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Variability among Isolates of *Fusarium oxysporum* f. sp. *chrysanthemi* Pathogenic to *Chrysanthemum*

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ABSTRACT

Fusarium oxysporum f. sp. *chrysanthemi* (FOC) is one of the most wide spread and destructive pathogen, causing infection to *Chrysanthemum* plants throughout the world. Qualitative evaluation of 10 FOC isolates was done for their ability to produce fusaric acid, using thin layer chromatography. A variation in FOC isolates was observed and isolate FO-1, FO-2 and FO-3 were very strong producers of fusaric acid. Isolate FO-1 produced highest biomass in liquid medium and radial diameter on solid medium and FO-2 was the slowest. Growth of FOC isolates was recorded maximum on Potato Dextrose Broth (PDB), followed by Czapex Dox Broth (CDB) and FSM-Liquid. All the isolates of FOC showed significantly ($p<0.01$) higher growth on pH 5 followed by 7, 9, 3 and 11. FOC isolates growth was highest at temperature of 25°C followed by 30, 20, 15, 35 and 40°C. None of FOC isolates showed radial growth at 40 and 45°C. Results of this study has revealed that a temperature of 25°C, pH of 5.0 and Potato dextrose medium was most suitable for the growth of FOC isolates.

Key words: *Fusarium oxysporum* f. sp. *chrysanthemi,* temperature, *Chrysanthemum,* fusaric acid, ornamental

INTRODUCTION

Chrysanthemum is cultivated throughout the world and it is ranked among the three top flowers. *Chrysanthemum* plants are infected by various plant pathogens of which Fusarium *oxysporum* f. sp. *chrysanthemi* (FOC) is one of the most destructive pathogen, causing infection and losses to plant. Severe losses to the *Chrysanthemum* caused by FOC have been reported from various part of the world (Garibaldi *et al*., 2009; Minuto *et al*., 2007; Murkar *et al*., 1994). The isolates of *Fusarium oxysporum* are very virulent and they cause severe losses to most of the commercial crops. Losses due to *Fusarium oxysporum* are reported by several workers (Gupta *et al*., 2010; Latiffah *et al*., 2009; Jegathambigai *et al*., 2009).

FOC have an ability to infect *Chrysanthemum* plants at any stage of its growth starting from nursery to flowering. It is reported that along with *Chrysanthemum* it also infects *Gerbera jamesonii,* Argyranthemum frutescens (Paris daisy) and Osteospermum sp. Recently disease occurrence on four major economically important ornamental crops belonging to the Compositae family was reported which indicates that isolates of FOC are very virulent and have wide range of plant host infection capability (Garibaldi *et al*., 2009).

Fusaric acid is produced by *Fusarium* spp. and formae speciales of *F. oxysporum*. The phytotoxic effects of fusaric acid have been proved in several *Fusarium* induced diseases. Production of fusaric acid by *Fusarium oxysporum* has been reported by several workers (Diniz and Oliveira, 2009;

Peterson and Rutherford, 1991). Fusaric acid is phyto-toxic and plays an important role in disease development (Wu *et al*., 2008; Liu *et al*., 2010).

A critical review of literature reveals that very little work has been done on the understanding of nature of FOC. Considering the importance of FOC, the present investigation was undertaken to find out its nutritional, pH and temperature requirements. Attempts were also made to characterize FOC isolates on the basis of fusaric acid.

MATERIALS AND METHODS

Rating of FOC isolates on the basis of fusaric acid: The present study was conducted during 2007 to 2009. FOC isolates were characterized on the basis of their ability to produce fusaric acid, using the method described by Peterson and Rutherford (1991).

