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

Vijai Kumar Gupta[1], Ashok Kumar Misra[2], Rajarshi Kumar Gaur[1]*

[1] Department of Biotechnology, MITS University, Laxmangarh-332311, Rajasthan
[2] Central Institute for Subtropical Horticulture, Lucknow-227017, UP, India

Received: January 5, 2010
Accepted: September 24, 2010

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

*Corresponding address:
vijaifzd@gmail.com

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):

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

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

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

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 ($4.0X10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $3.9X10^5$ per ml for macro-conidia for *F. solani* and $2.8X10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.2X10^5$ per ml for micro-conidia for *F. solani*) followed by CMA ($3.6X10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $3.4X10^5$ per ml for macro-conidia for *F. solani* and $2.4X10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.0X10^5$ 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 (macro-conidia), 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 ($4.5X10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $4.2X10^5$ per ml for macro-conidia for *F. solani* and $2.9X10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.9X0^5$ per ml for micro-conidia for *F. solani*) followed by PDB ($2.8X10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $4.0X10^5$ per ml for macro-conidia for *F. solani* and $2.2X10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.8X10^5$ 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 ($4.4x10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $4.8x10^5$ per ml for macro-conidia for *F. solani* and $2.8x10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.9x0^5$ per ml for micro-conidia for *F. solani*) followed by a pH of 6.0 ($3.8x10^5$ per ml for macro-conidia for both of *F. oxysporum* f. sp. *psidii* and *F. solani*; $2.4x10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.5x10^5$ 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 ($3.6x10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $3.9x10^5$ per ml for macro-conidia for *F. solani* and $2.6x10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.8x10^5$ per ml for micro-conidia for *F. solani*) followed by a temperature at 40°C temperature ($3.2x10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $3.3x10^5$ per ml for macro-conidia for *F. solani* and $2.2x10^5$ 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

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

No.	*Fusarium* sp. isolates	Media	Colony colour	Mean radial growth [mm]	Metabolite colour	No. of spore/ml	
						macro conidia	micro conidia
1	2	3	4	5	6	7	8
F. oxysporum f. sp. *psidii* (Fop)							
1	F10	PDA	orange-cottony white	71.60	light brown	$2.8X10^5$	$1.2X10^5$
		CDA	cottony white	67.63	no colour	$2.5X10^5$	$1.2X10^5$
		CMA	cottony white	70.13	no colour	$3.2X10^5$	$1.9X10^5$
		OA	cottony white	64.25	yellow	$3.8X10^5$	$2.3X10^5$
		CRBA	cottony white	62.25	no colour	$1.5X10^5$	$1.0X10^5$
2	F18	PDA	cottony white	74.38	light brown	$2.4X10^5$	$1.1X10^5$
		CDA	cottony white	68.00	pink colour	$3.1X10^5$	$1.6X10^5$
		CMA	white matty type	71.13	no colour	$3.4X10^5$	$2.1X10^5$
		OA	cottony white	65.50	cream colour	$3.5X10^5$	$2.4X10^5$
		CRBA	cottony pink	61.38	no colour	$1.5X10^5$	$1.1X10^5$
3	F24	PDA	cottony white	78.00	pale yellow	$2.4X10^5$	$1.0X10^5$
		CDA	cottony white	68.00	no colour	$2.8X10^5$	$1.4X10^5$
		CMA	dirty cottony white	72.25	no colour	$3.2X10^5$	$2.2X10^5$
		OA	dirty cottony white	71.00	cream colour	$3.6X10^5$	$2.6X10^5$
		CRBA	cottony white	62.88	cream colour	$1.7X10^5$	$1.1X10^5$
4	F30	PDA	cottony off-white	73.75	pale yellow	$2.3X10^5$	$1.5X10^5$
		CDA	cottony white	69.50	pink colour	$2.4X10^5$	$1.3X10^5$
		CMA	off white cottony	72.50	no colour	$3.6X10^5$	$2.4X10^5$
		OA	cottony white	69.50	no colour	$4.0X10^5$	$2.8X10^5$
		CRBA	cottony white	58.87	cream colour	$1.7X10^5$	$1.2X10^5$
5	F38	PDA	cottony white	73.38	dark yellow	$2.1X10^5$	$1.0X10^5$
		CDA	cottony white	68.38	no colour	$2.2X10^5$	$1.0X10^5$
		CMA	dirty cottony white	72.25	no colour	$2.4X10^5$	$2.1X10^5$
		OA	dirty white matty type	69.00	yellow colour	$3.4X10^5$	$2.3X10^5$
		CRBA	cottony pink	61.25	no colour	$1.2X10^5$	$1.0X10^5$
F. solani (Fs)							
6	F2	PDA	cottony white with bluish at centre	70.38	violet	$2.6x10^5$	$1.2x10^5$
		CDA	cottony white	66.12	light orange colour	$1.0x10^5$	$1.1x10^5$
		CMA	dirty cottony white	69.75	no colour	$2.8x10^5$	$1.8x10^5$
		OA	cottony white with violet ring at centre	55.00	pale yellow	$3.6x10^5$	$2.0x10^5$
		CRBA	cottony pink	56.5	no colour	$2.0x10^5$	$1.4x10^5$

