracts has been evaluated as a strategy of biological control. The antifungal activity of different extracts of *Justicia adhatoda* L., *Ocimum tenuiflorum* L., *Piper betle* L. and *Vitex negundo* L. against *F. oxysporum* f. sp. *cubense* was evaluated by well diffusion method. The results revealed that the plant extracts of *V. negundo* L. exhibited maximum antifungal activity. *In vitro* screening of plant extracts showed encouraging results, and hence they could be potentially used in the management of *Fusarium* wilt in banana plant caused by *F. oxysporum* f. sp *cubense*.

Key words : Fusarium wilt, *Musa paradisiaca* L., biological control, plant extracts

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INTRODUCTION

Banana (*Musa* sp.) is one of the first cultivated food crops. It is the fourth most important global food commodity after rice, wheat and maize in terms of gross value production. At present, it is grown in more than 150 countries throughout the tropical and subtropical regions, and it is the staple food for more than 400 million people (Mohapatra *et al*., 2010). In India, main banana growing states are Tamil Nadu, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Bihar, Assam and Madhya Pradesh. Tamil Nadu is one of the leading banana producing states in India which contributes 19% of the total production (Indian Horticulture database, 2014).

Fusarium wilt of banana, also called Panama disease, is caused by *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder and Hansen, shortly as Foc (Ploetz, 2006). Fusarium wilt is known to be one of the most important and destructive diseases of banana. It has been reported in all banana-producing countries, including Asia, America, Africa, and Australia (Ploetz and Churchill, 2011) and it was responsible for losses of billions of dollars in the first export trades in the world (Jones, 2009; Ploetz *et al*., 2015).

The use of botanicals has a long history in the control of plant diseases. Plant biochemicals and crude extracts have been reported to have antimicrobial properties against plant pathogens including fungi, bacteria, nematodes and insects *in vitro* as well *in vivo*. Further, the use of botanicals is regarded as the best suited environmental friendly measure as they are easily biodegradable and safer (Pradhanang *et al.,* 2003). Consequently the present investigation was designed to assess the *in vitro* biological control of *F. oxysporum* f. sp. *cubense* casual agent of wilt disease in *Musa paradisiaca* L. by using some medicinal plants.

MATERIALS AND METHODS

Collection of Banana Plant

The Fusarium wilt disease affected banana pseudo stem (Banana variety - Poovan) was collected from banana cultivated field of Marungulam, Thanjavur district.

Isolation of plant pathogen

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

Collection of plants

Fresh plant leaves of *Justicia adhatoda* L., *Ocimum tenuiflorum* L., *Piper betle* L. and *Vitex negundo* L. were collected from the natural habitat of Marungulam,

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Thanjavur district, South India. The plant species was identified and confirmed with reference to Herbarium specimens available in the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. These plants were selected based on traditional knowledge.

Preparation of plant extracts

Plant extracts were prepared following the methodology of Indian Pharmacopoeia (Indian Pharmacopoeia, 1996). The plant leaves were shade dried and pulverized. Then, 50g of each leaf powder was packed separately in Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 mL of ethyl acetate, methanol, n-hexane and petroleum ether as solvents. The obtained extracts were concentrated by using a rotary flash evaporator. The plant extracts were preserved in airtight containers for further analysis.

Screening of antifungal activity

The antifungal activity of the selected medicinal plants was tested against *F. oxysporum* f. sp *cubense* by using agar well diffusion method (Perez *et al*., 1990). The freshly prepared and sterilized potato dextrose agar medium was supplemented with streptomycin (10 µg/mL) to prevent bacterial contamination, poured into each Petri plate 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 ethyl acetate, methanol, n-hexane and petroleum ether extracts of selected medicinal plants were transferred to wells in separate plates. The commercial fungicide carbendazim (mg/ml) and solvents (ethyl acetate, methanol, n-hexane and petroleum ether) were used as positive and negative controls respectively. Then these plates were incubated at 28 °C for 48-72 h. After incubation period the results were observed and the diameter of inhibition zone around each well was measured. All the tests were performed under aseptic condition, three replicates were maintained and the activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

RESULTS AND DISCUSSION

In the present study, the plant pathogen, *F. oxysporum* f. sp *cubense*, was isolated from infected stem of banana plant using PDA medium. The pathogen macroscopically appeared as white myulial mat over the medium and microscopic structures revealed the presence of macro conidia and micro conidia (Fig. 1 and 2).

Figure 1. Macroscopic view of *F. oxysporum* f. sp. *cubense*

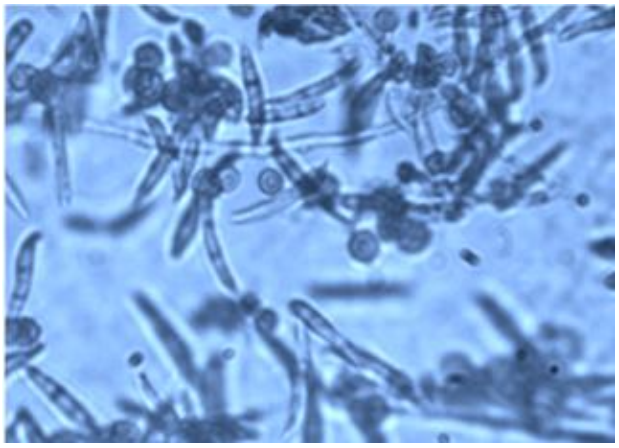

Figure 2. Microscopic view of *F. oxysporum* f. sp. *cubense*

In the present day constraints of chemical pesticides on the environment and human health attempts have been made to find alternative methods to manage the plant diseases and pathogens, the biological methods including use of antagonists, soil augmentation with organic residues, green manures, etc. have been recognized as viable alternatives.