Effect of nutrient medium on growth of FOC: Four isolates FO-1, FO-2, FO-3 and FO-4 were selected for growth and cultural studies using 3 liquid (Potato Dextrose Broth-PDB, Czapex Dox Broth-CDB and Fusarium Selective Medium-FSM) and 3 solid (Potato Dextrose Agar-PDA, Czapex Dox Agar-CDA and Fusarium Selective Medium-FSM) medium. PDB was prepared by adding 24 gm of powder (Make-Himedia Lab.) per liter of distilled water. CDB medium was prepared by adding Sodium nitrate 2 g, Di potassium hydrogen phosphate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g and Sucrose 30 g per liter of distilled water. FSM-Liquid (Nash and Snyder, 1962) was prepared by adding Peptone 15.0 g, KH_2PO_4 1.0 g, $MgSO_4$.7H2O 0.5 g, Pentachloronitrobenzene 1.0 g and Streptomycin 0.3 g per liter of distilled water. For preparing solid medium (PDA, CDA and FSM) agar was added to the above three liquid medium @ 20 g L^{-1}.

One hundred milliliter of freshly prepared liquid medium were poured in 250 mL of conical flasks (Make-Borosil) and sterilized in autoclave for 15 min at 121°C temperature and 15 p.s.i pressure. Solid media were poured in the Petri plates after sterilization as per above method. Five mm bits of FOC isolates were cut with the help of a sterilized cork borer from seven days old culture, inoculated in flasks and Petri plates and incubated at 25±2°C in BOD-incubator. Data on radial growth and biomass was recorded periodically up to 14 days. All the isolates were inoculated in triplicates.

Effect of pH on growth of FOC: Growth characteristics of FOC were studied to evaluate the suitable pH required or preferred by the wilt pathogen for its growth and multiplication. PDB and PDA media were prepared by the method described above and pH of media was maintained at 3, 5, 7, 9 and 11 using hydrochloric acid and sodium hydroxide solutions. Hundred mL of PDB was poured in 250 mL of conical flasks and sterilized. PDA was poured in Petri plates after its sterilization. Bits of 5 mm were cut with a cork borer, inoculated in PDB flask and PDA Petri plates and incubated at 25±2°C in a BOD-incubator under stationary phase. The data of radial growth and biomass was taken periodically up to 14 days. All the isolates were inoculated in triplicates.

Effect of temperature on growth of FOC: PDB and PDA were prepared by above methods, inoculated with a single bit (5 mm) of fungus and incubated at 15, 20, 25, 30, 35, 40 and 45°C in BOD-incubator. Data on radial diameter and biomass were recorded periodically up to 14 days.

Statistical analysis: Data of radial growth and mycelial biomass of FOC isolates were analyzed for standard deviation and Standard Error (SE) using Excel 2003. Analysis of Variance (ANOVA) was carried out for all the data obtained from cultural studies (pH, medium and temperature).

RESULTS

Production of fusaric acid by isolates of FOC: All the 10 isolates of FOC show presence of the fusaric acid based on qualitative screening on TLC plate. There was variation in the content of fusaric acid produced by the isolates. Isolate FO-1, FO-2 and FO-3 were very strong in producing the fusaric acid as compared to others. Remaining all the isolates ranged within strong to medium fusaric acid producers (Table 1).

Growth of FOC isolates on nutrient medium: Among all the FOC isolates, maximum significant ($p<0.01$) growth was recorded with FO-1 both in liquid medium and solid medium. FO-2 was the slowest among all the four isolates (Fig. 1). Amongst the 3 liquid medium maximum growths was recorded on PDB, followed by CDB and FSM-Liquid. Results of solid media were comparable with the liquid media and PDA was found to be more suitable for growing the isolates of FOC. Slowest growth of the pathogen was observed on FSM. On CDA medium there was no significant ($p<0.01$) difference in radial diameter of FO-3 and FO-4. FO-1 and FO-3 isolate were

Table 1: Production of fusaric acid on TLC plate by different isolates of FOC

FOC Isolates	Qualitative rating FOC on the basis of Fusaric acid*
FO–1	+++++
FO–2	+++++
FO–3	+++++
FO–4	++++
FO–5	+++
FO–6	+++
FO–7	++++
FO–8	++++
FO–9	+++
FO–10	++++

