J. Mycopathol, Res, ***50(1)*** *: 27-33 (2012)*

First report on the cultural and pathogenicity variations of wilt pathogen infecting tobacco in Karnataka

K. SUMANA[1], S. S. SREENIVAS[2] AND N. S. DEVAKI[1]
[1]Department of Molecular Biology, Yuvaraja's college (Autonomous), University of Mysore, Mysore 570 005, Karnataka
[2]Central Tobacco Research Institute, Research Station, Hunsur 571 105, Karnataka.

Received : 24.06.2011 Accepted : 18.11. 2011 Published : 30.04.2012

Fusarium oxysporum Schlecht. is a devastating pathogen on tobacco and is reported for the first time during 2002 in Karnataka Light Soil (KLS) region of Karnataka State, India. Fourteen isolates collected from different diseased samples were grown on PDA. They were grouped into five based on cultural and morphological characteristics. The representative five isolates were grown on twelve different media. All the isolates showed variation in their cultural characteristics when grown on the same nutrient medium. Growth characteristics of every isolate exhibited variations when grown on different media. The media that supported the maximum growth and sporulation were Czapek Dox Agar (CDA) and Potato Sucrose Agar (PSA), while the growth was poor in all the other media tested. Colony diameter was restricted up to 7.5 cm on PDA in 9 cm diameter Petriplates. Scanty growth and very poor sporulation was recorded in Spezieller Nahrstoffarmer Agar (SNA) and Potato Carrot Agar (PCA). Media for obtaining good pigmentation was found to be OMA and PDA where pigmentation of the substratum ranged from magenta pink to dark brown. To obtain both good growth and pigmentation of *F. oxysporum* OMA is recommended. Sector formation was induced in PSA and OMA. All the isolates that exhibited variations in the cultural studies were tested for pathogenicity. Wilt symptoms started on fourth day of inoculation and isolate 5 *Fo* was recorded to be highly virulent and isolate 3 *Fo* was least virulent among the isolates tested. This is the first report on the cultural and pathogenicity variations of tobacco wilt pathogen in KLS. This is also a first report on the existence of variations in the number of days taken for initiating the disease symptom by different isolates of the pathogen.

Key words: *Fusarium oxysporum*, mycelial growth, pathogenicity, pigmentation, sector formation, sporulation.

INTRODUCTION

Fusarium oxysporum Schlecht. the causal agent of Fusarium wilt is one of the most important fungal pathogens which can significantly reduce the production of the crop in several areas. It is more prevalent where growing season is relatively dryer and warmer. It causes complete loss in yield if the disease occurs in the vegetative and reproductive stages of the crop (Haware and Nene, 1980; Haware *et al.*, 1990; Navas-Cortex *et al.*, 2000). Shenoi *et al.*, (2002) have reported for the first time wilt disease caused by *Fusarium oxysporum* in Flue Cured Virginia (FCV) tobacco crop of Karnataka Light Soil (KLS) during 2002. FCV tobacco is extensively cultivated as a rain fed commercial crop in Hunsur tract of Karnataka. The type of soil (sandy loams) present in Karnataka where FCV tobacco is grown is referred to as Karnataka Light Soil (KLS) (Shenoi and Nagarajan, 2000) and is ideal for perpetuation and development of soil borne pathogens under favourable environmental conditions (Garner, 1951; Gopalachari, 1984; Lucas, 1975). Few growth media for *F. oxysporum* are earlier tested by Chittem and Kulkarni, (2008). They have reported that PDA is the ideal medium for culturing *Fusarium* spp. *F. oxysporum* is known to exhibit cultural and morphological variations (Ho and Varghese, 1988; Lucas, 1975). Hence, identification of an optimum media is necessary for the growth of a fungal species isolated from a specific host to carry out biochemical, molecular and pathogenicity studies. This can be done by comparing the growth of the fungus in different media. Fungi are known to show variations in different media (Devaki, 1991). Different isolates may show cul-

tural and morphological differences when grown on the same media (Leslie and Summerell, 2006). Such work will help to know the extent of variations a pathogen exhibits and also the variations existing among the isolates which are collected from a particular geographical area (Shenoi *et al.,* 2004). Such type of work has not been carried out to this region. This is the first work reported on tobacco wilt pathogen. Different isolates may vary in their pathogenicity (Dudley *et al.,* 2007). Thus to identify this variation pathogenicity study is also carried out.

