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Eco-friendly management of *Fusarium oxysporum* f.sp. *cubense* causing *Fusarium* wilt on Banana under *In vitro* condition

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Abstract

Fusarium wilt (Panama wilt) of banana (Musa paradisiaca) is caused by *Fusarium oxysporum* f. sp. cubense was considered as a serious threat in the rasthali cultivar of banana in recent years throughout India. The use of plant extracts and is gradually becoming a choice of method in management of plant diseases as these are ecofriendly and safe. Several plant extracts such as Chilli, Datura, Custard apple, *Aloe*, Castor and Notchi are used against *Fusarium oxysporum* f.sp. cubense which helps in the organic cultivation of banana. Among these several botanical extracts screened, ethanol leaf extract of Datura followed by custard apple are effectively retard the growth of panama wilt pathogen under *in vitro* condition.

Keywords: Fusarium wilt of banana, plant extracts, ethanol leaf extracts

Introduction

Banana is one of the most important fruit crop belongs to the family of Musaceae. It has been evolved from humid tropical regions of Southeast Asia is considered as an centre of origin. Modern edible varieties have evolved from two species Musa acuminate and Musa balbisiana. Banana is the rich source of carbohydrates and vitamins, particularly vitamin - B. Banana is mostly affected by various fungal, bacterial and viral diseases reflecting negatively on plant growth and yield. Among these, the pathogenic soil borne fungi especially Fusarium oxysporum f.sp. cubense causes vascular wilt disease, is great challenging task in terms of management. It causes yield reduction of about 80-90 per cent in areas of susceptible cultivars are grown in Tamilnadu (Thangavelu et al., 2012)^[11]. The symptoms become evident after 5-6 months of planting and are expressed both externally and internally. The disease causes yellowing of leaf margin on oldest leaves, hanging down of leaves around pseudo-stem and splitting of pseudo-stem, it causes severe losses in terms of yield in later stages. Plants which were affected by wilt pathogen generally produce unmarketable bunches and the disease ultimately destroys the entire plant (Akila et al., 2011)^[1]. Fungus persist in soil as resting structure of chlamydospore for several years, and thus soil becomes unfit for cultivation of susceptible genotypes for more than 30 years (Ploetz., 2000)^[8]. Application of fungicides to control wilt disease is not practical and environmentally unsafe to human beings and animals, the present objective was focused on eco-friendly management to promote the organic cultivation of Banana and reduce the pesticide usage.

Material and methods

Isolation and Morphological confirmation of Pathogen

Fusarium oxysporum f.sp. *cubense* is isolated from wilt infected banana pseudostem samples. Brown discolored vascular tissues are cut into small pieces, infected tissues are surface sterilized by using sodium hypochlorite 0.1 per cent. The sterilized tissues were placed on Potato Dextrose Agar medium (PDA) and incubated at 25±20c for its growth. The pure culture of pathogen was maintained on PDA medium for the further studies (Plate 1).

Morphological conformation of *Fusarium oxysporum* f. sp. *cubense* by using microscopic studies. Under the microscopic studies we observed the macroconidia and microconidia it gives the confirmation of the pathogen (Plate 1a).

Pathogenicity test

Banana leaves were detached from healthy plant and surface sterilized by using 70 per cent ethyl alcohol.

Each sterilized healthy leaves are cut in to small pieces of $(5\text{cm}\times5\text{cm})$ and placed over sterile petridish containing moistened filter paper to maintain higher humidity. By using sterile needle make artificial wounding at center of the leaf pieces for easy access of the fungus. Mycelial disc of 7 days old culture *Fusarium oxysporum* f.sp. *cubense* was inoculated on wounded surface, seal the petriplate by using parafilm, and incubate under room temperature for 7 days and observed the macroconidia and microconidia under microscope (Mongkutkarn Udompongsuk and Kasem Soytong., 2016)^[7].

