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GROWTH CHARACTERISTICS OF FUSARIUM SPP. CAUSING WILT DISEASE IN PSIDIUM GUAJAVA L. IN INDIA

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Abstract: Fusarium oxysporum f. sp. psidii and F. solani, causal agents of wilt in guava are highly variable pathogens. This study was conducted on cultural and physiological (temperature and pH) characters. The data revealed that maximum mycelial growth was obtained in potato dextrose agar as semi-solid media i.e. 78.00 mm for F. oxysporum f. sp. psidii; 73. 83 mm for F. solani, while malt extract broth as liquid broth media i.e. 1 385 mg mycelia for F. oxysporum f. sp. psidii; 1491 mg for F. solani. Maximum sporulation was recorded in oatmeal agar and mycological broth. The optimum temperature and pH for growth of both Fusarium spp. isolates was 28°C and 5.5. The isolates differed in their colony growth; mycelial mass, macro-conidia, and micro-conidia produced. These variations were characters of each of the isolates with respect to cultural and physiological characters.

Key words: Fusarium oxysporum f. sp. psidii, Fusarium solani, cultural characters, physiological characters

INTRODUCTION

Guava (Psidium guajava L.) is an important fruit crop in subtropical countries. In India, it is grown almost in all the states. Wilt is the most destructive disease of guava and causes a 5-60 per cent loss (Misra 2006) in guava production in India. Although various pathogens are encountered to cause guava wilt, two species of Fusarium (F. oxysporum f. sp. psidii and F. solani) are widely reported (Prasad et al. 1952; Edward 1960; Chattopadhyaya and Bhattachariya 1968). Reports reveal wide variations in cultural and morphological characteristic of different isolates of Fusarium oxysporum f. sp. psidii and F. solani. There is variation in the different isolates of *F. oxysporum* f. sp. psidii and F. solani, when tested in vitro against different cultural and physiological parameters (Chattopadhyay and Sengupta 1955; Dwivedi and Dwivedi 1999). Booth (1977) reported potato dextrose agar, potato sucrose agar and oatmeal agar as good media for the growth of F. oxysporum and F. solani. Bilay's medium modified by Joffe was better for sporulation. Armstrong's Fusarium medium was good for increasing the inoculum potential of Fusarium species. Paulkar and Raut (2004) studied variability among *F. oxysporum* f. sp. *ciceri* isolates on five media viz. Ashby's medium; Asthana and Hawker's medium; Czapek's medium; Krichoff's agar medium, Potato dextrose agar medium and Richard's agar medium and observed that potato dextrose agar supports maximum growth, while poor growth was in Krichoff's agar. Several species of Fusarium have been reported to grow and sporulate in pH ranges of 5.0 to 6.0 (Cochrane 1958). Agarwal and Sarbhoy (1978) reported acidic pH favouring growth of all *Fusarium* spp. *F. oxysporum* and *F. solani* grew best at pH 4.5 and 6.0 while *F. graminearum* and *F. equiseti* at pH 3.5 and 6.5, respectively. Farkya *et al.* (1996) observed maximum growth and sporulation of *F. solani* at 5.5 pH. The fungus could grow and sporulate under a wide range of pH from 4.0 to 8.0. However, pH 6.5 and 7.0 proved optimum for growth and sporulation of the fungus *F. solani* (Chauhan 1997). Pandav (2002) found pH 5.5 to 7.0 optimum for the growth and sporulation of *F. solani*.

Ahamad *et al.* (2002) grew *Gibberella fujikuroi* and observed that excellent growth and sporulation took place at 30°C followed by 25°, 20° and 35°C. Moderate sporulation was recorded at 25° and 35°C. Also, Sharma *et al.* (2005) studied the effect of temperature on the growth and sporulation of *F. oxysporum* f. sp. *lini*.

Hence, considering the importance of variability in *Fusarium* spp., the present study was undertaken to evaluate the cultural and physiological parameters of guava wilt pathogens viz. *F. oxysporum* f. sp. *psidii* and *F. solani* representing different agro-ecological regions of India.

MATERIALS AND METHODS

Isolation of pathogens

A total of ten selected representative isolates of *Fusarium* sp. (5 isolates each of *F. oxysporum* f. sp. *psidii* and *F. solani*) isolated from wilted guava roots from different guava growing areas of India were used in the present study. Five isolates of *F. oxysporum* f. sp. *psidii* F10, F18, F24, F30, and F38 were collected from Chandigarh, Ranchi, Kanpur, Unnao and Rewa respectively. Five isolates

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of F. solani F2, F12, F15, F20, and F29 were collected from West Bengal, Chandigarh, Ajmer, Puskar and Unnao, respectively (Table 1). These isolates were isolated on potato dextrose agar (PDA, Hi-Media, India) and incubated at 28±1°C for 6 days. The morphological and cultural characterizations of the cultures grown on PDA were studied and compared with those mentioned by Booth (1971). The reference pure culture of the fungus was also sent to Indian Type Culture Collection (ITCC), Division of Mycology and Plant Pathology, I.A.R.I. (Indian Agricultural Research Institute), New Delhi-110 012, India, for identification of F. solani [ITCC No. 5208 (F20) and 5212 (F15)]. F. oxysporum f. sp. psidii [MTCC No. 3326 (F24) and 3327 (F30)] was sent for identification to the Microbial Type Culture Collection, IMTECH, Chandigarh, India. These identified cultures were used as reference cultures and thus, Fusarium sp. in the study were identified and confirmed. Pure cultures of the isolates were maintained on PDA slants under controlled temperature (Table 1). Pathogenicity of these isolates was also confirmed in a separate study (Misra and Gupta 2010).

