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Isolation and pathogenicity evaluation for causal agent of Panama Wilt in Banana

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Panama wilt, induced by *Fusarium oxysporum* f. sp.*cubense*, is one of the most significant banana plant diseases. The disease, characterised by the yellowing of older leaves at the margins and then towards the midrib, the draping of decaying leaves as a skirt, and ultimately the death of the plant, causes economic loss. To prevent economic loss and boost banana production, FOC must be characterised. The banana (*Musa paradisiaca*) is a member of the Musaceae family, which is a commercial crop and universally nutrient-rich food source. For this research, eight samples of infected banana plants were obtained from eight agricultural fields in four districts. In total, eight isolates were obtained and subcultured from the samples using the direct plate and serial dilution methods. The isolates were identified as *Fusarium* spp. using cultural and microscopic characteristics, and 18s rRNA sequencing was used to confirm the species. The fragmentary nucleotide sequences of eight isolates have been submitted to Genbank, NCBI. The triplicate container culture of banana plants was prepared, and the eight isolates were inoculated into the plants. The plant growth parameters and wilt symptoms were observed and recorded at planting, after one, two, and three months of planting, for a total of three months, in conjunction with standard *Fusarium* culture. During the first and second months after planting, the fewest symptoms were recorded. During the third month, however, the greatest number of symptoms, including leaf discoloration, leaf curling, and reduced plant growth in contrast with the control plants, were observed. By this work, it can be concluded that *Fusarium oxysporum* is the causal agent of Panama wilt disease in the banana crop; consequently, this research provides the framework for the development of disease management and control strategies for Panama wilt in banana crops.

Keywords: *Fusarium oxysporum, Musa paradisiaca*, Panama wilt, pathogenicity

INTRODUCTION

Banana is one of the world's most important fruit crops, widely grown in tropical and subtropical countries for its valuable applications in the food sector (Chand *et al*. 2016). The banana is considered to be the world" fourth greatest fruit crop. With an annual production of 33 million tonnes, India was the world's largest banana farmer, accounting for 20% of the total global output of 125 million tonnes in 2021 (World Data Atlas). Bananas (*Musa* sp.) are fast-growing monocotyledonous herbaceous plants in the Zingiberales group (Pradeep *et al*. 2020).

Furthermore, bananas are susceptible to the disease because they are propagated vegetatively. This makes the crop vulnerable to disease transmission, such as Panama wilt, which is easily transmitted by infected plant material or soil. Panama wilt is a deadly banana disease caused by the soil-dwelling fungus *Fusarium oxysporum* (Dita *et al*. 2018; Goncalves *et al*. 2019). This fungus infiltrates the root system as well as the xylem vessels (Mongkutkarn *et al*. 2016). *Fusarium* wilt of banana is the most serious vascular wilt disease, producing massive crop losses (Vishwanath *et al.* 2011).

Fusarium wilt, often known as Panama disease, causes two forms of external symptoms in bananas:

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"yellow leaf syndrome" and "green leaf syndrome." (Perez-Vicente, 2004). A susceptible banana plant infected with *Fusarium* wilt seldom recovers; growth is low, and the mother plant produces a large number of infected suckers before dying. Internal symptoms include vascular discoloration, which starts with yellowing of the root and rhizome vascular tissue and develops to the development of continuous yellow, red, brownish threads in the pseudostem. Reddish-colored vessels can also be seen in petioles of vulnerable cultivars (Akila *et al.* 2011; Sudha *et al.* 2019). Bananas are a good source of fibre, potassium, vitamin B6, vitamin C, antioxidants and phytonutrients. The banana's health-promoting properties are linked to its high concentration of bioactive substances such as phenolic compounds. Bananas also aid in the elimination of infections and disorders, as well as in the stabilisation of the body's electrolyte balance. It contributes significantly to the body's immunity. Significant losses in banana output cause farmers to face economic difficulty. As a result, it is critical to preserve the long-term viability of banana production and the livelihoods of those who rely on it. Controlling banana wilt is critical since it is one of the most important food crops in tropical climates, supplying food and income to millions of people. To manage the panama wilt in bananas, biological methods, chemical methods, and consortia methods were applied. The aim of the present research had been to isolate *Fusarium oxysporum* from pseudostem samples of infected plants collected from various agricultural fields. Using morphological and molecular characteristics, the isolated fungus was identified. The isolated *Fusarium oxysporum* was tested for pathogenicity using sterile pot cultivation of plants.

