

GROWTH CHARACTERISTICS OF DIFFERENT ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* ON SABOURAUD'S AND POTATO DEXTROSE AGAR MEDIA

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ABSTRACT

Fourteen different isolates of *Fusarium oxysporum* f. sp. *cubense* studied *in vitro* to know their growth and morphological characteristics on Sabouraud's and Potato dextrose agar media. Among fourteen isolates studied Maddur (Kowdle) isolate showed best growth and sporulation on Sabouraud's medium with pinkish to purple colour colony growth and maximum macro and micro conidial spore size of 31.4 × 3.8 and 10.8 × 3.2 respectively. Whereas in Potato Dextrose Agar Mysore (Thandavapura), Maddur (Koppa) and Mysore (Konnur) isolates revealed maximum growth and sporulation with whitish to pink colour colony growth. The maximum spore size of macro (31.4 × 3.7) and micro (10.5 × 3.4) conidia was found best in Bangalore (Shivakote) isolate.

INTRODUCTION

The Karnataka is the major banana producing state after Tamil Nadu and Maharashtra because of its diverse range of climatic conditions banana can be grown throughout the year. In the Karnataka, 102.71 thousand hectare area comes under banana cultivation which produces 2675.63 thousand million tonnes of banana annually (Anon., 2014).

The main hurdle in increasing the productivity is the threat posed by pest and diseases. Among the diseases *Fusarium* wilt also known as Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* is the major constraint to banana production and the disease has been ranked as No.1 fungal disease of banana in India. In southern India, incidence of Panama wilt is widespread in some districts the disease incidence is as high as 80-90% (Sivamani, 1987). The disease occludes the xylem vessels causing water blockage. It survives in soil for longer periods and thus susceptible genotypes cannot be grown in an infested field for up to 30 years (Plotez, 2000).

Fusarium wilt of banana or Panama disease is one of the most economic important and harmful diseases of *Musa*. During the first half of the last century was the cause of destruction of more than 50,000 ha of Gros Michel and the substitution for Cavendish cultivars together with important transformations of the banana export industry. Every living being requires food for its growth and reproduction; fungi are not exception to it.

Fungi secure food from the substrate upon which they live in. In order to culture the fungus in the laboratory, it is necessary to furnish the essential elements and compounds in the medium, for their growth and other life processes. All media are not equally good for all fungi, nor there is a universal substrate or artificial medium, upon which all fungi can grow (Lilly and Barnett, 1951). Various culture media showed differential effects on the growth and cultural characteristics of different fungal pathogen on various host plants (Singh and Kaiser, 1994). In the same way different isolates of same species show difference in their growth and morphological characters when they grow on same medium or different medium with respect to their spore size, shape and mycelia colour. Hence, the present study aimed in identification of variability among different isolates of *Fusarium oxysporum* f. sp. *cubense* two different media.

MATERIALS AND METHODS

An experiment was carried out during 2011 at K. R. C. College of Horticulture, Arabhavi to find out growth and morphological characters among different isolates of *Fusarium oxysporum* f. sp. *cubense* *in vitro*. The experiment was designed in Complete Randomized Design (CRD) with three replications and fourteen treatments.

Plants showing wilting symptoms were collected from the

farmer's field during the survey. Infected rhizomes were cut into small bits and washed well in running water to remove the adhering soil particles. The cut pieces were surface sterilized by immersing the pieces in 1 per cent mercuric chloride solution for one minute. The bits were washed thoroughly in sterile distilled water 3 times to remove traces of mercuric chloride and dried between 2 sterilized filter paper and then aseptically transferred to sterile potato dextrose agar (PDA) amended with streptomycin sulphate (0.01 per cent) in petriplates and incubated at $28 \pm 1^\circ\text{C}$ for 7 days. The growing fungi were individually transferred to PDA medium. Pure culture of *Fusarium oxysporum* f.sp.cubense was obtained by using single spore or hyphal tip technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 4°C for further use. Based on the pathogenesis test, highly virulent isolate of *Fusarium oxysporum* f.sp.cubense was used for further studies (Pradeep et al., 2013)

