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GROWTH CHARACTERISTICS OF DIFFERENT ISOLATES, NITROGEN SOURCES AND EFFICACY OF BIOLOGICAL AGENT ON *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* CAUSING PANAMA WILT IN BANANA

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ABSTRACT
Fourteen different isolates of *Fusarium oxysporum* f. sp. *cubense* studied *in vitro* to know there growth and morphological characteristics on Richard's medium and Asthana and Hawkers medium.Among fourteen different isolates grown in Richard's agar, Maddur (Kowdle) and Mysore (Konnur) isolates produced maximum growth of 90.00mm and good sporulation (>75conidia per microscopic field) with pinkish to purple colour colony growth and maximum macro and micro conidial spore size of 33.4x3.8 and 13.1x3.3μm respectively.In Asthana and Hawkers medium Channapatna (Abbur)and Bangalore (Hessaraghatta)isolates showed best growth of 90.00 and 87.67 mm respectively with good sporulation and isolates produced whitish to purple colour mycelium and maximum macro and micro conidial spore size of 30.3x3.7 and 12.3x3.0μm respectively. Among the different nitrogen sources tested in Richard's medium, ammonium nitrate was better utilized by Bangalore and Mysore isolates, sodium nitrate was better utilized by Channapatna isolate, the mean dry weight of mycelium was 351.77, 356.03 and 435.47 mg respectively. In Czapek's medium all the three isolates of Bangalore, Channapatna, and Mysore produced maximum growth in sodium nitrate the mean dry weight of mycelium was 439.07, 436.50 and 452.13 mg respectively. Seven biological agents were tested against FOC, among them *Trichoderma viride* (NBAIT), *Trichoderma viride*-27 and *Trichoderma harzianmum*-55 showed maximum Per cent inhibition of 70.42, 76.60 and 65.25 respectively over the control.

INTRODUCTION

Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) 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).

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.Nitrogen is an important element for protein synthesis and like carbon it is used by fungi for functional as well as structural purpose. Developing appropriate formulation and delivery systems is the prerequisite for implementing biological control using microbial antagonists (Lumsden and Lewis, 1989). Hence there is a need to know about the variability produced by different isolates on different media and nitrogen sources, at a same time to obtain effective bio agent against FOC.

The objective of the study is to screen the different isolates of FOC on different media to know the variability among the isolates for their growth and morphological characteristics and also to evaluate different isolates for best nitrogen source.

MATERIALS AND METHODS

An experiment was carried out during 2011-12 at Department of Plant Pathology K. R. C. College of Horticulture, Arabhavi to know growth and morphological characters among different isolates of FOC,best nitrogen source for FOC and different bioagents were evaluated to find out which bioagent is effective against FOC. The experiment was designed in Complete Randomized Design (CRD).

Growth characteristics of different isolates of *Fusarium oxysporum* f. sp. *cubense*

Fourteen different isolates were studied on two media *viz.*,Richard's agar and Asthana and Hawker's medium.All 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 pertidishes.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 ± 1ºC.Observations were recorded 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).

Effect of nitrogen source on growth of *Fusarium oxysporum* f. sp. *cubense*

This experiment was conducted to find out the sources of nitrogen, which is used most efficiently by the fungus for its growth.Several nitrogen sources were tried by incorporating them in Richard's and Czapek's broth for different isolates *viz.,* Bangalore, Channapatna and Mysore. The quantity of each nitrogen compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of nitrogen as was of potassium nitrate present in the basal medium.The nitrogen compounds used were Ammonium chloride, Ammonium nitrate, Asparagine, Potassium nitrate, Sodium nitrate, Ammonium sulphate and Ammonium orthophosphate.30 ml of each media were poured into 100 ml flasks plugged with nonabsorbent cotton and autoclaved at 1.1 kg/cm^2 pressure for 15 min.Each of the treatment was replicated thrice. All the flasks were aseptically inoculated with 5 mm of prepared suspension of 10 days old culture of *Fusarium oxysporum*. f. sp. *cubense*.Inoculated flasks were incubated at 26 ± 1ºC for nine days. The mycelia were harvested and dried to a constant weight.Results were analyzed statistically.(Ramteke and Kamble, 2011).