Radial growth and sporulation studies of *Fusarium* sp. isolates in semi solid medium

In this study, five solid media viz. Potato Dextrose Agar (PDA), Czapek's Dox Agar medium (CDA), Corn Meal Agar medium (CMA), Cooke's Rose Bengal Agar medium (CRBA) and Oatmeal Agar medium (OA) were used. All the media were prepared according to the manufacturer instructions (HiMedia, India). Each Petri dish was poured with 20 ml sterilized medium for solidification. Equal discs of a 5 mm in diameter of each test isolate [5 isolates each of F. oxysporum f. sp. psidii (Fop) and F. solani (Fs)] grown from the 7-day-old pre-cultured Petri dishes on potato dextrose agar, were taken out with the help of a cork borer and placed at the centre of each set of Petri dishes containing different medium. After inoculation, Petri dishes were incubated at 28±2°C. The diameter of the each of the test isolates was recorded in millimeters in two directions at right angles to each other, and then average colony diameter in millimeters was calculated and recorded. Measurement of growth was made at the interval of 24 hours, till the full expansion of growth. Studies of sporulation on different solid media used, was also undertaken. A five mm disc of the culture was cut from the near center portion of the plate and put in sterilized water (10 ml) and shaken well, so that the spores were dislodged. One drop of this spore suspension was placed on a haemocytometer and the number of spores in 5 squares at random, were counted. The number of spores per ml was calculated with a haemocytometer, using the formula given by Pathak (1984):

No. of spores per ml =
$$\frac{N \times 1000}{X}$$

where:

N = Total No. of spores counted/No. of squares, X = Volume of mounting solution between the cover glass and above the squares counted.

Mycelial growth and sporulation studies of *Fusarium* sp. isolates in liquid broth medium

For conducting this study, five broth media viz. Potato Dextrose Broth medium (PDB), Czapek's Dox Broth medium (CDB), Malt Extract Broth medium (MEB), Mycological Broth medium (MB) and Oatmeal Broth medium (OB) were used. All the media were prepared using the standard method. One hundred fifty sterilized 250 ml conical flask were taken. Each flask had 100 ml sterilized medium poured into it. Equal discs measuring 5 mm in diameter of each test isolate (5 isolates each of Fop and Fs) grown from the 7-day-old pre-cultured Petri dishes on Potato Dextrose Agar, were taken out with the help of a cork borer and placed in each set conical flask containing different medium. After inoculation, flasks were incubated at 28±2°C for seven days and were shaken twice every day. Mycelial growth of each of the test isolates was harvested in preweighed moistureless whatmen filter paper No. 42, oven dried at 60°C and weighed again to record mycelial growth in milligrams. Studies of sporulation on different liquid media used, was also undertaken.

Effect of temperature on growth and sporulation of *Fusarium* sp. isolates

The observations on the colony growth of ten isolates of *Fusarium* sp. were determined at 10, 16, 22, 28, 34 and 40±1°C. Twenty ml of PDA were poured in all 240 plates (10 isolates, six temperatures, 4 replicate) and used for different temperature studies. Mycelial discs of 5 mm in diameter were transferred from the margins of the 7 day old growing colony of each test isolate (5 isolates each of Fop and Fs) to the centre of each PDA plate. Each treatment was replicated four times. Observations on colony growth in diameter were recorded on the seventh day after inoculations. Studies of sporulation at different temperatures were also undertaken as per above mentioned method.

Effect of pH on growth and sporulation of *Fusarium* sp. isolates

Mycelial growth of the ten isolates of *Fusarium* sp. (5 isolates each of *F. oxysporum* f. sp. *psidii* and 5 isolates each of *F. solani*) were studied at 8 pH level 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8. In all 320, 250 ml conical flask (10 isolates, 8 pH, 4 replicates) were poured with 100 ml PDB. The desired pH levels of PDB medium were maintained by adding the required amount of buffer 0.1 N Citric acid or 0.1 N NaOH with the help of a digital pH meter. Then medium was sterilized in autoclave at 120°C for 15 min. A five mm mycelial disc was transferred from the margin of the 7-day-old growing colony, to the flask with PDB. Mycelium harvested after seven days were measured according to the above mentioned method. Studies of sporulation on different pH were also undertaken according to the above mentioned method.

Table 1. Fusarium spp. isolates of guava used for the study

4					Jour	nal of P	lant Pro	tection	Research	50 (4),	2010					
		Metabolite in culture			light brown	brown	pale yellow	pale yellow	dark yellow		violet	pinkish colour	pinkish	pinkish	pink yellow	
		Septation in macroconidia			3–5	5–7	5–7	5–7	4–5		3-4	3–5	3–5	3–5	3-5	
		Micro			Д	Ъ	M	Ţ	M		M	T	M	Ţ	M	
		Macro			M	Cl Cl	M	IJ	IJ		ď	M	ď	M	Ы	
		micro	W		4.85	4.96	4.69	8.32	5.48		8.98	7.56	5.91	6.70	5.66	
	Spore Size [µm]	, ig	ų	F. oxysporum f. sp. psidii	12.49	10.05	13.73	9.61	9.56	F. solani	18.16	13.76	16.94	14.39	14.86	
	Spore S	macro	W	E. oxysporu	4.76	4.51	5.12	6.87	6.18	E	10.09	7.50	8.91	10.35	8.13	
		ma	Ţ		31.72	44.82	38.61	39.50	40.88		35.54	23.79	35.62	36.08	33.27	
		Identification			Booth, 1971	Booth, 1971	MTCC 3326	MTCC 3327	Booth, 1971		Booth, 1971	Booth, 1971	ITCC 5212	ITCC 5208	Booth, 1971	
		Wilt [%]		-	50	100	50	50	50		100	50	100	50	100	,
		Location			Chandigarh	Ranchi	Kanpur	Unnao	Rewa		West Bengal	Chandigarh	Ajmer	Puskar	Unnao	
		Isolate			F10	F18	F24	F30	F38		F2	F12	F15	F20	F29	