Variation among fourteen different isolates was studied on two media viz., Sabouraud's medium and potato dextrose agar medium. The media were sterilized at 1.1 kg cm^{-2} pressure

for 15 min. To carry out the study, 30 ml of each of this medium was poured into 90 mm diameter petri dishes. After solidification 5 mm discs of *Fusarium oxysporum* f. sp. cubense were prepared and a single disc was placed at the center of the plate. Each set was replicated thrice and plates were incubated at $26 \pm 1^\circ\text{C}$. Observations were recorded after eight to nine days of incubation with respect to mycelial colour, substrate colour, margin of the colony, topography, centre of the colony, colony size, sporulation and spore size. The data on radial growth was analyzed statistically. (Khan et al., 2011)

RESULTS AND DISCUSSION

Based on growth and sporulation of the pathogen, sabouraud's medium and potato dextrose agar medium were chosen to study the 14 different isolates of *Fusarium oxysporum* f. sp. cubense.

Sabouraud's agar medium

Results revealed that Sabouraud's medium was best for the growth of Maddur (Kowdle) isolate with mean maximum

Table 1: Effect of growth of different isolates of *Fusarium oxysporum* f. sp. cubense on Sabouraud's agar medium

Isolate	Colony diameter (mm)				
	2 nd day	4 th day	6 th day	7 th day	8 th day
Bangalore (Hessaraghatta)	22.67	37.00	51.00	55.33	60.00
Bangalore (Mathikere)	22.00	40.67	53.00	60.00	64.67
Bangalore (Shivkote)	23.00	42.33	55.00	59.00	63.67
Channapatna (Abbur)	20.33	40.00	53.00	60.00	64.67
Channapatna (Chikkanahalli)	22.33	40.67	50.67	55.33	60.33
Channapatna (Tagachagere)	21.00	41.33	53.00	59.67	64.67
Maddur (Koppa)	22.33	41.67	54.00	59.00	65.00
Maddur (Kowdle)	24.67	43.67	56.33	62.00	71.00
Mandya (Kotigere)	22.67	41.33	52.33	61.00	65.00
Mandya (Bukanakere)	19.33	40.00	51.67	58.33	62.33
Mysore (Nanjanagudu)	25.33	41.33	53.00	57.33	62.33
Mysore (Devarasanahalli)	21.33	38.67	48.67	53.67	59.33
Mysore (Konnur)	24.67	41.67	52.00	56.67	62.67
Mysore (Thandavapura)	22.67	39.67	51.33	60.00	64.67
S.Em \pm	0.63	0.91	1.09	1.38	1.47
CD @ 1%	2.46	3.55	4.26	5.40	5.76

Table 2: Morphological characters of different isolates of *Fusarium oxysporum* f. sp. cubense on Sabouraud's agar medium

