

Original Research Article

Fusarium Wilt of Banana at Panchgaon

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ABSTRACT

Keywords

Fusarial wilt,
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Rhizoplane,
Infected stem

Wilted plants of banana were collected from Panchgaon. The isolation of fungi from rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted banana were done in order to know the dominance of the pathogen. Fungal species were isolated from the rhizosphere soils by serial dilution plate technique. In the present soil types a total of 19 fungal species belonging to 13 genera and two mycelia sterile were isolated. The *Fusarium* showed maximum percent occurrence in rhizosphere soil (83.4) and minimum in rhizoplane(46.3) while *Fusarium oxysporum* showed 71.6 per cent occurrence in infected stem and 51.6 per cent in collar region. The pathogenicity of this dominant pathogen confirmed that this is responsible for banana wilting causing similar wilting symptoms.

Introduction

Banana is a perennial herbaceous plant that grows from the underground rhizome. It flourishes well under tropical and moisture farmlands. Banana has unique growth, in fact the whole plant is a false stem. This false stem is consisting of broad leaves with their long petioles in disc like fashion. Banana is locally consumed fruit and is major staples for millions. Fusarium wilt of banana, popularly known as Panama disease, is a lethal fungal disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). The fungus enters the plant through the roots and colonizes the xylem vessels thereby blocking the flow of water and nutrients (Ploetz and Churchill,

2011). Since no systematic work on Banana wilting has been done at Panchgaon so wilted samples were brought in Laboratory in order to find out causal organism for banana wilting. So far that dominant fungal species and their pathogenicity were studied.

Materials and Methods

Survey of Panchgaon for severity of disease

A thorough survey of banana growing spots of Panchgaon was carried out in different months (March to June) from 2014 to 2015 to find the symptoms and severity of wilted

plants of banana. The natural diseased plants showing the typical symptoms of disease were selected from the field.

Soil preparation and collection

The rhizospheric soil samples were collected by digging out soil roots of banana plants. Randomly selected 5 diseased plants growing in one kilometer area were uprooted carefully and the excess of soil was removed by gentle shaking and the soil adhering to roots formed composite samples. The collected samples were placed in presterilized polyethylene bags and kept at 4°C in the laboratory until processed. Five such samples were collected from each diseased spot and mixed together to prepare a single polyethylene bag.

Mycobiota analysis of rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted banana

Fungal species were isolated from the rhizosphere soils by serial dilution plate technique. A known amount (10g) of material was suspended or agitated in a known volume of sterile water blank (90ml so as to make the total volume to 100ml) to make a microbial suspension. Serial dilutions 10^{-2} , 10^{-3} , 10^{-7} were made by pipetting measured volumes (usually 10ml) into additional dilution blanks (having 99ml or 90ml sterile water). Finally 1ml aliquot of various dilutions were added to sterile petridishes (triplicate for each dilution) to which were added 15ml (approximately) of the sterile, cool, molten (45°C) media. Potato Dextrose Agar media was used for mycobiota analysis and incubated at $28 \pm 2^\circ\text{C}$ for 2–7 days.

Per cent occurrence of each fungal species associated with root sample was calculated as per formula-

Percent occurrence = $\frac{\text{Number of colonies of a particular fungus}}{\text{Total number of colonies of all the fungi}} \times 100$

Identification of fungi

Fungal identifications were confirmed on following keys and description given by Raper and Thom (1949), Gilman (1967), Raper and Fennell (1965), Booth (1971) and Ellis (1971, 1976).

When *Fusarium* species is present, the isolate was grown on Synthetic Nutrient-Poor Agar (SNA) to analyse the shape of macroconidia and microconidia. Identification was done by comparing the morphology with the atlas of *Fusarium* (Leslie and Summerell, 2006; Samson *et al.*, 2008). A single spore of *Fusarium oxysporum* was used for further test (as master isolate).