L = less; M = moderate; P = profuse sporulation

RESULTS AND DISCUSSION

There were five representative isolates of *F. solani* (F2, F12, F15, F20, F29) and 5 of *F. oxysporum* f. sp. *psidii* (F10, F18, F24, F30, F38). The results on colony/cultural characters, growth and sporulation of the fungus are presented in tables 2, 3, 4, 5.

Effect of semi solid media

Five solid media (PDA, CDA, CMA, CRBA and OA) were used for the cultural studies. The data revealed that maximum mycelial growth was obtained in PDA (78.00 mm for F. oxysporum f. sp. psidii; 73. 83 mm for F. solani) followed by CMA (72.50 mm for F. oxysporum f. sp. psidii; 70.38 mm for F. solani) among all solid media tested during the experiment. It was clearly indicated that F. oxysporum f. sp. psidii is a faster growing pathogen of guava wilt than F. solani and potato dextrose agar was best for the growth of the Fusarium spp. isolates among the semi solid media. Colony colour had a cottony white range of colour. Variable colony character was designated as ideal colony character. Metabolite colour ranged from yellowish to no colour for both F. oxysporum f. sp. psidii and F. solani. An ideal colony growth pattern (i.e. cottony white) of F. oxysporum f. sp. psidii isolates was recorded when they were grown on CDA while. F. solani isolates when grown on CRBA media showed a cottony pinkish type growth. Both F. oxysporum f. sp. psidii and F. solani isolates produces no metabolite colour when grown on CMA media (Table 2).

Excellent sporulation was recorded in OA media (4.0X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.9X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 2.2X10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.2X10⁵ per ml for micro-conidia for *F. solani*) followed by CMA (3.6X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.4X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 2.0X10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.0X10⁵ per ml for micro-conidia for *F. solani*).

Effect of liquid broth media

Five liquid broth medias viz. PDB, CDB, MB, MEB and OB were tested for quantification of sporulation (macroconidia), and dry wt. of mycelial mass produced were recorded (mg/100ml). The results revealed that among the liquid media tested, maximum mycelial growth was obtained in MEB (1 385 mg for F. oxysporum f. sp. psidii; 1 491 mg for F. solani) followed PDB (1 160 mg for F. oxysporum f. sp. psidii; 1225 mg for F. solani). These results were optimized by production of mycelial mass in mg per 100 ml media. Which obviously indicates that F. solani is a faster growing pathogen than F. oxysporum f. sp. psidii, when they were cultured on liquid media. This is contrary to the results obtained when using semi-solid media. F. oxysporum f. sp. psidii is a faster growing pathogen of guava wilt than F. solani. when cultured on semi-solid media. Among the liquid media, malt extract broth was the best for the growth of *Fusarium* spp. isolates (Table 3).

Excellent sporulation was recorded in MB media (4.5X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp.

psidii; 4.2X10⁵ per ml for macro-conidia for *F. solani* and 2.9X10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. psidii; 2.9X0⁵ per ml for micro-conidia for *F. solani*) followed by PDB (2.8X10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. psidii; 4.0X10⁵ per ml for macro-conidia for *F. solani* and 2.2X10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. psidii; 2.8X10⁵ per ml for micro-conidia for *F. solani*).

Effect of pH level

Mycelia growth of five isolates each of *F. oxysporum* f. sp. *psidii* and *F. solani* were studied at 8 pH level 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.

The data revealed that the pH level significantly differentiates the mycelia growth. The maximum growth was recorded when the pH was at the level of 5.5 (1208 mg for *F. oxysporum* f. sp. *psidii*; 1275 mg for *F. solani*) followed by a pH of 5.0 (956 mg for *F. oxysporum* f. sp. *psidii*; 1083 mg for *F. solani*) and then a pH of 6.0 (952 mg for *F. oxysporum* f. sp. *psidii*; 958 mm for *F. solani*) (Table 4).

Maximum sporulation of *F. oxysporum* f. sp. *psidii* isolates was recoded at a pH of 6.5 for (4.4x10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 4.8x10⁵ per ml for macro-conidia for *F. solani* and 2.8x10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.9x0⁵ per ml for micro-conidia for *F. solani*) followed by a pH of 6.0 (3.8x10⁵ per ml for macro-conidia for both of *F. oxysporum* f. sp. *psidii* and *F. solani*; 2.4x10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.5x10⁵ per ml for micro-conidia for *F. solani*).

Effect of temperature

Colony growth of 5 isolates of each isolate of *F. oxysporum* f. sp. *psidii* and *F. solani* was studied at six different temperature viz. 10°, 16°, 22°, 28°, 34°, and 40°C. The data indicate that radial growth at six temperature levels was maximum when the temperature was 28°C (72.50 mm for both *F. oxysporum* f. sp. *psidii* and *F. solani*) followed by a temperature at 34°C (66.5 mm for *F. oxysporum* f. sp. *psidii*; 69.5 mm for *F. solani*) (Table 5).