1	2	3	4	5	6	7	8
7	F12	PDA	creamy	70.37	pinkish colour	$2.2x10^5$	$1.0x10^5$
		CDA	cottony white	62.37	light orange colour	$2.9x10^5$	$1.9x10^5$
		CMA	white matty type	68.12	no colour	$3.2x10^5$	$2.1x10^5$
		OA	dirty cottony white	61.75	pale yellow	$3.8x10^5$	$2.2x10^5$
		CRBA	cottony pink	59.31	no colour	$2.6x10^5$	$1.2x10^5$
8	F15	PDA	cottony pink	73.83	pinkish	$2.1x10^5$	$1.8x10^5$
		CDA	dirty cottony white	60.75	dark yellow	$2.2x10^5$	$1.0x10^5$
		CMA	dirty white matty type	64.00	no colour	$3.4x10^5$	$2.0x10^5$
		OA	dirty cottony white	61.12	pale yellow	$3.9x10^5$	$2.2x10^5$
		CRBA	cottony pink	59.63	no colour	$2.6x10^5$	$1.2.8x10^5$
9	F20	PDA	white matty	70.35	pinkish	$2.4x10^5$	$1.2x10^5$
		CDA	cottony white	65.00	cream colour	$2.6x10^5$	$1.1x10^5$
		CMA	dirty cottony white	70.38	no colour	$2.9x10^5$	$1.6x10^5$
		OA	dirty cottony white	66.38	no colour	$3.1x10^5$	$2.2x10^5$
		CRBA	cottony pink	62.25	no colour	$2.8x10^5$	$1.8x10^5$
10	F29	PDA	white matty	69.35	pink yellow	$1.8x10^5$	$1.2x10^5$
		CDA	cottony white	61.50	cream colour	$1.8x10^5$	$1.1x10^5$
		CMA	dirty cottony white	63.38	no colour	$2.4x10^5$	$1.6x10^5$
		OA	dirty white matty type	57.50	no colour	$3.9x10^5$	$2.2x10^5$
		CRBA	cottony pink	59.47	no colour	$2.1x10^5$	$1.4x10^5$
Statistical analysis		CD (p = 0.05)		2.44			