Pathogenicity

Four healthy plants of banana were selected from nursery. A cut was made in the stem of 3 healthy plants with the help of a sterilized knife and inoculated with 1×2 cm block of the isolated culture of *Fusarium oxysporum* isolated from naturally infected diseased plants in the field.

Following this method a cut was made in the remaining one healthy plant to serve as a control and inoculated with 1×2 cm block of only PDA and wrapped with parafilm. Plants were monitored for the development of disease symptoms and pathogens were reisolated from stem of the test plants after 25 days to confirm the pathogenicity.

Results and Discussion

It is evident from figure 1, that there is discolouration in root and pseudostem. The

fungus invades the vascular tissue through the root hairs causing discolouration from pale yellow in the early stages to dark red or almost black in later stages and finally wilting eventually killing the plant. The fruit of the plant do not exhibit any symptom.

Fusarium oxysporum are in direct contact with root and root hairs (Figure 2). Due to infection the collapsed leaves hang down the pseudostem like a skirt. Eventually, all the leaves fall down and dry up (Figure 3).

Table.1 Per cent occurrence of isolated fungi from rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted banana

Fungal species	Per cent occurrence of isolated fungi			
	rhizosphere soils	rhizoplane	infected stem	infected collar region
<i>Aspergillus aculeatus</i> Iizuka,	1.9	1.3	1.4	1.5
<i>A. flavus</i> Link,	3.1	3.3	0.3	1.4
<i>A. fumigatus</i> Fresenius	1.9	2.5	3.1	2.9
<i>A. niger</i> Van Teighmer	3.0	2.2	2.3	14.0
<i>A. ochraceous</i> K. Wilh.	3.7	4.1	1.5	1.5
<i>A. sulphureous</i> (Fresen.) Wehmer,	2.1	2.2	3.2	1.6
<i>A. tamari</i> Kita	1.4	1.5	3.5	3.6
<i>A. versicolor</i> (Vuillemin) Tiraboschi.	2.5	3.6	1.2	1.7
<i>Alternaria solani</i> Keissler	0.4	4.3	2.1	2.1
<i>Cladosporium cladosporioides</i> (Fres.) de Vries,	3.9	2.4	3.5	3.0
<i>Colletotrichum</i> sp.	10.7	14.4	16.1	14.3
<i>Curvularia lunata</i> (Wakker) Boedijn var	3.1	4.1	6.0	3.6
<i>Fusarium oxysporum</i> Schl	83.4	46.3	71.6	51.6
<i>Helminthosporium oryzae</i> Breda de Hann	0.5	6.9	5.3	4.1
<i>Humicola grises</i> Omvik	10.0	11.4	13.4	14.1
<i>Paecilomyces</i> sp	0.5	10.5	11.5	12.0
<i>Penicillium oxalicum</i> Currie & Thorn	6.8	3.9	2.5	4.1
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	11.5	13.4	12.4	11.4
Sterile mycelium black	9.5	10.4	11.4	13.4
Sterile mycelium white	0.5	1.5	0.5	1.0
<i>Trichoderma viride</i> Persand Fris	0.0	0.5	0.5	0.0

Figure.1 Discolouration in root and pseudostem



Figure.2 Colonisation of fungus with root and root hairs of banana



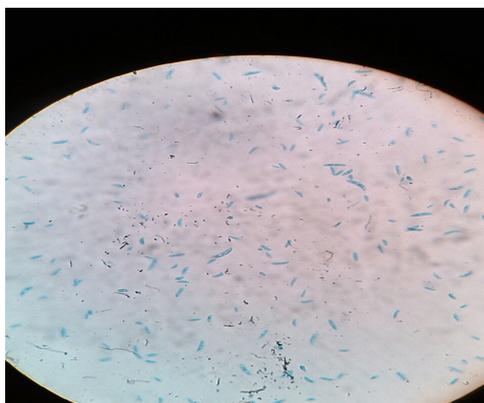
Figure.3 Collapsed leaves of banana due to fungal infection