Optimum sporulation was recorded at the temperature level of 34°C (3.6x10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.9x10⁵ per ml for macro-conidia for *F. solani* and 2.6x10⁵ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.8x10⁵ per ml for micro-conidia for *F. solani*) followed by a temperature at 40°C temperature (3.2x10⁵ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.3x10⁵ per ml for macro-conidia for *F. solani* and 2.2x10⁵ per ml for micro-conidia for both *F. oxysporum* f. sp. *psidii* and *F. solani*).

Among the semi solid media, PDA was best for the growth of the *Fusarium* spp. isolates. The data revealed that maximum radial growth was 78.00 mm for *F. oxysporum* f. sp. *psidii* followed 73. 83 mm for *F. solani* which indicates that *F. oxysporum* f. sp. *psidii* is faster growing than *F. solani*. Colony colour ranged from cottony white to variable colony character, and metabolite colour from yellowish to no colour for both *F. oxysporum* f. sp. *psidii* and *F. solani*. An ideal colony growth pattern (i.e. cottony white) of *F. oxysporum* f. sp. *psidii* isolates was recorded when they were grown on CDA. The *F. solani* isolates when grown on CRBA media had a pinkish cottony type

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Table 2. Progressive radial growth and sporulation of Fusarium spp. isolates on semi-solid media

5											Jou	rnal	of .	Plai	nt F	rote	ectio	n I	(ese	arci	150	(4)	, 20)10											
	No. of spore/ml	mcro conidia	8		1.2X10 ⁵	$1.2X10^{5}$	$1.9X10^{5}$	2.3X10 ⁵	$1.0X10^{5}$	$1.1X10^{5}$	$1.6X10^{5}$	2.1X10 ⁵	$2.4X10^{5}$	$1.1X10^{5}$	1.0X10 ⁵	$1.4 X 10^5$	$2.2X10^{5}$	$2.6X10^{5}$	$1.1X10^{5}$	1.5×10^{5}	$1.3X10^{5}$	$2.4X10^{5}$	$2.8X10^{5}$	$1.2X10^{5}$	$1.0X10^{5}$	$1.0X10^{5}$	$2.1X10^{5}$	$2.3X10^{5}$	$1.0X10^{5}$		$1.2x10^5$	$1.1x10^{5}$	$1.8x10^5$	$2.0x10^5$	1.4×10^5
***		macro conidia	7		2.8X10 ⁵	$2.5X10^{5}$	$3.2X10^{5}$	3.8X10 ⁵	1.5X10 ⁵	2.4X10 ⁵	3.1X10 ⁵	3.4X10 ⁵	3.5×10^{5}	1.5X10 ⁵	2.4X10 ⁵	$2.8X10^{5}$	3.2×10^{5}	$3.6X10^{5}$	1.7×10^{5}	$2.3X10^{5}$	$2.4X10^{5}$	$3.6X10^{5}$	4.0×10^{5}	1.7×10^{5}	$2.1X10^{5}$	$2.2X10^{5}$	$2.4X10^{5}$	3.4×10^{5}	1.2×10^{5}		2.6×10^{5}	1.0×10^5	2.8×10^{5}	3.6×10^{5}	2.0×10^{5}
	Metabolite	colour	9		light brown	no colour	no colour	yellow	no colour	light brown	pink colour	no colour	cream colour	no colour	pale yellow	no colour	no colour	cream colour	cream colour	pale yellow	pink colour	no colour	no colour	cream colour	dark yellow	no colour	no colour	yellow colour	no colour		violet	light orange colour	no colour	pale yellow	no colour
	Mean radial	growth [mm]	2	F. oxysporum f. sp. psidii (Fop)	71.60	67.63	70.13	64.25	62.25	74.38	68.00	71.13	65.50	61.38	78.00	68.00	72.25	71.00	62.88	73.75	69.50	72.50	05.69	58.87	73.38	68.38	72.25	00.69	61.25	E. solani (Fs)	70.38	66.12	69.75	55.00	56.5
	Colony	colour	4	F. oxysporum 1	orange-cottony white	cottony white	cottony white	cottony white	cottony white	cottony white	cottony white	white matty type	cottony white	cottony pink	cottony white	cottony white	dirty cottony white	dirty cottony white	cottony white	cottony off-white	cottony white	off white cottony	cottony white	cottony white	cottony white	cottony white	dirty cottony white	dirty white matty type	cottony pink	E. sol	cottony white with bluish at centre	cottony white	dirty cottony white	cottony white with violet ring at centre	cottony pink
	M. d.	Media	3		PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA		PDA	CDA	CMA	OA	CRBA
-	Fusarium sp. isolates					F10					F18					F24					F30					F38						F2			
	Ž	NO.	1				Н					2					3					4					2						9		