*Qualitative rating of fusaric acid: +++++: Very strong, ++++: Strong, +++: Medium, ++: Low intensities, +: Only detectable, as seen on TLC

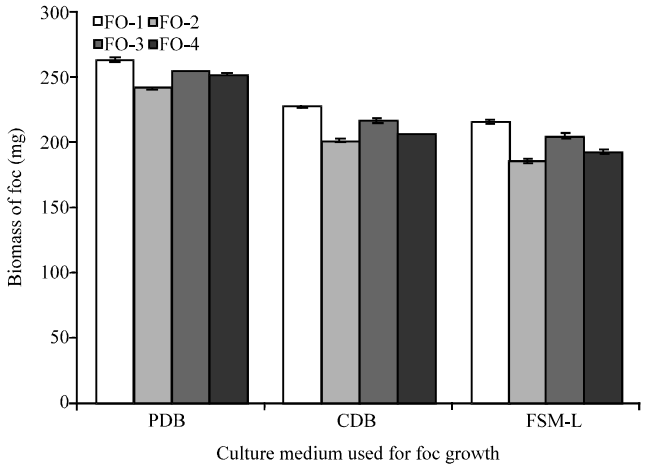

Fig. 1: Effect of liquid media on growth of *Fusarium oxysporum* f. sp. *chrysanthemi*. Values represent the mean of replicates with standard error

at par at FSM medium and they do not differ significantly ($p<0.01$) with each other (Fig. 2). There was also a variation in growth characteristics (colour, sporulation, colony appearance etc.) of the pathogen on different medium. The colonies of pathogen formed on PDA and CDA medium were fluffy and showed a consistent growth as compared to FSM medium.

Growth of FOC isolates at different pH: All the four isolates of FOC showed significantly ($p<0.01$) higher growth at pH 5 followed by pH-7, 9, 3 and 11 (Fig. 3). At acidic pH (3 and 5) isolate FO-4 produced maximum biomass and radial diameter which was significantly ($p<0.01$) different than all others. At neutral and basic pH isolate FO-1 produced significantly ($p<0.01$) higher biomass and radial diameter as compared to others (Fig. 4).

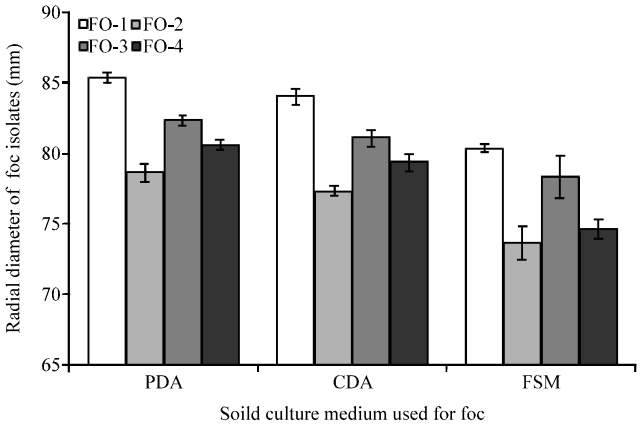

Fig. 2: Effect of Solid media on growth of *Fusarium oxysporum* f. sp. chrysanthemi. Values represent the mean of replicates with standard error

