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VARIABILITY STUDIES OF *FUSARIUM OXYSPORUM* F. SP. *VANILLAE* ISOLATES

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ABSTRACT
In vitro studies were conducted on, carbon and nitrogen sources, temperature and pH levels on mycelia growth of *F. oxysporum* f. sp. *vanillae* isolates. The fungus isolates showed best growth on Richard's agar and potato dextrose agar media among five culture media that were tried. Sucrose was found to be the best source of carbon whereas, L- asparagines was the best source of nitrogen. Growth of *F. oxysporum* was maximum at 25°C after seven days of inoculation, which was reduced drastically below 15°C and showed zero growth at 40°C. The most suitable pH level for growth of fungus was 5.0 and 6.0.

KEY WORDS: *Fusarium oxysporum; In vitro;* Culture media; pH; Carbon; Nitrogen; Mycelia growth; isolates

INTRODUCTION

Vanilla planifolia Andrews (Salisb) (Ames) is a native of humid tropical rain forests of South-eastern Mexico, Central America, the West Indies and northern part of South America and now it accounts for an about 0.75 percent of total world trade in spices (Madhusoodanan *et al.*, 2003). Now the global cultivation of vanilla is estimated to be about 40846 hectares from which production is about 5583 metric tonnes and in India it is grown in an area of 2545 hectares covering Karnataka, Kerla and Tamil Nadu, with the Production of about 100 metric tonnes (Kuruvilla, *et al.*, 2004). Although a number of biotic and abiotic factors contribute for low vanilla production but endemic occurrence of stem rot disease caused by *Fusarium oxysporum* f.sp. *vanillae* is of significant importance.

The wilt pathogen is soil-borne and survives in infected seedling and dead plant debris in soil (Haware *et al*, 1978). Since, the fungus can survive in the soil for several years, it is difficult to control the disease after invasion. Field control of these diseases was possible to a limited extent, with the help of bio-control agents and fungicides alone. These methods had not given satisfactory results, so there is an urgent need to formulate integrated disease management module.These includes introduction of antagonistic microorganisms into field especially *Trichoderma* spp. (Papavizas, 1984) and bacterial antagonists (Campbell and Faull, 1979); application of organic amendments to soil to stimulate resident antagonist and chemical control methods that help enormously in enhancing crop growth and disease management. (Joseph Thomas and Susheela Bhai, 2000). The present investigation was conducted to study the effect of physiological factors on the mycelial growth of the fungus.

MATERIALS AND METHODS

Studies of the following physiological aspects of *F.oxysporum* f. sp. *vanillae* isolates were conducted *in vitro*.

Effect of Culture Media: Following five culture media were used to find out the most suitable one for the mycelial growth of the pathogen isolates. Each culture medium was prepared in 1 liter of water and autoclaved at 120°C at 15 psi for 20 min. These were cooled to 45°C and then poured in 8 cm Petri dishes for solidification. Potato Dextrose Agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-Agar 20g), Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-Agar 20g, Czapeks dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g, Sucrose 30g, Agar-agar 20g), Brown's agar (BA) medium (Glucose 2g, L-Asparagines 2g, Di potassium hydrogen phosphate 1.25g, Magnesium sulphate 0.75g, Agar-agar 20g) and Yeast extract agar (YEA) medium (Mannitol 10g, Yeast extract 1.0g, Dipotassium hydrogen phosphate 0.5g, Magnesium sulphate 0.2g, Sodium chloride 0.1g, Congo Red (1%Solution) 2.5ml, Agar-agar 20g).

Effect of Different Carbon and Nitrogen Sources

Nitrogen sources: four nitrogen sources *viz*., sodium nitrate, ammonium nitrate, L-asparagines, and ammonium sulphate was added to the Richards agar medium in place of potassium nitrate prior to autoclaving and the quantity of nitrogen sources added was determined on the basis of their molecular weights so as to provide an equivalent amount of nitrogen present in the basal medium, as described by Lily and Barnet (1951).