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8	1.0x10 ⁵	1.9×10^5	2.1×10^5	2.2×10 ⁵	1.2×10 ⁵	1.8x10 ⁵	1.0x10 ⁵	2.0x10 ⁵	2.2x10 ⁵	1.2.8×10 ⁵	1.2x10 ⁵	1.1x10 ⁵	1.6x10 ⁵	2.2×10^5	1.8x10 ⁵	1.2x10 ⁵	1.1×10^5	1.6×10^5	2.2×10^5	1.4×10^5	
7	2.2×10 ⁵	2.9×10^{5}	3.2×10^5	3.8×10^{5}	2.6×10^{5}	2.1x10 ⁵	2.2×10 ⁵	3.4×10 ⁵	3.9×10^{5}	2.6×10 ⁵	2.4×10^5	2.6×10^5	2.9×10 ⁵	3.1×10^{5}	2.8×10 ⁵	1.8×10^5	1.8×10^{5}	2.4×10^{5}	3.9×10^{5}	2.1×10^{5}	
9	pinkish colour	light orange colour	no colour	pale yellow	no colour	pinkish	dark yellow	no colour	pale yellow	no colour	pinkish	cream colour	no colour	no colour	no colour	pink yellow	cream colour	no colour	no colour	no colour	
5	70.37	62.37	68.12	61.75	59.31	73.83	60.75	64.00	61.12	59.63	70.35	65.00	70.38	96.38	62.25	69.35	61.50	63.38	57.50	59.47	2.44
4	creamy	cottony white	white matty type	dirty cottony white	cottony pink	cottony pink	dirty cottony white	dirty white matty type	dirty cottony white	cottony pink	white matty	cottony white	dirty cottony white	dirty cottony white	cottony pink	white matty	cottony white	dirty cottony white	dirty white matty type	cottony pink	
3	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	PDA	CDA	CMA	OA	CRBA	CD (p = 0.05)
2	щ			F12				F15					F20					F29			Statistical analysis
1			\sim					∞		1	Į		6			ļ		10			

PDA - Potato Dextrose Agar, CDA - Czapek's Dox Agar, CMA - Corn Meal Agar, OA - Oatmeal Agar, CRBA - Cooke's Rose Bengal Agar

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Table 3. Dry mycelial weight and sporulation of Fusarium spp. isolates on liquid broth media

No.	Fusarium sp.	Media	Mycelial growth	No. of s	Ì	
	isolates		[mg]	macro conidia	micro conidi	
			rum f. sp. psidii (Fop)			
		PDB	1 002	2.8x10 ⁵	1.6x10 ⁵	
		CDB	974	1.5x10 ⁵	0.6x10 ⁵	
1	F10	MB	562	4.5x10 ⁵	2.9x10 ⁵	
		MEB	1 269	1.2x10 ⁵	0.4x10 ⁵	
		OB	654	1.1x10 ⁵	0.3x10 ⁵	
		PDB	963	2.4x10 ⁵	1.3x10 ⁵	
_		CDB	676	1.2x10 ⁵	0.8x10 ⁵	
2	F18	MB	538	4.1×10 ⁵	2.9x10 ⁵	
		MEB	1 263	1.5x10 ⁵	0.8x10 ⁵	
		OB	567	1.5x10 ⁵	0.8x10 ⁵	
		PDB	1 044	2.4×10 ⁵	1.3x10 ⁵	
		CDB	638	1.2x10 ⁵	0.5x10 ⁵	
3	F24	MB	809	4.2x10 ⁵	2.6x10 ⁵	
		MEB	1 369	1.2x10 ⁵	0.8x10 ⁵	
		OB	473	1.5x10 ⁵	0.3x10 ⁵	
		PDB	1 108	2.8x10 ⁵	2.2x10 ⁵	
		CDB	668	1.9x10 ⁵	1.8x10 ⁵	
4	F30	MB	858	4.5x10 ⁵	2.9x10 ⁵	
		MEB	1 385	1.6x10 ⁵	1.1x10 ⁵	
		OB	604	1.8x10 ⁵	1.3x10 ⁵	
		PDB	1 160	2.5x10 ⁵	1.4x10 ⁵	
		CDB	651	1.5×10^5	1.0x10 ⁵	
5	F38	MB	890	4.0x10 ⁵	2.2x10 ⁵	
		MEB	1 384	1.4×10^5	0.9x10 ⁵	
		OB	631	1.5×10^5	1.3x10 ⁵	
			arium solani (Fs)		,	
		PDB	1 141	2.4×10^5	1.4x10 ⁵	
		CDB	646	1.8x10 ⁵	1.0x10 ⁵	
6	F2	MB	929	2.5x10 ⁵	1.8x10 ⁵	
		MEB	1 491	1.8×10^5	1.0x10 ⁵	
		OB	575	1.2x10 ⁵	0.5x10 ⁵	
		PDB	1 172	2.4x10 ⁵	1.4x10 ⁵	
		CDB	710	1.5x10 ⁵	0.8x10 ⁵	
7	F12	MB	807	2.5x10 ⁵	1.9x10 ⁵	
		MEB	1481	2.0x10 ⁵	1.1x10 ⁵	
		OB	642	1.6x10 ⁵	1.2x10 ⁵	
		PDB	1 102	2.2x10 ⁵	2.3x10 ⁵	
		CDB	647	1.4×10^5	0.6x10 ⁵	
8	F15	MB	851	$3.2x10^5$	2.5x10 ⁵	
		MEB	1479	1.5x10 ⁵	0.9x10 ⁵	
		OB	508	2.5×10^5	1.4x10 ⁵	
		PDB	1 225	4.0x10 ⁵	2.8x10 ⁵	
		CDB	655	1.5x10 ⁵	0.9x10 ⁵	
9	F20	MB	185	4.2x10 ⁵	2.9x10 ⁵	
		MEB	1 443	2.5x10 ⁵	1.7×10 ⁵	
		ОВ	634	2.5x10 ⁵	1.2x10 ⁵	
		PDB	1 211	2.5x10 ⁵	1.8x10 ⁵	
		CDB	646	1.9x10 ⁵	1.0x10 ⁵	
10	F29	MB	723	2.8x10 ⁵	1.9x10 ⁵	
-		MEB	1 299	2.2x10 ⁵	1.6x10 ⁵	
		OB	636	1.5×10 ⁵	1.0x10 ⁵	
	1	CD (p = 0.05)	2.08		1.0/10	