Figure.4 Pure colony of *Fusarium oxysporum*



Figure.5 Spores of *Fusarium oxysporum*



In the present soil types a total of 19 fungal species belonging to 13 genera and two mycelia sterile were isolated (Table 1). Among total 19 fungal species, 8 species were *Aspergillus*, 1 species each of

Fusarium, *Colletotrichum*, *Cladosporium*, *Alternaria*, *Curvularia*, *Humicola*, *Penicillium*, *Paecilomyces*, *Rhizopus*, *Trichoderma*, *Mycelia sterilia black*, *Mycelia sterile white*. The species of

Aspergillus isolated were *A. flavus* Link, *A. fumigatus* Fresenius *A. niger* van Tieghan, *A. ochraceus* K. Wilh. *A. sulphureus* (Fresen.) Wehmer, *A. tamari* Kita *A. versicolor* (Vuillemin) Tiraboschi. The species of *Aspergillus* showed per cent occurrence in the range of 0.3-4.1. *Cladosporium cladosporioides* (Fres.) de Vries was in the range of 2.4–3.9 in different districts. The species of *Fusarium* isolated were *F. oxysporum* Schl Saccardo. The fungus *F. oxysporum* Schl are highly alarming on the basis of percent occurrence. The *Fusarium* showed maximum percent occurrence in rhizosphere soil (83.4) and minimum in rhizoplane (46.3) while *Fusarium oxysporum* showed 71.6 per cent occurrence in infected stem and 51.6 in collar region on the basis of percent occurrence. The *Trichoderma viride* Pers and Fris showed lowest percent occurrence so its low per cent occurrence is highly alarming. It indicates that biocontrol agents are decreasing in the soil. Other fungal species showed moderate or lower percentage occurrence.

The mycelia of *Fusarium oxysporum* are delicate white and are sparse to abundant. The fungus showed three types of spores: microconidia, macroconidia and chlamydospores. Microconidia are borne on simple phialides arising laterally and are abundant, oval-ellipsoid, straight to curved, 4-11 x 2.1-3.4 μm , and nonseptate. Macroconidia, sparse to abundant, are borne on branched conidiophores or on the surface of sporodochia and are thin walled, three- to five-septate, fusoid-subulate and pointed at both ends, have pedicellate base. Three-septate conidia measure 26–45 x 3–4 μm while five-septate conidia measure 32–57 x 3–5 μm . Three-septate spores are more common. Chlamydospores, both smooth and rough walled, are abundant and form terminally or on an intercalary basis (Figure

4 & 5). *Fusarium oxysporum* usually produces pale violet to dark red color pigments in PDA (Stover, 1962; Ploetz, 1990; Pérez-Vicente *et al.*, 2003). Similarly in present investigation this also produced similar pigments.

This can be placed as –Domain: Eukaryota; Kingdom: Fungi; Phylum: Ascomycota; Class: Ascomycetes; Subclass: Sordariomycetidae; Order: Hypocreales

It was found that during pathogenicity *Fusarium oxysporum* showed similar wilting symptoms in all four inoculated plants while uninoculated plants remained healthy. On account of wide occurrence and their pathogenicity these were selected as test organisms.

The report on wilt exists from 1934, as *Fusarium cubense* causing a severe wilt of bananas in Fiji (Parham, 1935). '*Fusarium oxysporum-musae*' was isolated from bananas in Guam in the early 1980s (Russo *et al.*, 1985). *Fusarium* wilt is responsible for the demise of the export. The first losses followed soon after *Fusarium* wilt was reported in Panama and Costa Rica in the 1890s (Soluri, 2002).

Recently, Mark (2014) mentioned that *Fusarium* wilt or Panama disease of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is among the most destructive plant diseases.

In conclusion, based on the findings of this investigation, it may be concluded that population of *Fusarium oxysporum* in terms of percent occurrence is increasing in soil and this is responsible for wilting of banana. This is present in rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted banana.

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