Evaluation of bio-agents against *Fusarium oxysporum* f. sp. *cubense*

Seven biocontrol agents such as *Trichoderma harzianum*-55, *Trichoderma viride*-27, *Trichoderma viride* (NBAIT), *Trichoderma viride*(Local), *Pseudomonas fluorescens, Pseudomonas virideflava* and *Bacillus subtilis* were tested against *Fusarium oxysporum* f. sp. *cubense*. Both biocontrol agents and test fungus were cultured on potato dextrose agar in orderto get fresh and active growth of fungus.

The 5 mm of fungal disc of the antagonist along with test fungus were kept on the potato dextrose agar medium in opposite direction.The plates were incubated for a week.The observation on interaction zone or inhibition zone was measured.The growth of antagonistic fungus and growth of pathogen were also recorded separately.The bacterial antagonist *viz.,Bacillus subtilis,Pseudomonas fluorescens* and *Pseudomonas virideflava* were streaked on side of the potato dextrose agar medium and on other side *Fusarium oxysporum* f. sp. *cubense* disc was placed.The inhibition zone was measured.After the period of incubation, the growth of the *Fusarium oxysporum* f.sp. *cubense* colony was recorded and the per cent inhibition of the colony over control was calculated. (Thangavelu *et al.,*2004)

RESULTS AND DISCUSSION

In the present study, the growth characteristics of different isolates and sporulation of the pathogen were studied on Richard's agar, Asthana and Hawker's medium and results are presented in Table 1-4.Seven nitrogen sources on the growth of three different isolates of FOC were studied by incorporating in to Richard's broth and Czapek's broth.The data on dry weight of mycelium was noted after nine days of incubation. The results are furnished in Table 5 and 6. Bio-agents were evaluated against FOC data on per cent inhibition over control was recorded and presented in table 7.

Growth characteristics of different isolates of *Fusarium oxysporum* f. sp. *cubense*

Richard's agar medium

The Maddur (Kowdle) isolate produced maximum growth of 90.00 mm on Richard's agar at 8th day of inoculation followed by Mysore (Konnur) (90.00 mm), Mysore (Thandavapura) (84.00 mm), Channapatna (Tagachagere) (84.00 mm), Channapatna (Chikkanahalli) (84.00 mm), Channapatna (Abbur) (83.33 mm), Bangalore (Mathikere) (83.33 mm), Bangalore (Hessaraghatta) (82.67 mm), Mysore

Table 1: Studies on Radial growth of different isolates of *Fusarium oxysporum* f. sp. *cubense* on Richards's media

Isolate	Colony diameter (mm)				
	2nd day	4th day	6th day	7th day	8th day
Bangalore (Hessaraghatta)	23.33	43.00	61.00	72.33	82.67
Bangalore (Mathikere)	25.00	44.67	64.00	74.33	83.33
Bangalore (Shivakote)	22.33	42.00	62.00	72.67	82.33
Channapatna (Abbur)	25.33	43.00	64.00	74.67	83.33
Channapatna (Chikkanahalli)	24.33	42.33	62.67	73.67	84.00
Channapatna (Tagachagere)	23.67	42.67	63.33	73.67	84.00
Maddur (Koppa)	21.67	42.00	61.33	72.00	81.33
Maddur (Kowdle)	26.67	51.00	70.67	80.33	90.00
Mandya (Kotigere)	21.67	41.67	59.00	68.33	77.67
Mandya (Bukanakere)	24.00	43.67	61.67	71.00	81.00
Mysore (Nanjanagudu)	23.00	41.67	62.33	72.00	81.33
Mysore (Devarasanahalli)	20.67	41.33	61.00	71.67	82.67
Mysore (Konnur)	27.67	54.00	72.67	83.00	90.00
Mysore (Thandavapura)	24.33	43.67	62.33	72.67	84.00
S.Em ±	0.67	1.07	0.97	0.82	0.85
CD @ 1%	2.61	4.16	3.80	3.21	3.34