 $PDB-Potato\ Dextrose\ Broth;\ CDB-Czapek's\ Dox\ Broth;\ MEB-Malt\ Extract\ Broth;\ OB-Oatmeal\ Broth$

Table 4. Effect of pH level on mycelia growth and sporulation of Fusarium spp. isolates

No.	Fusarium sp.	pH value	Dry mycelial weight	No. of s	
1	isolates 2	3	4	macro conidia 5	micro conidia 6
1			F. oxysporum f. sp. psidii (Fop		D D
		4.5	245	1.9 x10 ⁵	1.0x10 ⁵
		5.0	896	2.3 x10 ⁵	1.2x10 ⁵
		5.5	1 208	2.9x10 ⁵	1.2x10 ⁵
		6.0	880	3.8x10 ⁵	2.4x10 ⁵
1	F10	6.5	653	4.2×10 ⁵	2.8x10 ⁵
		7.0	509	3.0x10 ⁵	1.6x10 ⁵
		7.5	455	2.8x10 ⁵	1.1x10 ⁵
		8.0	298	2.2x10 ⁵	1.0x10 ⁵
		4.5	214	1.2x10 ⁵	0.6x10 ⁵
		5.0	882	2.9x10 ⁵	1.5x10 ⁵
		5.5	1 112	2.9x10 ⁵	1.0x10 ⁵
		6.0	802	3.6x10 ⁵	2.2x10 ⁵
2	F18	6.5	661	4.2×10 ⁵	2.6x10 ⁵
		7.0	558	3.0×10^5	1.8x10 ⁵
		7.5	412	2.8x10 ⁵	1.6x10 ⁵
		8.0	228	2.5x10 ⁵	1.0x10 ⁵
		4.5	255	1.4x10 ⁵	1.0x10 ⁵
		5.0	956	2.8x10 ⁵	1.8x10 ⁵
		5.5	1 059	2.9x10 ⁵	1.0x10 ⁵
		6.0	952	3.8x10 ⁵	2.2x10 ⁵
3	F24	6.5	756	4.4x10 ⁵	2.6x10 ⁵
		7.0	662	3.1x10 ⁵	1.8x10 ⁵
		7.5	450	2.5x10 ⁵	1.2x10 ⁵
		8.0	342	2.8x10 ⁵	1.1x10 ⁵
		4.5	243	1.4x10 ⁵	1.5x10 ⁵
		5.0	893	2.8x10 ⁵	1.4x10 ⁵
		5.5	1 092	3.0x10 ⁵	1.1×10 ⁵
		6.0	874	3.6x10 ⁵	2.1x10 ⁵
4	F30	6.5	625	4.2x10 ⁵	2.5x10 ⁵
		7.0	512	3.2x10 ⁵	1.8×10 ⁵
		7.5	482	2.2x10 ⁵	0.8x10 ⁵
		8.0	351	2.6x10 ⁵	0.5x10 ⁵
		4.5	347	1.5x10 ⁵	1.5x10 ⁵
		5.0	954	3.0x10 ⁵	1.1x10 ⁵
		5.5	1 187	3.0x10 ⁵	0.9x10 ⁵
		6.0	910	3.8x10 ⁵	2.0x10 ⁵
5	F38	6.5	782	4.2x10 ⁵	2.6x10 ⁵
		7.0	612	3.2x10 ⁵	1.9x10 ⁵
		7.5	582	2.5x10 ⁵	1.8x10 ⁵
		8.0	383	2.1x10 ⁵	0.8x10 ⁵
		0.0	F. solani (Fs)	2.17(10	0.0.710
		4.5	330	1.9x10 ⁵	1.2x10 ⁵
		5.0	980	2.2x10 ⁵	0.9x10 ⁵
		5.5	1 180	2.6x10 ⁵	0.5x10 ⁵
		6.0	915	3.8x10 ⁵	2.2x10 ⁵
6	F2	6.5	745	4.4x10 ⁵	2.8x10 ⁵
		7.0	540	3.0x10 ⁵	1.8x10 ⁵
		7.5	475	2.2x10 ⁵	1.9x10 ⁵
		8.0	332	2.5x10 ⁵	1.3x10 ⁵
	+	4.5	342	1.8x10 ⁵	2.2x10 ⁵
		5.0	1 005	2.1x10 ⁵	1.0x10 ⁵
		5.5	1 240	2.1x10 ⁵ 2.6x10 ⁵	0.8x10 ⁵
		6.0	958	3.5x10 ⁵	2.2x10 ⁵
7	F12	6.5	788	4.8x10 ⁵	2.6x10 ⁵
		7.0	562	3.0x10 ⁵	2.0x10 ⁵
		7.5	510	2.5x10 ⁵	1.5x10 ⁵
		8.0	389	2.6x10 ⁵	1.1x10 ⁵