Preparation of plant extracts (Ethanol extract)

Different botanical leaves were collected from medicinal field of VIA campus, Pollachi. Leaves were washed with running tap water for twice the times to remove soil, dust particles and Shade dried for three days. After shade drying, leaves were crushed with the help of pestle and mortar by using ethanol in 1:10 ratio. Extracts were passed through muslin cloth and collect the filtrate, then filtrate are passed through Whatman No.1 filter paper. The extracts were centrifuged at 5000 rpm for 30 minutes and collected the filtrate separately. Finally, filtrate was passed through syringe filter of 0.2µm pore size for sterilization. The filtrate was served as 100 per cent standard concentrate. (Chowdappa et al., 2018)^[3]. The filtrate was diluted in to 3 Per cent, 5 Per cent and 10 per cent concentration by using sterile distilled water for check its efficiency of antagonism against Foc. The standard concentrate solution was stored at 4°C for further use.

Table 1: List of Botanicals used against *Fusarium oxysporum* f.sp.

 cubense

S. No	Common name	Scientific name	Parts used
1	Aloe	Aloe vera	Leaf
2	Castor	Ricinus communis	Leaf
3	Datura	Datura metel	Leaf
4	Chilli	Capsicum annum	Leaf
5	Notchi	Vitex negundo	Leaf
6	Custard apple	Annona reticulate	Leaf

In vitro screening of plant extracts against Fusarium oxysporum f.sp. cubense

Botanical extracts such as Aloe, Datura, Chilli, Castor, Notchi and Custard apple of 3, 5 and 10 per cent concentrations were prepared separately and maintained as a stock. Sterile 100 ml of Potato Dextrose Agar medium was prepared and add 3 ml, 5 ml and 10 ml of extracts from stock solution; it provides 3, 5 and 10 per cent concentrations respectively. The PDA medium without botanical extract served as a control. Later media was poured in to sterile petri- plate at the rate of 15ml per plate and allowed to solidify. Seven days old culture disc of Fusarium oxysporum f.sp. cubense is inoculated in center of the petriplate and incubate at 25±20c.Three replication were maintained for each treatment (Schmitz, H., 1930)^[10]. The radial mycelial growth (in mm) in each treatment was measured after the control plate are completely covered by the mycelium of Foc. The inhibition percentage of radial mycelial growth was calculated by using the formula, (Vincent., 1927).

Per cent of inhibition over control =
$$\frac{C-T}{C}$$

C = Mycelial growth of the pathogen in control. T = Mycelial growth of the pathogen in treatment. Efficacy of different Botanical extracts were statistically analyzed and treatment means were compared by FCRD (Factorial Completely Randomized Design).

Results

In vitro screening of Plant extracts (Ethanol extract) against of *Fusarium oxysporum* f.sp. *cubense*.

The results revealed that higher concentration (10%) of plant extracts shows maximum inhibition of mycelial growth compare to lower concentrations (3% and 5%) in all three replicates. Among different extracts, Datura shows maximum inhibition percentage (85.5%), followed by custard apple (85%) where, other leaf extracts show inhibition percentage at lower extent (Plate 2a, 2b, 2c, 2d, 2e, 2f) (Fig 1.1, 1.2) (Table 1).