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

Isolate	Mycelial colour	Substrate colour	Margin of the colony	Topography	Center of the colony	Spore size (μm)		Sporulation
						Marco-conidia	Micro-conidia	
Bangalore (Hessaraghatta)	Pinkish-orange	Pinkish-orange	Irregular	Aerial mycelium	Pinkish	25.0x3.6	9.7x2.8	+ + + +
Bangalore(Shivakote)	pinkish -purple	Pinkish-purple	Irregular	Aerial mycelium	Pinkish	22.3x3.1	5.4x2.4	+ + + +
Bangalore(Muthikere)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Pinkish	28.1x3.8	7.3x3.0	+ + + +
Channapatna (Abbur)	Pinkish-white	Pinkish- white	Irregular	Aerial mycelium	Purple	28.4x3.6	6.4x2.6	+ + + +
Channapatna(Tagachagere)	Pinkish- purple	Pinkish-purple	Irregular	Aerial mycelium	Pinkish	27.6x3.1	6.0x2.3	+ + + +
Chanpatna(Chikkanahalli)	Pinkish-white	Pinkish-white	Irregular	Aerial mycelium	Whitish-pink	24.7x3.2	6.0x2.4	+ + + +
Maddur(Konnur)	Pinkish-orange	Pinkish-orange	Irregular	Aerial mycelium	Pinkish	20.2x3.0	8.2x3.1	+ + + +
Maddur(Kowdle)	Pinkish-purple	Pinkish-purple	Irregular	Aerial mycelium	Pinkish	33.4x3.8	13.1x3.3	+ + +
Mandya (Kotigere)	Whitish-pink	Whitish-orange	Irregular	Aerial mycelium	Orange	30.2x3.7	10.2x3.4	+ + + +
Mandya(Bukanakere)	Purple-pink	Purple-pink	Irregular	Aerial mycelium	Pinkish	22.4x3.1	11.6x2.8	+ + + +
Mysore(Nanjungudu)	Whitish	Whitish	Irregular	Aerial mycelium	Whitish-purple	22.1x3.5	10.5x3.0	+ + + +
Mysore(Devartasanahalli)	Pinkish-white	Pinkish-white	Irregular	Aerial mycelium	White	31.4x3.8	12.7x3.1	+ + + +
Mysore(Konnur)	Whitish-orange	Whitish-orange	Irregular	Aerial mycelium	Orange	28.1x3.0	10.1x2.8	+ + + +
Mysore(Thandavapura)	Purplish-pink	Purple-pink	Irregular	Aerial mycelium	Purplish-pink	33.2x3.8	9.6x3.2	+ + + +

+ + + + = >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: Studies on Radial growth of different isolates of *Fusarium oxysporum* f. sp. *cubense* on Asthana and Hawker's media

Isolate	Colony diameter (mm)				
	2nd day	4th day	5th day	6th day	7th day
Bangalore (Hessaraghatta)	24.33	51.00	64.33	76.67	87.67
Bangalore (Mathikere)	25.33	54.00	65.00	77.33	85.33
Bangalore (Shivakote)	23.00	46.67	56.33	67.33	76.00
Channapatna (Abbur)	26.33	53.33	65.00	78.33	90.00
Channapatna (Chikkanahalli)	24.67	48.33	58.33	69.00	80.33
Channapatna (Tagachagere)	19.67	44.67	56.33	66.33	75.67
Maddur (Koppa)	22.33	46.67	58.00	69.00	79.00
Maddur (Kowdle)	23.00	47.67	58.33	69.33	79.00
Mandya (Kotigere)	19.33	44.67	56.00	69.00	83.33
Mandya (Bukanakere)	19.00	44.67	56.67	69.67	82.33
Mysore (Nanjanagudu)	21.00	44.33	56.00	67.33	80.67
Mysore (Devarasanahalli)	24.33	47.33	57.00	69.33	79.00
Mysore (Konnur)	24.67	47.00	59.33	68.67	79.33
Mysore (Thandavapura)	24.00	46.33	58.33	67.00	76.33
S.Em ±	0.64	1.11	1.04	0.89	1.21
CD @ 1%	2.49	4.33	4.07	3.48	4.71