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1	2	3	4	5	6
		4.5	312	1.5x10 ⁵	1.1x10 ⁵
		5.0	955	2.0x10 ⁵	1.1x10 ⁵
		5.5	1 152	1.8x10 ⁵	1.2x10 ⁵
0	F15	6.0	905	3.6x10 ⁵	2.4x10 ⁵
8	F15	6.5	721	4.8x10 ⁵	2.8x10 ⁵
		7.0	546	3.0x10 ⁵	1.9×10^{5}
		7.5	508	2.8x10 ⁵	1.6×10^{5}
		8.0	310	2.4x10 ⁵	1.2×10^{5}
		4.5	356	1.9x10 ⁵	1.1×10^{5}
		5.0	1 083	2.2x10 ⁵	1.2×10^{5}
		5.5	1 275	2.9x10 ⁵	1.4×10^{5}
9	F20	6.0	953	3.8x10 ⁵	2.5x10 ⁵
9	F20	6.5	784	4.8x10 ⁵	2.9x10 ⁵
		7.0	563	3.2x10 ⁵	2.0×10^{5}
		7.5	554	2.2x10 ⁵	1.6×10^{5}
		8.0	390	2.2x10 ⁵	1.2×10^{5}
		4.5	326	1.6x10 ⁵	1.2×10^{5}
		5.0	911	2.3x10 ⁵	1.6×10^{5}
		5.5	1 158	2.8x10 ⁵	1.0×10^{5}
10	F29	6.0	923	3.8x10 ⁵	2.4×10^{5}
10	F29	6.5	718	4.7x10 ⁵	2.9x10 ⁵
		7.0	522	3.2x10 ⁵	1.9x10 ⁵
		7.5	504	2.8x10 ⁵	2.0×10^{5}
		8.0	321	2.2x10 ⁵	1.3x10 ⁵
Statis	tical analysis	CD (p = 0.05)	1.52		

Table 5. Effect of temperature on growth and sporulation of Fusarium spp. isolates

	Fusarium sp.	Temperature value	Mean colony	No.	of spore/ml
No.	isolates	[°C]	diameter [mm]	macro conidia	micro conidia
1	2	3	4	5	6
			xysporum f. sp. psidii		
		10	11.50	0.5x10 ⁵	1.0×10^{5}
		16	26.50	0.9x10 ⁵	1.0×10^{5}
1	F10	22	44.50	1.4x10 ⁵	$1.1x10^{5}$
1	1.10	28	71.00	2.2x10 ⁵	1.6×10^{5}
		34	64.50	3.3x10 ⁵	2.1×10^{5}
		40	58.50	3.0x10 ⁵	$1.8x10^{5}$
		10	11.25	0.4x10 ⁵	$0.9x10^{5}$
		16	25.50	0.8x10 ⁵	1.0×10^{5}
2	F18	22	44.50	1.6x10 ⁵	$1.1x10^{5}$
_	1.10	28	71.50	2.2x10 ⁵	1.6×10^{5}
		34	62.50	3.2x10 ⁵	2.3x10 ⁵
		40	60.50	3.0x10 ⁵	1.9×10^{5}
		10	11.50	0.4x10 ⁵	$1.0x10^{5}$
		16	30.50	0.6×10^{5}	1.0×10^{5}
3	F24	22	46.50	1.5x10 ⁵	1.0×10^{5}
3	Γ24	28	71.00	2.4x10 ⁵	1.5×10^{5}
		34	63.50	3.6x10 ⁵	2.6×10^{5}
		40	59.50	3.0x10 ⁵	2.1×10^{5}
		10	10.25	0.6×10^5	$1.0x10^{5}$
		16	32.50	1.0×10^5	1.0×10^{5}
4	F30	22	44.50	1.8x10 ⁵	0.9×10^{5}
4	150	28	72.50	2.6x10 ⁵	1.5×10^{5}
		34	66.50	3.4x10 ⁵	2.6×10^{5}
		40	59.50	3.1x10 ⁵	2.2x10 ⁵
		10	11.50	0.5x10 ⁵	1.0×10^{5}
		16	24.50	0.8x10 ⁵	1.5×10^{5}
5	F38	22	42.50	1.5x10 ⁵	$1.1x10^{5}$
3	150	28	70.50	2.8x10 ⁵	1.5×10^{5}
		34	65.50	3.5x10 ⁵	2.4×10^{5}
		40	60.50	3.2x10 ⁵	2.2x10 ⁵
			F. solani (Fs)		
		10	10.50	0.5x10 ⁵	1.1x10 ⁵
		16	24.50	0.8x10 ⁵	1.2x10 ⁵
6	F2	22	42.50	1.6x10 ⁵	1.0×10^{5}
6	FZ	28	69.50	2.6x10 ⁵	1.2x10 ⁵
		34	65.50	3.2x10 ⁵	2.2x10 ⁵
		40	60.50	2.8x10 ⁵	1.6x10 ⁵