Isolate	Mycelial colour	Substrate colour	Margin of the colony	Topography	Center of the colony	Spore size (μm)		Sporulation
						Macroconidia	Microconidia	
Bangalore (Hessaraghatta)	Purple	Purple	Irregular	Aerial mycelium	Purple	25.1x3.8	6.0x2.3	++++
Bangalore (Shivakote)	Whitish-purple	Purple	Irregular	Aerial mycelium	Purple	26.4x3.1	7.1x3.1	++++
Bangalore (Muthikere)	purple	whitish-purple	Irregular	Aerial mycelium	Purple	22.8x2.9	5.8x2.3	++++
Channapatna (Abbur)	Whitish-purple	Whitish-purple	Irregular	Aerial mycelium	Whitish-Purple	23.6x3.1	6.0x2.6	++++
Channapatna (Tagachagere)	Grey-purple	Grey-purple	Irregular	Aerial mycelium	Purple	28.4x3.8	10.2x3.1	++++
Channapatna (Chikkanahalli)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Purple	21.6x2.9	8.1x2.9	++++
Maddur (Konnur)	Pink-purple	Pinkish-purple	Irregular	Aerial mycelium	Dark Pink	31.4x3.8	10.8x3.2	++++
Maddur (Kowdle)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Purple	28.1x3.5	7.8x2.8	+++
Mandya (Kotigere)	Purple	Purple	Irregular	Aerial mycelium	Purple	28.7x3.7	8.6x3.3	++++
Mandya (Bukanakere)	whitish-purple	whitish-purple	Irregular	Aerial mycelium	Purple	25.1x3.1	10.4x2.8	++++
Mysore (Nanjanagudu)	Purple-pink	Purple-pink	Irregular	Aerial mycelium	Whitish-purple	28.7x3.2	7.1x2.7	++++
Mysore (Devarasanahalli)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Purple	25.4x3.6	6.8x2.6	++++
Mysore (Konnur)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Purplish-pink	31.3x3.0	11.3x3.1	++++
Mysore (Thandavapura)	Whitish-purple	Purple	Irregular	Aerial mycelium	Purple	27.2x3.3	8.1x2.4	++++

++++ = >75 conidia per microscopic field ; +++ = 50-75 conidia per microscopic field; ++ = 25-50 conidia per microscopic field; + = 1-25 conidia per microscopic field.

Table 3: Effect of growth of different isolates of *Fusarium oxysporum* f. sp. *cubense* on potato dextrose agar medium

Isolate	Colony diameter (mm)				
	2 nd day	4 th day	6 th day	8 th day	9 th day
Bangalore (Hessaraghatta)	23.67	42.67	62.33	78.67	88.00
Bangalore (Mathikere)	23.00	45.00	59.67	79.33	89.00
Bangalore (Shivakote)	23.67	45.33	64.00	82.33	89.33
Channapatna (Abbur)	22.67	41.67	56.67	74.33	84.67
Channapatna (Chikkanahalli)	22.33	46.00	60.33	78.33	88.67
Channapatna (Tagachagere)	21.33	43.67	61.67	80.67	88.67
Maddur (Koppa)	23.33	44.33	66.00	85.33	90.00
Maddur (Kowdle)	20.33	44.33	58.67	78.33	87.00
Mandya (Kotigere)	20.33	36.33	48.67	65.00	75.67
Mandya (Bukanakere)	21.33	39.00	54.67	76.00	87.33
Mysore (Nanjanagudu)	21.00	35.33	51.00	64.00	76.00
Mysore (Devarasanahalli)	22.67	42.67	61.67	77.67	88.33
Mysore (Konnur)	23.00	45.67	64.00	82.33	90.00
Mysore (Thandavapura)	25.00	46.33	66.67	85.67	90.00
S.Em ±	0.64	1.172	1.643	1.817	1.218
CD @ 1%	2.51	4.579	6.419	7.101	4.761

growth of 71.00 mm followed by Maddur (Koppa) and Mandya (Kotigere) isolates showed 65.00 mm radial growth. Bangalore (Mathikere), Channapatna (Abbur), Channapatna (Tagachagere) and Mysore (Thandavapura) isolates showed mean growth of 64.67 mm next best isolates was Bangalore (Shivakote) and Mysore (Nanjanagudu) showed 62.33 mm growth. Channapatna (Chikkanahalli) and Bangalore (Hessaraghatta) isolates showed 60.33 mm and 60.00 mm mycelia growth respectively. Whereas, lesser radial growth of 59.33 mm was observed in Mysore (Devarasanahalli) isolate after eight days of inoculation. The results are shown in the Table 1.