Table 4: Morphological characters of different isolates of *Fusarium oxysporum* f. sp. *cubense* on Asthana and Hawker's media

Isolate	Mycelial colour	Substrate colour	Margin of the colony	Topography	Center of the colony	Spore size (μm)		Sporulation
						Marco-conidia	Micro -conidia	
Bangalore (Hessaraghatta)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	27.2x3.0	8.1x2.3	+ + + +
Bangalore(Shivakote)	Whitish	White	Irregular	Aerial mycelium	Whitish	28.4x3.4	10.5x2.8	+ + + +
Bangalore(Muthikere)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	24.7x3.8	9.5x3.0	+ + + +
Channapatna (Abbur)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	30.3x3.7	12.3x3.0	+ + + +
Channapatna(Tagachagere)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	20.3x3.1	10.2x2.9	+ + + +
Chanpatna(Chikkanahalli)	Whitish	White	Irregular	Aerial mycelium	Whitish	26.2x3.6	7.4x2.3	+ +
Maddur(Konnur)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	26.8x3.4	7.8x2.5	+ + + +
Maddur(Kowdle)	Whitish	White	Irregular	Aerial mycelium	Purple	23.4x3.7	6.1x2.3	+ + + +
Mandya (Kotigere)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	28.2x3.1	6.4x2.6	+ + + +
Mandya(Bukanakere)	Whitish	White	Irregular	Aerial mycelium	Purple	30.1x3.0	11.4x3.0	+ + + +
Mysore(Nanjungudu)	Whitish	White	Irregular	Aerial mycelium	White	28.6x2.9	10.8x3.2	+ + + +
Mysore(Devartasanahalli)	Whitish-purple	White	Irregular	Aerial mycelium	Purple	30.7x3.8	10.2x3.0	+ + + +
Mysore(Konnur)	Whitish	White	Irregular	Aerial mycelium	Whitish	28.1x3.2	6.8x2.8	+ + +
Mysore(Thandavapura)	Whitish	White	Irregular	Aerial mycelium	Whitish	30.4x3.7	8.6x3.2	+ + +

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

(Devarasanahalli) (82.67 mm), Bangalore (Shivakote) (82.33 mm), Mysore (Nanjanagudu) (81.33 mm), Maddur (Koppa) (81.33) and Mandya (Bukanakere) (81.00 mm). Least growth was seen in Mandya (Kotigere) isolate (77.67 mm). Maximum sporulation was produced by all the fourteen isolates.The results are presented in Table 1.

Table 5: Effect of different nitrogen sources on growth of different isolates of *Fusarium oxysporum* f. sp.*cubense* on Richard's media

Nitrogen source	Mean dry weight of mycelium (mg)		
	Bangalore isolate	Channapatna isolate	Mysore isolate
Ammonium chloride	157.03	218.20	76.27
Ammonium nitrate	351.77	338.83	356.03
Aspargine	135.73	347.33	321.97
Potassium nitrate	115.07	277.33	226.23
sodium nitrate	255.07	435.47	211.13
Ammonium sulphate	154.80	279.60	155.03
Ammonium orthophosphate	133.60	288.47	217.43
S.Em ±	2.66	1.74	1.38
CD @ 1%	11.19	7.31	5.83

Table 6: Effect of different nitrogen sources on growth of different isolates of *Fusarium oxysporum* f. sp. *cubense* on Czapek's media