MATERIALS AND METHODS

Collection of infected samples

Infected pseudostem, leaves, and rhizosphere soil samples were collected from agricultural fields in the districts of Chitradurga, Shivamogga, Davanagere, and Chikkamagaluru in the Indian state of Karnataka. The collected samples were transported aseptically to the laboratory in sterile polythene containers and stored at 4°C (Raja and Ebenezar, 2021; Gnanasekaran *et al*. 2015).

Isolation of Fusarium oxysporum

The infected plant samples were washed with sterile distilled water, sliced into small sections, and placed on PDA medium. The soil samples were then inoculated with streptomycin (30mg/L) using the serial dilution method to prevent bacterial growth. After inoculation, all Petri dishes were incubated at room temperature for four to seven days. After the incubation period, the colonies were purified by sub-culturing on fresh media for identification purposes (Kubura *et al*. 2018; Gnanasekaran *et al*. 2015).

Characterization of Fusarium oxysporum

C*ultural characterization*

Subculturing on fresh potato dextrose agar medium purified the isolated *Fusarium oxysporum*. Fungal colony form, arrangement, and colour were observed and recorded after growth (Kubura *et al*. 2018; Gnanasekaran *et al*. 2015; Aneja, 2007).

Microscopic Characterization

Fusarium oxysporum was identified using the lactophenol cotton-blue (LPCB) mounting method. The LPCB moist mount preparation is the most common technique for observing fungi under the microscope. Characters were noted by observing spore shape, spore size, spore arrangement, and hyphae arrangement and identified by referring to the standard manuals (Aneja,2007; Barnett 1975; Booth 1971; Funder 1961; Subramanian, 1983).

Molecular characterization by 18sr RNA Method

The genomic DNA was isolated using the Chromous Biotech gDNA mini spin reagent and the CTAB (Cetyltrimethyl ammonium Bromide) technique. The internal transcribed spacer (ITS) sections of the fungal genome were sequenced. The ITS region of the *Fusarium* strain genome was amplified using the forward and reverse primers ITS1-52 - CCGTAGGTGAACCTGCGG-32 and ITS4-52 - TCCTCCGCTTATTGATAGGC-32 . Using 50ng of DNA template, 1.5mM $MgCl_2$, and 0.4U Taq DNA polymerase, and amplification was performed in a

Table 1: Collection of infected banana samples

	District	Agricultural field	No. of samples
	Chitradurga	Gollarahatti	1
		Bharamasagara	2
	Shivamogga	Thammadihalli	1
		Gonibidu	1
	Davanagere	Doddabathi	1
		Belludi	2
	Chikkamagaluru	Rangenahalli	1
		Bhavikere	1

Table 2: Isolation of *Fusarium oxysporum* by infected banana samples

Agricultural field	Samples	No. of Isolates	Culture code
Gollarahatti	Rhizosphere soil	1	*Foc* 1
Bharamasagara	Psuedostem	1	*Foc* 2
		1	*Foc* 3
Thammadihalli	-	-	-
Gonibidu	Rhizosphere soil	1	*Foc* 4
Doddabathi	Psuedostem	1	*Foc* 5
Belludi	Psuedostem	1	*Foc* 6
		1	*Foc* 7
Rangenahalli	Leaf	1	*Foc* 8
Bhavikere	-	-	-

Table 3: 18s rRNA sequences deposited to GenBank, NCBI

Code No.	Samples	GenBank Accession No.
KUMBPJBT-68	*Fusarium oxysporum*	OL470899
KUMBNPJBT-69	*Fusarium oxysporum*	OL504748
KUMBPJBT-70	*Fusarium oxysporum*	OL679453
KUMBAMNPT-71	*Fusarium oxysporum*	ON323573
KUMBMNPABT-72	*Fusarium oxysporum*	ON479652
KUMBNPAMBT-73	*Fusarium odoratissimum*	ON496460
KUMBPAMNBT-74	*Fusarium oxysporum*	ON496459
KUMBNGBT-75	*Fusarium oxysporum*	OL825726

CG palm cycler, the band was recorded using a gel documentation machine, and the PCR product was purified using a gel extraction reagent. The obtained ITS sequences were analysed using NCBI Basic Local Alignment Search Tool (BLAST).

Preparation of inocula

In order to produce inoculum, Potato dextrose broth was inoculated with a 7-day-old pure culture grown on PDA broth and incubated on a rotary shaker (1000 rpm) at room temperature for seven days. When the culture showed complete growth by forming a mat on the broth surface, it was utilised further (Kubura *et al.* 2018).