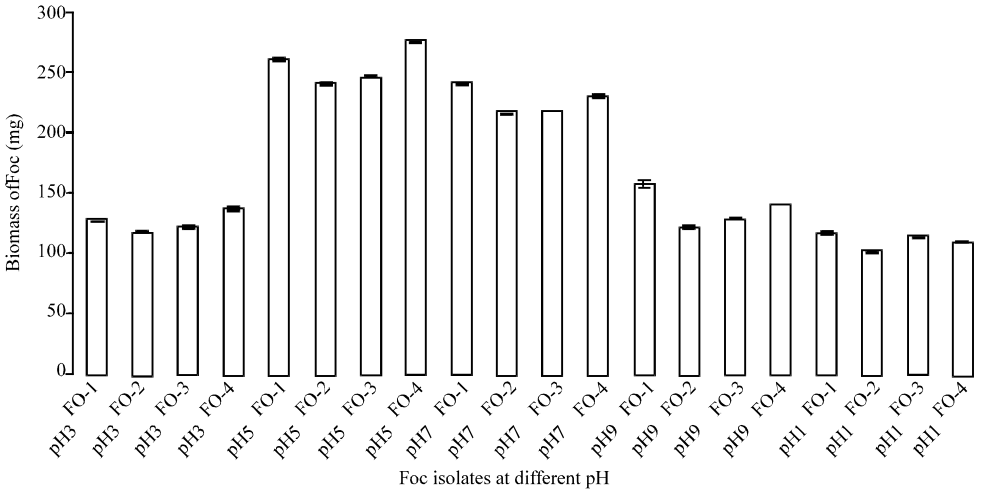

Fig. 3: Effect of pH on biomass growth of *Fusarium oxysporum* f. sp. chrysanthemi in PDB. Values represent the mean of replicates with standard error

Growth of FOC on different temperature: Growth of FOC isolates was recorded maximum when the temperature of the environment was kept at 25°C. On PDB Liquid medium biomass was recorded maximum on 25°C followed by 30, 20, 15, 35 and 40°C (Fig. 5). No mycelial growth was recorded at 45°C. At temperature of 15°C and 20°C FO-4 produced higher biomass than all the others isolates, however FO-1 produced significantly ($p<0.01$) higher biomass when it was grown on 25, 30, 35 and 40°C. On PDA medium, maximum radial diameter was produced on 25°C, followed by 30, 20, 15 and 35°C. FOC isolates do not show any mycelial growth at 40 and 45°C. Liquid medium was more supportive in the growth of pathogen (Fig. 6).

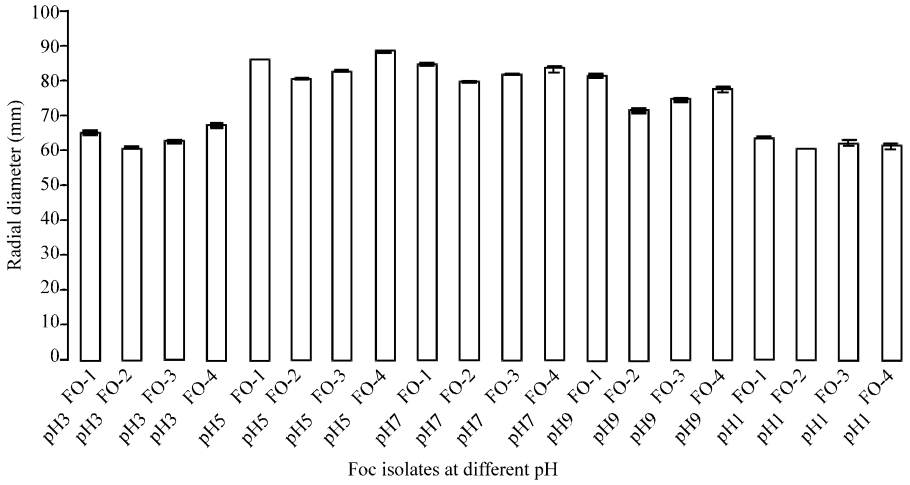

Fig. 4: Effect of pH on radial growth of *Fusarium oxysporum* f. sp. *chrysanthemi* on PDA. Values represent the mean of replicates with standard error