Discussion

Effect of plant extracts on growth of *Fusarium oxysporum* f.sp. *cubense*

In recent years, use of herbal plants extracts is becoming a method of choice in management of plant diseases as these are low mammalian toxicity, target specific, easy biodegradability and it contains different active ingredients in lower concentrations which possess antagonist activity against both insect pest and plant pathogens (Harish et al., 2008; Kalaycioglu et al., 1997) [4, 9]. Botanical extracts were used against both air and soil borne pathogens as foliar application, Pre and post inoculation for controlling of pathogens (Akila et al., 2011 and Kagale et al., 2004) [1, 5]. Datura metel leaf extract shows antifungal action against foliar and soil borne pathogens such as Alternaria alternata Curvularia lunata, Fusarium equiseti, Macrophomina phaseolina, Botryodiplodia theobromae and Colletrotrichum corchori (Begum et al., 2007)^[2]. Application of Datura metel leaf extract against fungal and bacterial pathogens it induces the resistance development by accumulation of two-fold to fivefold times increase in defense related compounds such as PR Proteins β -1,3-glucanase and chitinase (Kagale *et al.*, 2004) ^[5]. Datura leaf extract at 10 Per cent concentration it shows complete inhibition of Fusarium oxysporum f.sp. cubense (Akila et al., 2011)^[1]. The results of our present investigations shows that clear indication for the potential use of plant extracts to control fungal pathogens. It is evident from the result that all plant extracts show significantly inhibit the radial growth of the isolated fungus Foc. Among the different plant extracts Datura metel was found most effective followed by Custard apple, Chilli and Aloe.



Plate 1: Isolate of Fusarium oxysporum f.sp. cubense

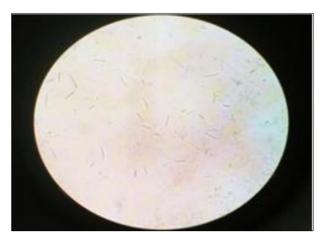


Plate 1a: Morphological characterization of Fusarium wilt

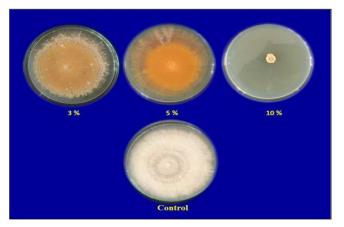


Plate 2: In vitro screening of Botanical extracts against Fusarium oxysporum f.sp. cubense



Plate 2a: In vitro screening of Aloe vera extract against Fusarium oxysporum f.sp. cubense

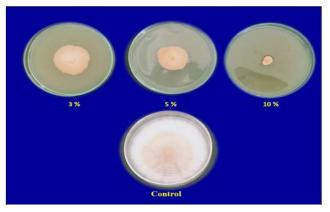


Plate 2c: In vitro screening of Chilli extract against Fusarium oxysporum f.sp. cubense



Plate 2d: In vitro screening of Custard apple extract against Fusarium oxysporum f.sp. cubense

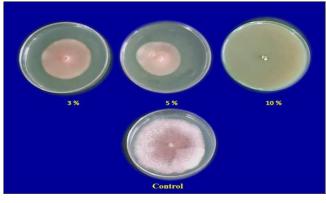


Plate 2e: In vitro screening of Datura extract against Fusarium oxysporum f.sp. cubense

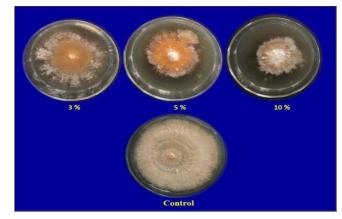


Plate 2f: In vitro screening of Notchi extract against Fusarium oxysporum f.sp. cubense

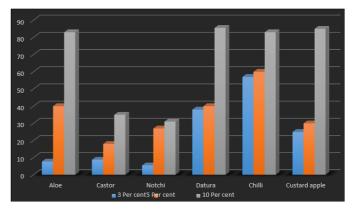


Fig 1: Inhibition percentage of botanical extracts against *Fusarium* oxysporum f.sp. cubense under *In vitro* condition

 Table 1: In vitro screening of ethanol extracts of plants against

 Fusarium oxysporum f.sp. cubense

S. No	Botanical extracts	Per cent inhibition over control			
Concentration		3%	5%	10%	
1	Aloe	7.7	40	83	
2	Castor	8.8	18	35	
3	Notchi	5.5	27	31	
4	Datura	38	40	85.5	
5	Chilli	57	60	83	
6	Custard apple	25	30	85	
7	Control	-	-	-	
SEd		1.09	0.82	0.65	
CD(p=0.05)		2.25	1.70	1.35	

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