Nitrogen source	Mean dry weight of mycelium (mg)		
	Bangalore isolate	Channapatna isolate	Mysore isolate
Ammonium chloride	190.07	233.90	183.93
Ammonium nitrate	362.07	293.47	324.60
Aspargine	311.27	318.37	262.53
Potassium nitrate	315.13	333.97	309.63
sodium nitrate	439.07	436.50	452.13
Ammonium sulphate	188.43	227.90	75.00
Ammonium orthophosphate	166.90	219.80	94.27
S.Em ±	2.61	2.20	2.91
CD @ 1%	11.01	9.28	12.26

Table 7: *In vitro* evaluation of bioagents against *Fusarium oxysporum* f.sp.*cubense* through dual culture technique

Sl. No	Bioagents	Growth of the Pathogen (mm)	Growth of the antagonistic (mm)	Per cent inhibition of colony growth over control
1	*Trichoderma harzianmum*-55	24.67	65.33	65.25
2	*Trichoderma viride*-27	23	67	67.6
3	*Trichoderma viride* (NBAIT)	21	69	70.42
4	*Trichoderma viride* (Local)	28	62	60.56
5	*Pseudomonas fluorescens*	38	52	46.47
6	*Bacillus subtilis*	42	48	40.84
7	*Pseudomonas virideflava*	41.67	48.33	41.3
8	Control	71	-	-
S.Em ±		1.03	1.08	-
CD @ 1%		4.27	4.56	-

Bangalore (Hessaraghatta) and Maddur (Koppa) isolates produced pinkish to orange coloured colonies. The growth of Bangalore (Shivakote), Bangalore (Mathikere), Channapatna (Tagachagere) and Maddur (Kowdle) was in the fashion of pinkish to purple coloured mycelia.Pinkish to white coloured colonies were seen in Channapatna (Abbur), Channapatna (Chikkanahalli) and Mysore (Devarasanahalli). Mandya (Kotigere) isolate produced whitish to pink coloured growth.Purple to pink coloured growth was seen in Mandya (Bukanakere) isolate.Mysore (Nanjanagudu) isolate produced whitish mycelial growth.Whereas, Mysore (Thandavapura) produced purplish to pink coloured colonies.Maximum sporulation was produced by all the fourteen isolates. The size of macro and micro conidia ranged from 22.3-33.4 x 3.0-3.8 µm and 5.4-12.7 x 2.4-3.3 µm respectively. Morphological characteristics are presented in Table 2.

The Maddur (Kowdle) isolate produced maximum growth on Richard's agar followed by Mysore (Konnur) isolate.Least growth was seen in Mandya (Kotigere) isolate. Colony growths of whitish and pinkish to orange, pinkish to white, pinkish to purple and pinkish to pink colours were observed.Maximum sporulation was produced in all the isolates.Earlier studies for this pathogen from have also suggested that Richard's medium was the best for growth of *Fusarium* spp. by Sowmya (1993), Naik *et al.* (2010) and Khan *et al.*(2011).

Asthana and Hawker's medium

In Asthana and Hawker's medium Chanapatna (Abbur) isolate showed best growth of 90.00 mm followed by Bangalore (Hessaraghatta) (87.67 mm), Bangalore (Mathikere) (85.33 mm), Mandya (Kotigere) (83.33 mm), Mandya (Bukanakere) (82.33 mm), Mysore (Nanjanagudu) (80.67 mm), Channapatna (Chikkanahalli) (80.33 mm), Mysore (Konnur) (79.33 mm),Mysore (Devarasanahalli) (79.00 mm), Maddur (Kowdle) (79.00 mm), Maddur (Koppa) (79.00 mm), Mysore (Thandavapura) (76.33 mm), Bangalore (Shivkote) (76.00 mm) after seven days of inoculation. Whereas, Chanapatna (Tagachagere) isolate produced less growth of 75.67 mm. The data are recorded in Table 3.