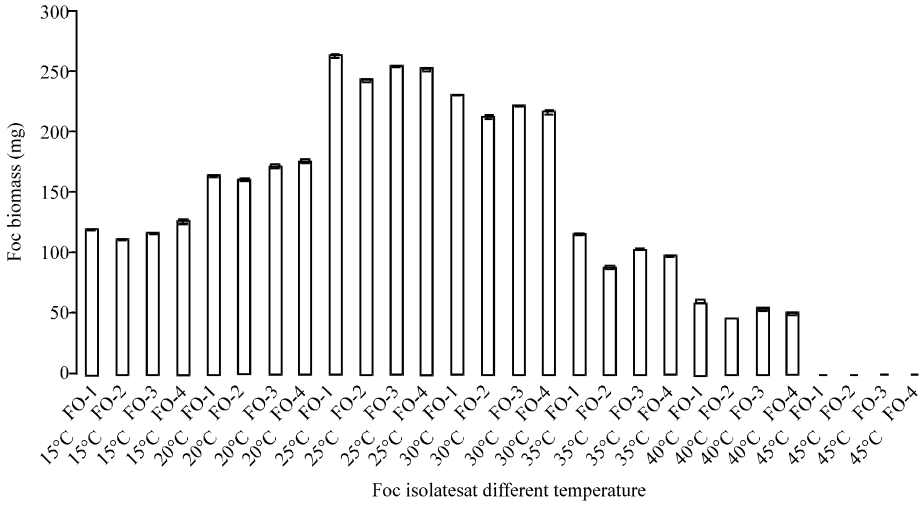

Fig. 5: Effect of Temperature on growth of *Fusarium oxysporum* f. sp. chrysanthemi in PDB. Values represents the mean of replicates with standard error

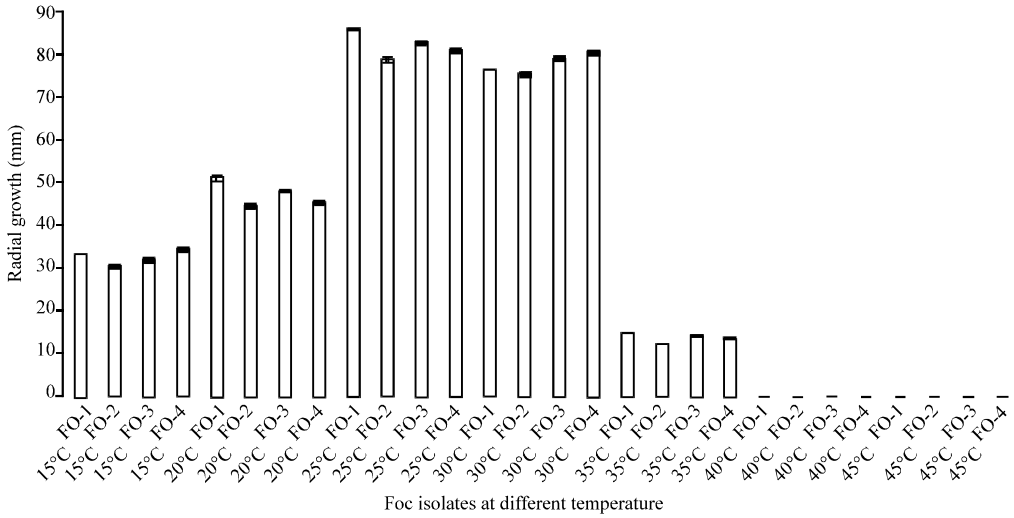

Fig. 6: Effect of Temperature on growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in PDB. Values represents the mean of replicates with standard error

DISCUSSION

Fusaric acid is an important mycotoxin which is produced by *Fusarium* species on the growth medium. Production of fusaric by strains of *Fusarium oxysporum* has been reported by several workers (Diniz and Oliveira, 2009; Notz *et al*., 2002) and is reported to involved in disease development (Liu *et al*., 2010). However, Curir *et al*. (2000) reported only a marginal importance of fusaric acid in the lily basal rot diseases.