Carbon sources: For comparison of isolates based on their carbon source assimilation, five carbon sources were added to the basal media in place of sucrose *viz.*, fructose, maltose, starch, lactose and glucose. The quantity of carbon compound added was determined on the basis of their molecular weights so as to provide an equivalent amount of carbon present in the basal medium, as described by Lily and Barnet (1951).

Effect of Temperature: Six F. *oxysporum* f. sp. *vanillae* isolates was inoculated in Richards's agar medium and temperature was maintained at 10, 15, 20, 25, 30, 35, and 40^{o}C.

Effect of Different pH Levels: The test fungus isolates was inoculated on Richards agar medium whose pH was adjusted to 4, 5, 6, 7, 8 and 9, respectively. All these experiments were conducted in five replicates. Plates were inoculated by placing 4 mm agar medium plugs containing active mycelium of the fungus and were placed in the centre of the Petri dishes. Plates were incubated at 27± 1^{o}C for 7 days (except for the study of temperatures). Observations on linear growth were recorded after seven days of inoculation.

RESULTS AND DISCUSSION

Effect of culture media: The results of the experiment revealed that, Richards agar and potato dextrose media were the best for the radial growth of *F. oxysporum* f. sp. *vanillae* isolates with a mean maximum growth of 61.58 and 59.30 mm, followed by Czapek's Dox Agar, Browns Agar and Yeast Extract Agar media with a mean maximum growth of 50.95, 52.55 and 43.41 mm, respectively. These results were in confirmation with Ingole (1995) who reported that PDA and Richard's agar supported best mycelial growth of *F. udum*. Major (1923) observed the profuse production of aerial mycelium of *F. solani* on Richards agar. Jamaria (1972) also reported maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* on potato dextrose agar, Richard's agar and Czapek's Dox agar. Anjaneya Reddy (2002) observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar.

Effect of different carbon and nitrogen sources: The results of this experiment indicated that all the carbon sources were suitable for the growth of *F. oxysporum* f. sp. *vanillae* isolates.

Table 1: Effect of carbon sources on the growth of *Fusarium oxysporum* f. sp. *vanillae* isolates

Colony diameter (mm)								
		Carbon sources						
Sl. no	Isolates	Sucrose	Fructose	Maltose	Starch	Lactose	Glucose	Mean
1	Fov-1	74.6	62.7^{a}	62.9^{ab}	56.2^{a}	55.8^{ab}	56.9^{a}	61.39
2	Fov-2	63.1^{a}	60.3^{a}	47.4^{c}	53.3^{a}	46.5^{b}	38.4^{b}	51.51
3	Fov-3	51.6^{b}	54.4^{b}	56.4^{bc}	50.3^{a}	57.5^{a}	44.4^{b}	52.46
4	Fov-4	55.7^{b}	51.2^{b}	65.3^{a}	57.3^{a}	58.4^{a}	57.1^{a}	57.55
5	Fov-5	50.9^{b}	51.5^{b}	51.3^{b}	52.9^{a}	51.2^{b}	43.9^{b}	50.30
6	Fov-6	64.3^{a}	61.7^{a}	58.2^{b}	54.6^{a}	58.5^{a}	57.1^{a}	59.06
	Mean	60.0	57.0	56.9	54.1	54.7	49.5	

	SE ±	CD@0.01
Isolates	0.867	2.40
Carbon sources	0.867	3.16
Isolates x Carbon sources	1.93	6.58

Treatments with same superscript in the respective column are statistically on par

However, isolates growth was best on sucrose followed by fructose and maltose with a mean maximum growth of 60.0, 57.0 and 56.9 mm, respectively, after seven days of inoculation wherein, all the six isolates, exhibited variation in the requirement of carbon sources. This could probably be due to triggering of some dormant mechanism, which helped in maximum utilization of sucrose and maltose (Desai *et al.*, 1994). The findings are in conformity with the reports on the variation among the isolates of *Fusarium* sp, as observed by Khare *et al.* (1994), Patel (1991) and Sowmya (1993). As is evident from Fig. 3, out of five different forms of nitrogen sources utilized; L-asparagines was found to be very good nitrogen source for the growth and development of all isolates. Among the isolates, Fov-1 showed highest growth of (64.7mm) and differed significantly compared to others followed by Fov-6, Fov-4, Fov-2, Fov-5, Fov-3 with 60.8mm, 59.6mm, 58.1mm, 54.6mm and 50.2 respectively and is on par with potassium nitrate. Sodium nitrate ($NaNO_3$) also had stimulatory effect on the growth of all the isolates. However, ammonium nitrate had moderate influence on the growth of the isolates and ammonium sulfate was the only nitrogen source that had least influence on the growth of *F. oxysporum* f.sp. *vanillae* isolates. From the results it was observed that, isolate Fov-6 efficiently utilized all the nitrogen sources with a mean of 52.02 followed by Fov-1 with 51.24mm growth and are on par