The plant world is a rich store house of natural chemicals that could be exploited for the use as fungicides. The presence of antifungal compounds in higher plants has long been recognized as important factors for controlling some plant diseases (Tapwal *et al*., 2011). Plant bio-fungicides are cheap, locally available, non toxic and are easily degradable. There is a great demand for them as alternative agents to control plant pathogenic fungi (Singh and Srivastava, 2013).

In the present investigation, four plants such as *J. adhatoda* L., *O. tenuiflorum* L., *P. betle* L. and *V. negundo* L. were screened *in vitro* for antifungal activity against phytopathogenic fungus, *F. oxysporum* f. sp *cubense.* The results of antifungal efficacy of ethyl acetate, methanol, n-hexane and petroleum ether extracts of *J. adhatoda* L., *O. tenuiflorum* L., *P. betle* L. and *V. negundo* L. are presented in Table 1. Among the four plants tested, the plant extracts *V. negundo* L. exhibited maximum antifungal activity against *F. oxysporum* f. sp *cubense* followed by *J. adhatoda* L. and *P. betle* L. extracts. *O. tenuiflorum* L. plant extracts exhibited least antifungal activity against the tested plant pathogen. The ethyl acetate extract of all the plants showed the maximum antifungal activity and petroleum ether extract showed minimum activity against the test pathogen. The results of antifungal effect of four solvents such as ethyl acetate, methanol, n-hexane and petroleum ether revealed no activity against test pathogen. The commercial fungicide carbendazim showed the highest antifungal activity against *F. oxysporum* f. sp *cubense* (23 mm). The antifungal activity of ethyl acetate extract of *V. negundo* L. was found to be comparable to the standard fungicide tested.

In the present study, *V. negundo* L. plant extracts exhibited highest antifungal activity against *F. oxysporum* f. sp *cubense*. The results of antifungal activity of *V. negundo* L. were in harmony with results of Siva *et al*. (2008) who reported that *V. negundo* L. plant extracts exhibited significant antifungal activity against *F. oxysporum* causing wilt disease of *Solanum melogena* L. in *in vitro.* Recently a study by Bapat *et al*. (2016) reported considerable antifungal activity of ethanolic and petroleum ether extracts of *V. negundo* L. against phytopathogenic fungus, *Sclerotium rolfsii* Sacc.

In the same way, antifungal activity of various medicinal plants against *F. oxysporum* has been reported by several researchers. Dwivedi and Enespa (2012) reported that *Moringa oleifera* extracts (leaves, bark and seeds) showed significant inhibition in the mycelial growth of *F. oxysporum* f. sp. *lycopersici*. In addition, Huang *et al.* (2012) reported the inhibitory effects of *Allium tuberosum* on *F. oxysporum* f. sp. *cubense.* Hadi *et al.* (2013) also accounted *Mentha piperita* plant extracts exhibited remarkable antifungal activity against *F. oxysporum*. Recently, Neela *et al*. (2014) who reported that *P. betle* L. plant extracts exhibited significant antifungal activity against *F. oxysporum* f. sp. *lycopersici* the causal agent of Fusarium wilt in tomato.

In the present study it was also found that the plant extracts of *J. adhatoda* L. showed moderate antifungal activity against *F. oxysporum* f. sp. *cubense.* This is in contrast to the finding of Siva *et al*. (2008) who reported strong fungicidal activity of *Adhatoda vasica* against *Fusarium oxysporum* causing wilt disease in *Solanum melogena* L.

The extracts of *O. tenuiflorum* L. were found to have least antifungal activity against *F. oxysporum* f. sp. *cubense*. On the contrary, mycelial growth of various plant pathogens such as *Botryodiplodia theobromae* causing fruit rot of mango (Patil *et al*., 1995), *B. theobromae* causing fruit rot of sweet orange (Godara and Pathak, 1995), *F. oxysporum* causing wilt disease of *Solanum melogena* L. (Siva *et al*., 2008), *F. solani* causing dry rot disease of potato (Bhardwaj, 2012) and *Alternaria solani* which causes early blight disease on tomato plants (Dheeba *et al*., 2015) was inhibited by *O. tenuiflorum* L. plant extracts. This could be due to the fact that there is variations in the sensitivity of micro organisms to different plant extracts.

The results indicated that all the tested plant extracts had different levels of antifungal activity against the phytopathogenic fungus *F. oxysporum* f. sp. *cubense*. The differences in the inhibitory effect of tested plant extracts may be due to qualitative and quantitative differences in the antifungal principles or compounds present in them.

Table 1: *In vitro* biological control of *F. oxysporum* f. sp *cubense* by using some medicinal plants

Sl. No.	Name of the Plant	Zone of inhibition (Diameter in mm)			
		Ethyl acetate extract	Methanol extract	n-Hexane extract	Petroleum ether extract
1	*J. adhatoda* L.	15.7 ± 2.52	14 ± 1	12.3 ± 0.58	11 ± 1
2	*O. tenuiflorum* L.	11.3 ± 1.12	11 ± 1	10 ± 1	9.3 ± 0.6
3	*Piper betle* L.	14.3 ±1.15	12.3±1.53	11.3± 0.58	10± 1
4	*V. negundo* L.	29 ± 0.5	17± 0.82	14.7 ± 1.53	12 ± 1

Results expressed as Mean ± Standard Deviation (n-3)

CONCLUSION

The findings of this study suggest that *V. negundo* L. plant extracts possessed broad spectrum antifungal activity against *F. oxysporum* f. sp. *cubense*. This potential could be exploited for the an ecofriendly management and control of wilt disease of banana plant. In addition, *V. negundo* L. plant extracts could be applied as agro-eco-friendly fungicides for various crops.

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