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

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

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

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

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

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

No.	*Fusarium* sp. isolates	Temperature value [°C]	Mean colony diameter [mm]	No. of spore/ml	
				macro conidia	micro conidia
1	2	3	4	5	6
F. oxysporum f. sp. *psidii* (Fop)					
1	F10	10	11.50	$0.5x10^5$	$1.0x10^5$
		16	26.50	$0.9x10^5$	$1.0x10^5$
		22	44.50	$1.4x10^5$	$1.1x10^5$
		28	71.00	$2.2x10^5$	$1.6x10^5$
		34	64.50	$3.3x10^5$	$2.1x10^5$
		40	58.50	$3.0x10^5$	$1.8x10^5$
2	F18	10	11.25	$0.4x10^5$	$0.9x10^5$
		16	25.50	$0.8x10^5$	$1.0x10^5$
		22	44.50	$1.6x10^5$	$1.1x10^5$
		28	71.50	$2.2x10^5$	$1.6x10^5$
		34	62.50	$3.2x10^5$	$2.3x10^5$
		40	60.50	$3.0x10^5$	$1.9x10^5$
3	F24	10	11.50	$0.4x10^5$	$1.0x10^5$
		16	30.50	$0.6x10^5$	$1.0x10^5$
		22	46.50	$1.5x10^5$	$1.0x10^5$
		28	71.00	$2.4x10^5$	$1.5x10^5$
		34	63.50	$3.6x10^5$	$2.6x10^5$
		40	59.50	$3.0x10^5$	$2.1x10^5$
4	F30	10	10.25	$0.6x10^5$	$1.0x10^5$
		16	32.50	$1.0x10^5$	$1.0x10^5$
		22	44.50	$1.8x10^5$	$0.9x10^5$
		28	72.50	$2.6x10^5$	$1.5x10^5$
		34	66.50	$3.4x10^5$	$2.6x10^5$
		40	59.50	$3.1x10^5$	$2.2x10^5$
5	F38	10	11.50	$0.5x10^5$	$1.0x10^5$
		16	24.50	$0.8x10^5$	$1.5x10^5$
		22	42.50	$1.5x10^5$	$1.1x10^5$
		28	70.50	$2.8x10^5$	$1.5x10^5$
		34	65.50	$3.5x10^5$	$2.4x10^5$
		40	60.50	$3.2x10^5$	$2.2x10^5$
F. solani (Fs)					
6	F2	10	10.50	$0.5x10^5$	$1.1x10^5$
		16	24.50	$0.8x10^5$	$1.2x10^5$
		22	42.50	$1.6x10^5$	$1.0x10^5$
		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
7	F12	10	11.25	$0.9x10^5$	$1.0x10^5$
		16	24.50	$1.2x10^5$	$1.0x10^5$
		22	41.25	$1.8x10^5$	$1.1x10^5$
		28	69.50	$2.8x10^5$	$1.6x10^5$
		34	66.50	$3.9x10^5$	$2.4x10^5$
		40	59.50	$3.3x10^5$	$2.2x10^5$
8	F15	10	10.25	$0.6x10^5$	$1.2x10^5$
		16	26.50	$1.0x10^5$	$1.0x10^5$
		22	40.50	$1.5x10^5$	$1.0x10^5$
		28	72.00	$2.4x10^5$	$1.6x10^5$
		34	68.50	$3.9x10^5$	$2.8x10^5$
		40	61.50	$3.3x10^5$	$2.2x10^5$
9	F20	10	10.25	$1.0x10^5$	$0.9x10^5$
		16	28.50	$1.6x10^5$	$1.0x10^5$
		22	40.50	$2.0x10^5$	$1.5x10^5$
		28	72.50	$3.2x10^5$	$1.8x10^5$
		34	69.50	$3.5x10^5$	$2.5x10^5$
		40	60.50	$3.2x10^5$	$2.1x10^5$
10	F29	10	10.50	$0.6x10^5$	$1.5x10^5$
		16	22.50	$1.1x10^5$	$1.0x10^5$
		22	41.50	$1.8x10^5$	$1.4x10^5$
		28	71.50	$2.8x10^5$	$2.3x10^5$
		34	69.50	$3.5x10^5$	$2.6x10^5$
		40	61.50	$3.1x10^5$	$2.2x10^5$
Statistical 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.

REFERENCES

Agarwal D.K., Sarboy A.K. 1978. Physiological studies on four species of *Fursarium* pathogenic to soyabean. Indian Phytopathol. 31 (1): 24–31.

Ahamad S., Agrawal D.K., Udit N., Chauhan S.S. 2002. Effect of temperature, pH, light and inoculation period on growth, sporulation, biomass and gibberellic acid production. Ann. Plant Protect. Sci. 10 (2): 343–348.

Booth C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, 276 pp.

Booth C. 1977. *Fusarium*, Laboratory Guide to the Identification of the Major Species. Commonwealth Mycological Institute, Kew, Surrey, England, 58 pp.