with each other. This is in conformity with the results reported by Agarwal (1958). Mathur (1960) also reported that L-asparagines and potassium nitrate were effective sources for the growth of *Fusarium* spp.

Table 2: Effect of nitrogen sources on the growth of *Fusarium oxysporum* f. sp. *vanillae* isolates

Colony diameter (mm)							
		Nitrogen sources					
Sl. no	Isolates	Sodium nitrate	Potassium nitrate	Ammonium nitrate	L- Asparagines	Ammonium sulphate	Mean
1	Fov-1	48.5[a]	64.7	41.7	59.9[ab]	41.4	51.24
2	Fov-2	45.1[b]	58.1[ab]	37.6	53.3[d]	21.7	43.16
3	Fov-3	47.5[a]	50.2	41.9[a]	56.3[c]	25.4[a]	44.26
4	Fov-4	50.7[a]	59.6[ab]	45.8	58.4[bc]	31.0	49.10
5	Fov-5	45.5[b]	54.6	40.3[a]	52.9[d]	24.7[a]	43.60
6	Fov-6	53.1[a]	60.8[a]	45.5	62.0[a]	38.7	52.02
	Mean	48.4	57.0	42.13	57.13	30.48	

	SE ±	CD@1%
Isolates	0.306	1.115
Nitrogen sources	0.273	0.997
Isolates x Nitrogen sources	0.612	2.231

Treatments with same superscript in the respective column are statistically on par

Table 3: Effect of different temperature levels on the growth of *Fusarium oxysporum* f.sp.*vanillae* isolates

Colony diameter (mm)								
				Temperature levels				
Isolates	10^0C	15^0C	20^0C	25^0C	30^0C	35^0C	40^0C	Mean
Fov-1	0.80 (1.587)	30.8[ab] (33.687)	51.3[a] (45.723)	72.3[a] (58.220)	60.9[ab] (51.273)	27.3[a] (31.520)	0.0 (0.330)	34.77
Fov-2	0.0 (0.330)	35.2[a] (36.390)	46.4[ab] (42.907)	63.3[b] (56.843)	48.3 (44.043)	25.1[ab] (30.116)	0.0 (0.330)	31.18
Fov-3	0.0 (0.330)	32.2[a] (34.550)	42.0[b] (40.133)	65.1[b] (53.733)	54.8[b] (47.833)	17.2 (24.550)	0.0 (0.330)	30.18
Fov-4	0.75 (4.967)	28.0[b] (31.967)	61.6 (53.623)	63.7[b] (52.670)	62.0[a] (53.937)	19.8[b] (26.467)	0.0 (0.330)	33.69
Fov-5	0.26 (2.960)	25.4[b] (30.270)	43.0[b] (41.010)	66.0[ab] (54.333)	56.7[b] (48.887)	19.1b (25.957)	0.0 (0.330)	30.06
Fov-6	0.0 (0.330)	28.2[b] (32.117)	42.2[b] (45.990)	72.0[a] (58.050)	64.6[a] (46.793)	30.5[a] (22.500)	0.0 (0.330)	33.92
Mean	0.30	29.96	47.75	67.06	57.88	23.17	0.0	

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	SEm ±	CD@ 1%
Isolates	1.031	2.567
Temperature levels	1.120	2.910
Isolates x Temperature levels	2.526	6.508

Figures in parenthesis are arc- sine transformed values

Treatments with same superscript in the respective column are statistically on par