Pathogenicity tests of Fusarium oxysporum

The pot culture approach was used to test pathogenicity in triplicate (Dinesh *et al*. 2014). The experiment was set up by planting the banana plantlets into sterile soil-filled pots and keeping them in the greenhouse for one week to get used

Fig.1: a. Infected Banana leaf, **b**. infected pseudostem of the banana plant, **c**. Rhizosphere soil of the infected banana plant, **d**. Pure colony of *Fusarium oxysporum* and **e**. Microscopic view of Micro conidia of *Fusarium oxysporum*.

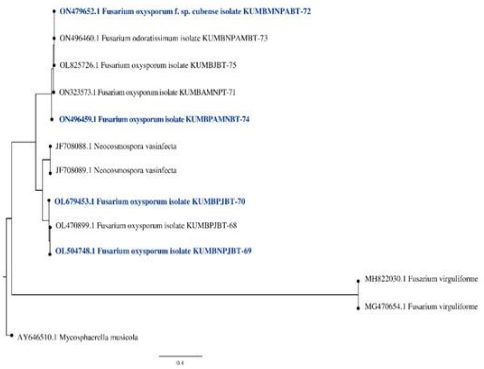

Fig.2: Phylogenetic analysis of *Fusarium oxysporum* using neighbor joining method at 100 boot strap

Fig.3: Pathogenicity test for selected FO isolates (**a** and **b**). Pathogenicity test result (**c**).

to the soil. The produced inoculums of *Fusarium oxysporum* culture were transplanted to the Banana plantlets grown in the greenhouse pots, demonstrating Koch's postulates. The ability of the

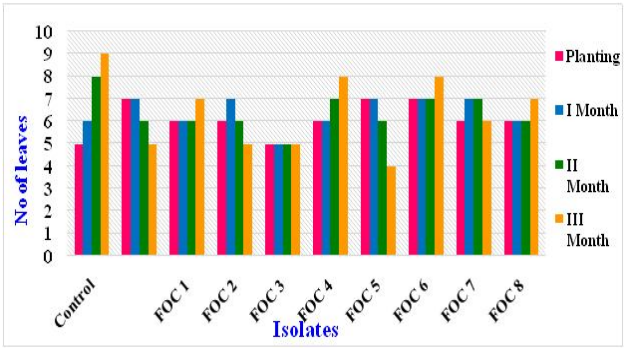

Fig.4: Effect of *Fusarium oxysporum* on number of leaves of banana plant.

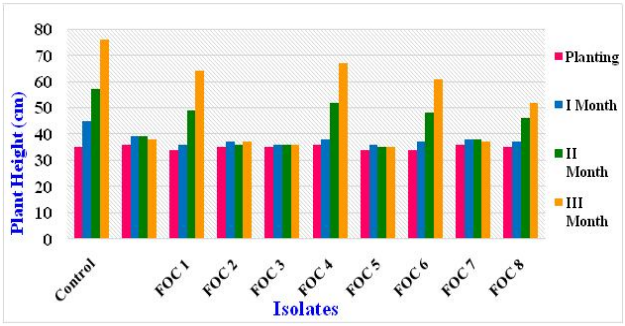

Fig.5: Effect of *Fusarium oxysporum* on Plant Height of banana plant.

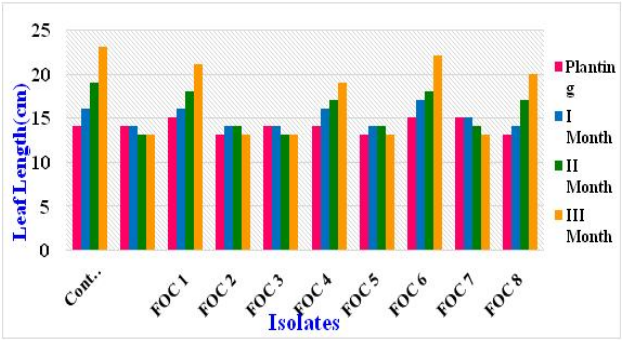

Fig.6: Effect of *Fusarium oxysporum* on leaf length of banana plant.

Fusarium oxysporum strain to infect and cause pathogenesis was determined by measuring plant height, leaf number, leaf length, and morphological changes such as wilting or shrinking of banana plants at one-month intervals up to three months (Kubura *et al.* 2018).