Purple coloured colony growth was seen in Bangalore (Hessaraghatta), Bangalore (Mathikere) and Mandya (Kotigere) isolates. Whitish to purple coloured growth pattern was observed in Bangalore (Shivakote), Channapatna (Abbur), Mandya (Bukanakere) and Mysore (Thandavapura) isolates. Grey to purple colour mycelium was seen in Channapatna (Tagachagere) isolates. In Channapatna (Chikkanahalli), Maddur (Kowdle), Mysore (Devarasanahalli), Maddur (Kowdle) isolates pinkish to purple coloured growth was observed. Whereas, pink to purple and purple to pink coloured growth was found in Maddur (Koppa) and Mysore (Nanjanagudu) isolates. Good sporulation was produced in all the isolates except Bangalore (Shivakote) and Channapatna (Chikkanahalli) followed by Mysore (Thandavapura). The size of macro and micro conidia ranged from 21.6-31.4 x 2.9-3.8 mm and 6.0-11.3 x 2.3-3.3 mm respectively. Morphological characteristics are shown in Table 2.

The result of the experiment revealed that Sabouraud's medium was best for the growth of Maddur (Kowdle) isolate. Whereas, lesser radial growth was observed in Mysore (Devarasanahalli) isolate. All the isolates produced whitish to purple, grey to purple, pinkish to purple and purple colour mycelium. Good sporulation was produced in all the isolates except Bangalore (Shivakote) and Channapatna (Chikkanahalli) followed by Mysore (Thandavapura). Nagaraj and Jahagirdar 2014 reported among the eleven isolates collected from six different states exhibited morphological variability with respect to colony colour varied from cottony white to dark gray, spore

ranging between 20.10-23.00 x 3.2-4.50 to 21.10- 24.20 x 3.8-4.10 μ m. The colony colour was dark gray in Bidar isolate of Karnataka, Ujjain isolate of Madhya Pradesh whereas Bagalkot isolate of Karnataka and Kota isolate of Rajasthan produced white colony colour. The rest of the other isolate produced distinct colony colour.

Potato dextrose agar medium

The best mycelial growth of 90.00 mm was produced on Potato Dextrose Agar by Mysore (Thandavapura), Maddur (Koppa) and Mysore (Konnur) isolates followed by Bangalore (Shivakote) (89.33mm), Bangalore (Mathikere) (89.00 mm), Channapatna (Tagachagere) (88.67 mm), Channapatna (Chikkanahalli) (88.67), Mysore (Devarasanahalli) (88.33 mm), Bangalore (Hessaraghatta) (88.00 mm), Mandya (Bukanakere) (87.33 mm), Maddur (Kowdle) (87.00 mm) and Channapatna (Abbur) (84.67) after nine days of inoculation. Lowest growth was recorded in Mandya (Kotigere) isolate of 75.67 mm and Mysore (Nanjanagudu) isolate of 76.00mm. The results are mentioned in the Table 3.

All the isolates produced whitish to pink coloured colony growth except Bangalore (Hessaraghatta) and Bangalore (Mathikere) isolates produced pinkish coloured mycelium growth and Bangalore (Shivakote) isolates produced pink colour colony growth. Excellent sporulation was produced in all other isolates except in Maddur (Kowdle), Channapatna (Abbur), Mysore (Nanjanagudu) and Channapatna (Tagachagere). The size of macro and micro conidia ranged from 23.7-31.4 x 2.8-3.9 mm and 6.0-10.5 x 2.4-3.4 mm respectively. Morphological characteristics are presented in Table 4.