Effect of temperature: Differences in the growth rate of all the isolates of *F. oxysporum* f. sp. *vanillae* were recorded at different temperature levels *viz.*, 10^0 C, 15^0 C, 20^0 C, 25^0 C, 30^0 C, 35^0 C and 40^0C. No growth was observed in any of

the isolates evaluated at the temperature of 40^0C. At 10^0C Isolates Fov-1 and Fov-5 produced 0.80 mm and 0.26mm growth respectively, however, other isolates showed no growth at 10^0C. At 15^0C maximum growth of 35.2mm and 32.2mm was recorded with Fov-2 and Fov-3 isolates respectively and at 20^0C Fov-4 isolate produced highest growth of 61.6mm and differed significantly compared to other isolates whereas, at 35^0C isolates Fov-6 produced highest growth of 30.5mm and differed significantly compare to other isolates. However, isolate Fov-1 and Fov-2 produced colony growth of 27.30 and 25.10 respectively and are on par with each other and differed significantly with rest of the isolates. At temperature 25^0C all the isolates showed very good growth and the maximum growth of 72.3 mm was recorded with Fov-1 which was on par with Fov-5 and Fov-6 with 66.0mm and 72.0 mm respectively and differed significantly with rest of the isolate. These studies are in confirmation with Anjaneya Reddy who reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 20^0 to 35^0C. *In vitro* studies conducted by Chi and Hansen (1964) indicated that *F. solani* isolates grew well at higher temperature of 28^0 C. the fungus grew at the temperature range of 10–35^0C. However, growth of the fungus was drastically reduced below 15^0C and started to decline above 30^0C and become zero at 40^0C, as these temperatures did not favour for growth of the fungus. It was observed that at 25^0C and 30^0C, the fungus attained the maximum growth 76.8 and 85.4 mm while at 25^0C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5^0C. Gupta *et al.* (1986) reported similar findings regarding temperature requirements to this fungus. Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30^0C (Chauhan, 1965).

Effect of different pH levels: Differences in the growth rates among isolates of Fov were recorded at different pH levels .All the *Fusarium* isolates grew well at all the pH levels; however maxmimum growth was recorded pH at 5.0, wherein Fov-3 and Fov-6 isolates showed highest growth of 62.4 and 62.1 mm respectively. Least growth of all the isolates was recorded at 9.0 pH with a maximum growth of 51.2mm by Fov-6 isolate and differed significantly compare to all other isolates. Among the Fov isolates studied, isolate Fov-6 found very efficient isolate in utilizing varied pH for its growth under *in vitro* compare to others. These results are in confirmation with the findings of Moore (1924) who reported that two strains of *F. coeruleum* could tolerate a P^H range of 3.0 to 11.0. The studies conducted by Jamaria (1972) on *F. oxysporum* f. sp. *nivium* indicated that, as the pH decreases or increases from the optimum, the rate of amount of growth gradually decreases.

Table 4: Effect of PH levels on the growth of *F.oxysporum* f.sp*. vanillae* isolates

Colony diameter (mm)								
Sl.no	isolates	p^H Levels						
		4.0	5.0	6.0	7.0	8.0	9.0	Mean
1	Fov-1	51.6^a	56.3^b	54.6^a	52.6^a	50.8^a	41.1^a	51.16
2	Fov-2	43.0^c	50.4^b	51.2^{ab}	44.4	46.0^b	29.0^b	44.83
3	Fov-3	45.3^b	62.4^a	49.8^{bc}	47.1^b	46.4^b	29.6^b	47.55
4	Fov-4	43.2^c	47.6	52.4^{ab}	48.2^b	51.4^a	35.7	46.41
5	Fov-5	46.3^b	53.2^b	46.0^c	53.0^a	51.0^a	39.1^a	48.1
6	Fov-6	53.9^a	62.1^a	64.3	65.2	58.4	51.2	60.1
	Mean	47.16	55.3	53.04	51.8	50.7	37.53	49.25

	SEm ±	CD@1%
Isolates	0.368	1.342
P^H levels	0.368	1.342
Isolates x Temperature levels	0.824	3.041

Treatments with same superscript in the respective column are statistically on par

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