RESULTS AND DISCUSSION

Isolation of Fusarium oxysporum

Agricultural land in the districts of Chitradurga, Davanagere, Shivamogga, and Chikkamagaluru

Karnataka, India were surveyed to obtain samples of the pseudostem, rhizosphere soil, and infected leaves of banana plants (Fig.1.a, b, c and Table 1, 2). Positive results for Panama wilt disease were found in every sample tested. The virulence of the isolated organisms was investigated by subculturing them. These findings were consistent with those of Raja and Ebeneza (2021), who collected wilt-infected root and rhizome samples from the surveyed areas (14 places) for pathogen isolation and found that 14 isolates of *Fusarium oxysporum* f. sp. *cubense* were present. Banana wilt disease is caused by *Fusarium oxysporum* f. sp. *cubense*, which was isolated from an infected banana stem by Gnanasekaran *et al.* (2015) using PDA media.

Characterization of Fusarium oxysporum
Cultural Characterization

Fusarium oxysporum was cultivated on potato dextrose agar medium and had radial development with uneven edges of 50-90 mm. Pinkish-white cottony growth occurs in the colony. It emits a pinkish-purple tint into the media (Fig.1d). Similar cultural characteristics of *Fusarium* were discovered in earlier work by Gnanasekaran *et al*. (2015), who isolated *Fusarium oxysporum* f. sp. *cubense* causal agent of wilt disease in the banana plant from the infected stem of the banana plant using PDA medium, revealing that the aerial mycelium first appeared as white and then changed to dark purple colour.

Microscopic characterization

After incubation at 28°C on potato dextrose agar plates, a whitish fungal colony was observed and identified as *Fusarium oxysporum* based on colony morphology and observation of separate macroconidia, randomly distributed microconidia, and short-chained chlamydospores under the microscope (Fig.1e).

Molecular characterization

The eight isolates were identified and confirmed as *Fusarium oxysporum* by 18s rRNA sequencing, and the partial nucleotide sequence was deposited at GenBank, NCBI. Table 3 provides the information. MEGA-Version 7.0.14 can build a phylogenetic tree using the neighbor-joining (NJ) method with nucleotide pairwise variation adjustments from ABI sequencing files (Fig. 2).

Pathogenicity test of Fusarium oxysporum

Four of the eight isolates exhibited pathogenic symptoms on banana plants. Several leaves, plant height, leaf length, yellowing leaves that extend from leaf margins to the midrib, eventual collapse at the petiole, and longitudinal splitting of the outer leaf sheaths in the pseudostem were recorded for these four isolates on banana plants grown in standard culture(Fig.3). In control plants, no symptoms were observed or recorded. The results were recorded at planting, after one, two, and three months. During the first and second months after planting, the fewest symptoms were documented. During the third month, however, a greater variety of symptoms, including yellowing, curling, and stunted plant growth, were observed and recorded (Figs.4, 5, 6). Dinesh *et al*. (2014) conducted a pot culture experiment to investigate the interaction of *Radopholus similis* and *Fusarium oxysporum* f. sp. *cubense* on wilting banana. Nanjanagud Rasabale exhibited wilt symptoms sixty days after vaccination. In contrast, the first symptoms of Nendran appeared 75 days after vaccination. The onset of wilt symptoms occurred 90 days after Foc alone inoculated Nendran and Nanjanagud Rasabale plants, while Gabrekiristos *et al*. (2018) studied untreated seedlings that exhibited no symptoms of wilting, inoculated seedlings exhibited varying degrees of withering 10 days after inoculation. Foc colonies were effectively re-isolated from inoculated poyo exhibiting yellowing symptoms. Kubura *et al*. (2018) found that two months after inoculation of the isolated organism into banana plantlets, the leaves become wilted, yellow, and eventually collapse at the petiole, and the outer leaf sheaths of the pseudostem split longitudinally. The older foliage exhibited these symptoms.

CONCLUSION

Panama wilt, induced by *Fusarium oxysporum* f. sp. *cubense*, is one of the most significant banana

plant diseases. The disease is characterised by the yellowing of older leaves at the margins and then along the midrib, the drooping of deceased leaves as a skirt, and ultimately the death of the plant, resulting in economic loss. *Fusarium oxysporum* was isolated from infected *Musa paradisica* leaves, pseudostem, and rhizosphere soil and identified through morphological and molecular analysis. The pathogenicity test confirmed Koch's postulates by revealing Panama wilt disease in banana plants. *Fusarium oxysporum* is the causative agent of the Panama wilt disease that affects banana plants. The objective of this study is to devise methods for disease management and control of this disease.

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