Colonies appeared whitish to purple colour in Bangalore (Hessaraghatta), Bangalore (Mathikere), Channapatna (Abbur), Channapatna (Tagachagere), Maddur (Koppa) and Mysore (Devarasanahalli).Whereas, Bangalore (Shivakote), Channapatna (Chikkanahalli), Maddur (Kowdle), Mandya (Bukanakere), Mysore (Nanjanagudu), Mysore (Konnur) and Mysore (Thandavapura) produced whitish mycelial colour colonies. Maximum sporulation was produced in all the isolates except Chanapatna (Chikkanahalli) and Mysore (Konnur) isolates. The size of macro and micro conidia ranged from 20.3-30.7 x 3.0-3.8 µm and 6.1-11.4 x 2.3-3.2 µm respectively. Morphological characteristics are presented in Table 4.

In Asthana and Hawker's medium Chanapatna (Abbur) isolate showed best growth followed by Bangalore (Hessaraghatta) isolate and Bangalore (Mathikere) isolate. Whereas, Channapatna (Tagachagere) isolate produced lesser growth. Whitish and whitish to purple colour mycelium was observed in the culture. Maximum sporulation was produced in all the isolates except Channapatna (Chikkanahalli) and Mysore (Konnur) isolates.Nagaraj and Jahagirdar2014 reported among the eleven isolates collected from six different states exhibited morphological variability with respect to colony colour.
Somu and Thammaiah(2015) reported Sabouraud's and Potato dextrose agar media are best for *Fusarium oxysporum f. sp. cubense*

Effect of nitrogen source on growth of *Fusarium oxysporum* f. sp. *cubense*

Growth of different isolates in Richard's broth

The result indicated that in presence of ammonium nitrate in Richard's media the Bangalore isolate produced maximum growth of 351.77 mg followed by sodium nitrate (255.07 mg), ammonium chloride (157.03 mg), ammonium sulphate (154.80 mg), aspargine (135.73 mg) and ammonium orthophosphate (133.60 mg). Least growth of 115.07 mg was observed in presence of potassium nitrate.

Channapatna isolate showed maximum growth in sodium nitrate (435.47 mg), followed by aspargine (347.33 mg), ammonium nitrate (338.33 mg), ammonium orthophosphate (288.47 mg), ammonium sulphate (279.60 mg) and potassium nitrate (277.33 mg).Minimum growth was observed in ammonium chloride (218.20 mg).

Mysore isolate produced maximum mycelial growth in ammonium nitrate (356.03 mg) followed by aspargine (321.97 mg), potassium nitrate (226.23 mg), ammonium orthophosphate (217.43 mg), sodium nitrate (211.13 mg) and ammonium sulphate (155.03 mg).In ammonium chloride fungus produced least mycelial growth of 76.27 mg.

Growth of different isolates in Czapek's broth

In Czapek's broth results revealed that Bangalore isolate produced maximum growth of 439.07 mg in sodium nitrate followed by ammonium nitrate (362.07 mg), potassium nitrate (315.13 mg), aspargine (311.27 mg), ammonium chloride (190.07 mg), and ammonium sulphate (188.43 mg). Lowest growth was observed in ammonium orthophosphate (166.90 mg).

Channapatna isolate utilized sodium nitrate as best nitrogen source and produced the maximum mycelial growth of 436.50 mg followed by potassium nitrate (333.97 mg), aspargine (318.37 mg), ammonium nitrate (293.47 mg), ammonium chloride (233.90 mg) and ammonium sulphate (227.90 mg),whereas, least growth was obtained in ammonium orthophosphate (219.80 mg).