Boughalleb N. 2001. Decay of watermelon in Tunisia: Identification of the causal organism and biology of *Fusarium* spp.

Mémoire de Diplôme d'Etudes Approfondies en Protection des Plantes et Environnement. Ecole Supérieure d'Horticulture et d'Elevage de Chott Mariam, 76 pp.

Chattapadhyay S.B., Bhattacharjya S.K. 1968. Investigations on the wilt disease of guava (*Psidium guajava* L.) in West Bengal I. Indian J. Agric. Sci. 38 (1): 65–72.

Chattopadhyay S.B., Sengupta S.K. 1955. Studies on wilt of *Psidium guajava* L. in West Bengal. Indian J. Hort. 12: 76–79.

Chauhan M.U. 1997. Investigations on wilt disease of bottle gourd caused by *Fuarium solani* (Mart.) Sacc. M.Sc. (Agric.) thesis. G.A.U., S.K. Nagar, India, 68 pp.

Chittem K., Kulkarni S. 2008. Effect of Media on the Growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum* f. sp. *dianthi.* Karnataka J. Agric. Sci. 21 (2): 303–304.

Cochrane V.W. 1958. Physiology of Fungi. Welly, New York, 524 pp.

Daami-Remadi M., El Mahjoub M. 2004. Appearance in Tunisia of *Fusarium oxysporum* f. sp. *tuberosi* causing vascular wilting and tuber dry rot of potato. Bull. OEPP/EPPO Bull. 34: 407–411.

Dammi-Remadi M., Jabnoun-Khiareddine H., Ayed F., EI Mahjoub M. 2006. Effect of temperature on aggerressivity of Tunisian *Fusarium* sp. causing potato (*Solanum tuberosum* L) tuber dry rot. J. Agron. 5 (2): 350–355.

Desai A.G., Dange S.R.S., Patel D.S., Patel D.B. 2003. Variability of *Fusarium oxysporum* f. sp. *ricini* causing wilt of castor. J. Mycol. Plant Pathol. 33 (1): 37–41.

Dwivedi S.K., Dwivedi P. 1999. Wilt disease of guava: a national problem. J. Appl. Hort. 1 (2): 151–154.

Edward J.C. 1960. Variation in the guava wilt pathogen, *Fusarium oxysporum* f. sp. *psidii*. Indian Phytopathol. 13: 30–36.

Farkya S., Pandey A.K., Rajak R.S. 1996. Mycoherbicides potential of *Fusarium* spp. against *Parthenium*: Factors affecting *in vitro* growth and sporulation. Bioved 7: 1–9.

Kulkarni S.P. 2006. Studies on *Fusarium oxysporum* Schlecht Fr f. sp. *gladioli* (Massey) Snyd. & Hans. causing wilt of *Gladiolus.* M. Sc. (Agric.) thesis, Univ. Agric. Sci., Dharwad, India, 245 pp.

Misra AK. 2006. Wilt of guava-a disease of national importance. Indian Phytopathol. 59 (3): 269–280.

Misra A.K., Gupta V.K. 2010. Pathogenicity of *Fusarium* spp. isolates of guava wilt. J. Mycol. Plant Pathol. 40 (1): 72–77.

Pandav F.J. 2002. Investigation on wilt [*Fusarium solani* (Mart.) Sacc.] on cowpea (*Vigna unguiculata* L. Walp) under South Gujarat condition. M.Sc. (Agric.) thesis, G.A.U., S.K. Nagar, India, 262 pp.

Pathak V.N. 1984. Laboratory Manual of Plant Pathology. Second edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi: 11–12.

Paulkar P.K., Raut B.T. 2004. Variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri*. J. Mycol. Plant Pathol. 34 (1): 20–23.

Prasad N., Mehta P.R., Lal S.B. 1952. *Fusarium* wilt of guava (*Psidium guajava* L.) in Uttar Pradesh, India. Nature 169, p. 753.

Sharma R.L., Singh B.P., Thakur M.P., Thapak S.K. 2005. Effect of media, temperature, pH and light on the growth and sporulation of *Fusarium oxysporum* f. sp. *lini.* Ann. Plant Protect. Sci. 13: 172–174.

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 mąki 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.