1	2	3	4	5	6
		10	11.25	0.9×10^{5}	1.0x10 ⁵
		16	24.50	1.2x10 ⁵	1.0×10^{5}
7	F12	22	41.25	1.8x10 ⁵	1.1x10 ⁵
/	F12	28	69.50	2.8x10 ⁵	1.6x10 ⁵
		34	66.50	3.9x10 ⁵	2.4×10^{5}
		40	59.50	3.3x10 ⁵	2.2x10 ⁵
		10	10.25	0.6×10^{5}	1.2x10 ⁵
		16	26.50	1.0x10 ⁵	1.0x10 ⁵
8	E15	22	40.50	1.5x10 ⁵	1.0×10^{5}
8	F15	28	72.00	2.4x10 ⁵	1.6x10 ⁵
		34	68.50	3.9x10 ⁵	2.8x10 ⁵
		40	61.50	$3.3x10^{5}$	2.2x10 ⁵
		10	10.25	1.0×10^{5}	0.9×10^{5}
		16	28.50	1.6x10 ⁵	1.0×10^{5}
9	F20	22	40.50	2.0x10 ⁵	1.5×10^{5}
9	F20	28	72.50	3.2x10 ⁵	1.8×10^{5}
		34	69.50	3.5x10 ⁵	2.5x10 ⁵
		40	60.50	3.2x10 ⁵	2.1×10^{5}
		10	10.50	0.6×10^{5}	1.5×10^{5}
		16	22.50	$1.1x10^{5}$	1.0×10^{5}
10	F29	22	41.50	1.8x10 ⁵	1.4×10^{5}
10	г29	28	71.50	2.8x10 ⁵	2.3x10 ⁵
		34	69.50	3.5x10 ⁵	2.6x10 ⁵
		40	61.50	3.1x10 ⁵	2.2x10 ⁵
Statis	tical analysis	CD (p = 0.05)	2.92		

growth. Both *F. oxysporum* f. sp. *psidii* and *F. solani* isolates did not produce a metabolite colour when grown on CMA media.

The present results confirm the reports of earlier workers doing their physiological studies related to suitable media for growth and sporulation of *Fusarium* spp. (Kulkarni 2006; Chittem and Kulkarni 2008).

F. oxysporum f. sp. *psidii* and *F. solani* put maximum dry mycelial weight on malt extract broth which was significantly superior to all the media tested. This was followed by PDB. Excellent sporulation was recorded in MB media followed by PDB. These results are somewhat similar to the results obtained by Kulkarni (2006) who found that, the maximum dry mycelial weight of fungus was obtained in potato dextrose broth.

Cultural studies of *F. oxysporum* f. sp. *dianthi* on solid media indicated that, the radial growth was maximum on potato dextrose agar which was significantly superior over all other media viz. Czapek's Dox Agar, Malt Extract Agar, Oatmeal Agar and Rose Bengal Agar. Profused sporulation was observed in the case of Oatmeal Agar (Chittem and Kukarni 2008).

The results revealed that a suitable pH for maximum mycelial mass production was 5.5 followed by 5.0 for both species of *F. oxysporum* f. sp. *psidii* and *F. solani* isolates. While maximum sporulation for *F. oxysporum* f. sp. *psidii* and *F. solani* was obtained at a pH of 6.5 followed by a pH of 6.0. Hence, it is clearly indicated that the tested *Fusarium* spp. favoured acidic pH for its growth and sporulation. Ahamad *et al.* (2002) grew *G. fujikuroi* in broth medium at 4 different pH and observed excellent growth and sporulation at a pH of 5. Many reports also support our presented data (Farkya *et al.*1996; Chauhan 1997). Sharma *et al.* (2005) studied the effect of pH on the growth and sporulation of *F. oxysporum* f. sp. *lini* and reported that tested *Fusarium* spp. could sporulate and grew well at 5.5 pH.

Radial growth was maximum at a temperature of 28°C (72.50 mm for both *F. oxysporum* f. sp. *psidii* and *F. solani*) followed by a temperature of 34°C (66.5 mm for *F. oxysporum* f. sp. *psidii*; 69.5 mm for *F. solani*). Optimum sporulation was recorded at a temperature of 34°C followed by 40°C. Therefore, optimum temperature for the best growth was 28°C, which was close to room temperature, however moderate growth and sporulation was recorded at 34°C because it showed maximum colony growth next to a temperature of 28°C and highest in conidia production.

Desai *et al.* (2003) reported that *F. oxysporum* f. sp. *ricini* showed maximum growth and sporulation at 27±2°C on PDA. Daami-Remadi *et al.* (2006) observed that thermal optimum of 25 and 30°C was suitable for maximum mycelial growth of *F. oxysporum* f. sp. *tuberose,* whereas, for that of *F. solani* it was at 30°C. Therefore, It is clear that temperature near 25–35°C favored the growth and sporulation of *Fusarium* which is supported by the results of other workers (Boughalleb 2001; Daami-Remadi *et al.* 2004) and confirmed the results of our present investigation.

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POLISH SUMMARY

CECHY WZROSTU FUSARIUM SPP. WYWOŁUJĄCYCH WIĘDNIĘCIE *PSIDIUM* **GUAJAVA**

Fusarium oxysporum f. sp. psidii i F. solani, czynniki sprawcze więdnięcia P. guajava, są wysoce zmiennymi patogenami. Przedstawione badania wykonano w celu poznania cech kulturowych i fizjologicznych (temperatura i pH). Wyniki wykazały, że maksymalny wzrost grzybni miał miejsce na agarze ziemniaczanym z glukozą, jako na półstałej pożywce, np. wynosił 78 mm dla *F. oxysporum* f. sp. psidii; 73,83 mm dla F. solani, podczas gdy w półpłynnej pożywce słodowej uzyskano 1 385 mg grzybni dla Fusarium oxysporum f. sp. psidii i 1 491 mg dla F. solani. Maksymalne zarodnikowanie stwierdzono na pożywce z maki owsianej i w półpłynnej pożywce mikologicznej. Optymalna temperatura i pH dla wzrostu obydwóch gatunków Fusarium, wynosiły 28°C i 5,5. Izolaty różniły się wzrostem kolonii, masą grzybni, makrokonidiami i mikrokonidiami. Te zróżnicowania były charakterystyczne dla każdego izolatu i dotyczyły zarówno cech kulturowych, jak i fizjologicznych.