All the isolates of FOC used in our study produced fusaric acid, evident by spots on thin layer chromatogram. Variability within the isolates was observed with respect to content of fusaric acid produced. *Fusarium oxysporum* isolate FO-1, FO-2 and FO-3 produced high amount of fusaric acid as compared to others. Isolate FO-5, FO-6 and FO-9 were weak producer of fusaric acid. Similar were the findings of Notz *et al*. (2002). They reported variability among 12 isolates of *Fusarium oxysporum* with respect to amount of fusaric acid produced. Among 12 strains few were weak in producing fusaric acid while few produced high amount of fusaric acid. The results of the present study reveals that Fusaric acid production by *Fusarium oxysporum* is strain dependent.

The results obtained from the cultural studies shows that there was a variation in the growth of FOC on different culture media. FO-1 produced maximum radial diameter and biomass, followed by FO-3, FO-4 and FO-2. PDA medium was found most suitable for the best radial diameter and biomass growth of FOC, followed by Czapex,s Dox and Fusarium Selective medium. Naik *et al*. (2004) reported PDA and Richards Agar as best growth medium for *Fusarium oxysporum* f. sp. *vanillae*. Variation among the 6 isolates of *Fusarium oxysporum* f .sp. *vanillae* was also reported when the isolates were grown on same or different culture media, temperature, pH and carbon source. Present results are also in the conformity with Jhamaria (1972) who reported PDA, CDA and Richards Agar as suitable medium for the growth of *Fusarium oxysporum*. However, maximum growth of *Fusarium* species has been also reported on Richards Agar followed by PDA (Anjaneya, 2002).

FOC isolates successfully grew over all the pH levels (3-11). However, maximum radial growth and biomass of *Fusarium oxysporum* f. sp *chrysanthemi* isolates was obtained on pH 5 and

minimum was recorded on pH 11. Acidic pH was found more suitable for the growth of all the isolates on all the culture medium used for the study. A decline in the radial growth and biomass of FOC was recorded when the pH was increased or decreased from 5. These results are in confirmation with the findings of Moore (1924) who reported that two strains of *F. coeruleum* could tolerate a pH range of 3.0 to 11.0. Similar were the research findings of Chi *et al.* (1964). They reported pH 5 to 5.5 as best for the radial and biomass growth of *Fusarium oxysporum*. Although isolates grew on all the pH levels but pH level above 5.5 and below 4.5 reduced biomass and radial growth.

Difference in the growth of FOC isolates was observed at different temperature levels (15-45°C). Radial growth and biomass of all the isolates were recorded maximum at the temperature of 25°C. On liquid culture medium, minimum growth was observed at 40°C and on solid medium minimum growth was observed at 35°C. All the isolates did not grew at 45°C on liquid and at 40 and 45°C on solid culture medium. Chi *et al.* (1964) indicated that *Fusarium oxysporum* and *Fusarium solani* isolates grew well at higher temperature of 28°C. The fungus grew at the temperature range of 10-35°C. However, growth of the fungus was drastically reduced below 15°C and started to decline above 30°C and become zero at 40°C, as these temperatures did not favour for growth of *F. solani*. Variability in the temperature requirements of *Fusarium* has been reported by Daami-Remadi *et al.* (2006) and Fayzalla *et al.* (2008).

CONCLUSION

FOC isolate have ability to produce fusaric acid and there is a variation among the isolates with respect to their ability to produce fusaric acid. Isolate FO-1, FO-2 and FO-3 were very strong in producing the fusaric acid. FO-1 was fast growing isolate both in liquid medium and solid medium. FO-2 was the slowest among all the four isolates. Maximum growth of FOC was recorded on PDB, followed by CDB and FSM-Liquid. All the four isolates of FOC showed significantly ($p<0.01$) higher growth at pH 5 followed by pH-7, 9, 3 and 11. Growth of FOC isolates was recorded maximum when the temperature of the environment was kept at 25°C. No mycelial growth was recorded at 45°C. Liquid medium was more supportive in the growth of pathogen.

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