The best mycelial growth was produced on Potato Dextrose Agar by Mysore (Thandavapura), Maddur (Koppa) and Mysore (Konnur) isolates followed by Bangalore (Shivakote), Bangalore (Mathikere), Channapatna (Tagachagere), Mysore (Devarasanahalli), Bangalore (Hessaraghatta), Mandya (Bukanakere) and Maddur (Kowdle). Lowest growth was recorded in Mandya (Kotigere) isolate and Mysore (Nanjanagudu) isolate. The mycelial colour was pinkish and whitish to pink in all the isolates. Excellent sporulation was

Table 4: Morphological characters of different isolates of *Fusarium oxysporum* f. sp. *cubense* on potato dextrose agar medium

Isolate	Mycelial colour	Substrate colour	Margin of the colony	Topography	Centre of the colony	Spore size (μm)		Sporulation
						Macro-conidia	Micro-conidia	
Bangalore (Hessaraghatta)	Pinkish	Pinkish	Irregular	Aerial mycelium	Pink	30.1x3.9	7.5x2.7	+++ +
Bangalore(Shivakote)	Pink	Pink	Irregular	Aerial mycelium	pinkish	31.4x3.7	10.5x3.4	+++ +
Bangalore(Muthikere)	Pinkish	Pink	Irregular	Aerial mycelium	pinkish	31.3x3.6	8.2x2.7	+++ +
Channapatna (Abbur)	Whitish -pink	Whitish-pink	Irregular	Aerial mycelium	pinkish	28.6x3.4	7.0x3.1	+++ +
Channapatna(Tagachagere)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	27.4x3.0	6.3x2.8	+++ +
Channapatna(Chikkanahalli)	Whitish- pink	pink	Irregular	Aerial mycelium	pinkish	27.8x3.1	8.3x3.2	+++ +
Maddur(Konnur)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	30.1x3.0	7.8x2.8	+++ +
Maddur(Kowdle)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	28.2x3.1	8.1x3.0	+++ +
Mandya (Kotigere)	Whitish- pink	pink	Irregular	Aerial mycelium	pinkish	24.5x3.2	6.5x2.7	+++ +
Mandya(Bukanakere)	Whitish- pink	pink	Irregular	Aerial mycelium	pinkish	27.8x2.8	8.2x2.8	+++ +
Mysore(Nanjungudu)	Whitish- pink	Whitish-pink	Irregular	Aerial mycelium	pink	23.7x2.9	6.0x2.4	+++ +
Mysore(Devartasanahalli)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	26.4x3.3	7.8x2.7	+++ +
Mysore(Konnur)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	28.5x3.4	9.3x3.0	+++ +
Mysore(Thandavapura)	Whitish- pink	pink	Irregular	Aerial mycelium	pink	25.4x3.6	6.8x2.8	+++ +

recorded in all the isolates except in Maddur (Kowdle), Channapatna (Abbur), Mysore (Nanjanagudu) and Channapatna (Tagachagere) isolates. Jamaria (1972) also reported maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* on potato dextrose agar. Khilare and Rafi Ahmed (2012) the fungus grew the best on Czapekdox agar and PDA media among six culture media were tested. Khan *et al.* (2011) tested for variation in growth and cultural characters on five different solid media, PDA found to be best for the growth of different isolates. Pradeep *et al.* (2013) reported that out of eight different solid and six liquid media studied, *Fusarium moniliforme* KUMBF1201 grew well on Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media.

Kumar *et al.* (2010) reported that most of the isolates produced aerial mycelium with violet pigmentation on PDA (Potato Dextrose Agar). Pigmentation varied from white to dirty white, pinkish to greenish violet in isolates FOC 7, FOC 3, FOC 4 and FOC 6 respectively. Patel *et al.* (2011) the size of microconidia ranged from 2.9-7.7 x 1.4-2.8 mm in FU-14 and 2.9-8.8 x 1.8-2.9 mm in FU-21 to 8.2-9.4 x 1.2-2.5 mm in FU-7. The size of macro-conidia ranged from 10.2-17.6 x 2.8-3.4 mm in FU-6 to 32.5-62.5 x 3.9-5.2 mm in FU-15 in *Fusarium udum*. Lal *et al.* (2014) reported that Potato dextrose agar (7.60 cm) and Host extract agar (6.73 cm) were the best for fungus growth as well as for sporulation.

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