Sodium nitrate was the best nitrogen source utilized by Mysore isolate with growth of 452.13 mg followed by ammonium nitrate (324.60 mg), potassium nitrate (309.63 mg), aspargine (262.53 mg), ammonium chloride (183.93 mg) and ammonium orthophosphate (94.27 mg).Least growth was recorded when ammonium sulphate (75.00 mg) was used as nitrogen sources. In the present study, among the nitrogen sources tested, ammonium nitrate was found best for Bangalore and Mysore isolates of *Fusarium oxysporum* f. sp. *cubense* in Richard's medium and least growth of these isolates was observed in potassium nitrate and ammonium chloride, Channapatna isolate produced maximum growth in sodium nitrate and lowest the lowest growth in ammonium chloride.In Czapek's media all the three isolates of Bangalore, Channapatna and Mysore produced maximum growth in sodium nitrate and least in ammonium orthophosphate.

Fusarium oxysporum f. sp. *cubense* could utilize ammonium nitrate and sodium nitrate more efficiently and it is a better nitrogen source than any other nitrogen source.The nitrate compounds are excellent nitrogen source for imperfect fungi and also ascomycetes. Present studies are in agreement with the reports of Moore and Chupp (1952) reported that ammonium ion, urea, peptone, proteose-peptone and aspargine were utilized as nitrogen sources by *Fusarium oxysporum*f.sp. *lycopercisi*.Of the seven nitrogen sources, maximum growth of *Fusarium oxysporum* f.sp.*niveum* was obtained on potassium nitrate, followed by calcium nitrate, sodium nitrate, ammonium nitrate, ammonium oxalate, ammonium sulphate and ammonium phosphate (Jhamaria, 1972).Four nitrogen sources were amended in Czapek'sdox agar medium among them calcium nitrate was found to be best source of nitrogen for the growth of *Fusarium solani*.It was followed by sodium nitrate, ammonium nitrate and potassium nitrate after six days of inoculation (Ramteke and Kamble, 2011).

Evaluation of bio-agents against *Fusarium oxysporum* f. sp. *cubense*

Trichoderma viride (NBAIT) showed strong antagonistic activity by inhibiting 70.42 per cent of *Fusarium* colony as compared to control followed by *Trichoderma viride*-27 (66.60 %), *Trichoderma harzianum*-55 (65.25 %), *Trichodermaviride* (Local) (60.50 %), *Pseudomonas fluorescens* (46.47 %) and *Pseudomonas virideflava*(41.30%). Whereas, least parasitic activity was noticed in case of *Bacillus subtilis* which inhibited 40.84 per cent of *Fusarium* colony. Data presented in table 7.

In the light of present day constraints in plant disease management practices especially those on the use of pesticides, biological control is increasingly occupying the minds of scientists all over the world as they are eco-friendly and cost effective. In recent years, the use of *Trichoderma* has gained more importance. These antagonistic organisms act on the pathogen by different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1999). Formulations of antagonistic organisms

are available at cost effective rates and these organisms once introduced into the soil survive for a longer period. There is also circumstantial report that native antagonists are more efficient than introduced antagonists (Kulkarni and Sagar, 2006).

In the present study, maximum reduction in colony growth of *Fusarium oxysporum*f.sp.*cubense* was observed in *Trichoderma viride* (NBAIT) which was significantly superior to all the bioagents tested.Next best were *Trichoderma viride*-27 and*Trichoderma harzianum*-55.There was minimum inhibition of *Fusarium oxysporum* f. sp. *cubense* by *Bacillus subtilis* and *Pseudomonas virideflava*.Species of *Trichoderma viz.,Trichoderma harzianum*-55, *Trichoderma viride*-27, *Trichoderma viride* (NBAIT), *Trichoderma viride*(Local), showed more mycelial inhibition of organism compared to bacterial antagonists.This can be attributed to higher competitive ability of these*Trichoderm*a spp.The present results corroborate with the findings of Rani *et al*. (2009) and Ram and Pandey (2011) who reported that *Trichodermaviride* was effective against *Fusarium* spp.Zapata *et al*. (2001) and Thangavelu *et al*. (2004) reported *Trichodermaharzianum* was effective against *Fusarium* spp.Asha *et al*. (2011) reported that *Pseudomonas fluorescens* have an excellent potential to be used as biocontrol agents of *Fusariumoxysporum* in tomato greenhouses at the field level.

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