MATERIALS AND METHODS

Isolation and establishment of the pathogen

Wilt affected plants were collected from Hunsur, Piriyapatna and H. D. Kote. Fungal pathogen was isolated by plating root bits of infected plant which were (0.5 cm length) surface sterilized with 0.1 % mercuric chloride for 1 minute and plated on Potato Dextrose Agar (PDA) medium amended with 100 ppm of Ampicillin. Plates were incubated at 26 ± 2 °C for 7 days. Fourteen fungal isolates growing on separate plates were brought into pure culture by subculturing on PDA. These cultures are preserved in sterile soil (Nene and Thapliyal, 1979). Isolates were also sent to Fusarium Research Centre, Penn State University, USA for identification. The five representative isolates were named as 1 *Fo*, 2 *Fo*, 3 *Fo*, 4 *Fo* and 5 *Fo*. These isolates were used for all the experiments. Pure cultures were maintained on PDA medium and PSA medium.

Cultural studies

The isolates were grown on twelve different media *viz.,* Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Czapek Dox Agar (CDA), Corn Meal Agar (CMA), Potato Carrot Agar (PCA), Oat Meal Agar (OMA), Spezieller Nahrstoffarmer Agar (SNA), Yeast Extract Dextrose Chalk Agar (YEDCA), Sabouraud Dextrose Agar (SDA), Dextrose Agar (DA), Potato Sucrose Peptone Agar (PSPA) and Emerson's Yeast Phosphate Soluble Starch Agar (EYPSSA) at 26 ± 2°C in 9.0 cm diameter Petriplates (Booth, 1975). All media were sterilized at 121 °C for 15 minutes. Each treatment had three replications and the experiment was repeated thrice. Observations were taken on the seventh day of inoculation. The fungal mycelial growth, pigmentation, sporulation and colony diameter were recorded and compared. The number of spores per ml was quantified by haemocytometer using the formula given by Pathak (1984). : Number of spores per ml = N x 1000 / X where, N-Total no. of spores counted per no. of squares, and X-Volume of mounting solution between cover glasses and above squares counted.

Length and breadth of micro and macroconidia, diameter of chlamydospores were measured by micrometry and all the above data were analyzed by ANOVA analysis of variance.

Statistical Analysis

Data on colony diameter, length and breadth of micro and macroconidia and diameter of chlamydospores were analyzed by ANOVA analysis of variance. This was done to compare all the five isolates of *F. oxysporum.* All analysis was programmed using SPSS statistical software after ensuring that the data were normally distributed.

Establishment of pathogenicity

Pathogenicity test was conducted as per Koch's postulates with 5 isolates of *Fusarium oxysporum* under green house conditions. Healthy seedlings were inoculated by pin prick method (Jahagirdar *et al.*, 2001). The pots were filled with sterilized soil and the inoculum grown on sorghum seeds @ 30 g/kg of soil (Prameela *et al.,* 2005). The roots of 60-day-old healthy seedlings were punctured with a sterile needle and transplanted to the pots. The experiment was conducted with five replications and repeated thrice. Uninoculated pots served as control. The wilt pathogen was re-isolated in pure culture from the diseased seedling and again identified (Agrios, 2005). The relative humidity in the greenhouses during the experiment was in the range of 70 to 85%, the daily temperature was between 23 and 25 °C and the nocturnal temperature interval was between 17 and 20 °C. The incidence of wilt symptoms were recorded at 24 hrs interval. Wilt symptom developed was graded as least, minimum, moderate, maximum and high.

RESULTS AND DISCUSSION

Morphological characteristics of 5 isolates on 12 different media showed variations in colony diameter (Table 1), mycelial growth, sporulation (Table

Table. 1: Colony diameter of *Fusarium oxysporum* on different media

Media	1*Fo*	2*Fo*	3*Fo*	4*Fo*	5*Fo*
PDA	5.00±0.000 **a**	5.50±0.000 **a**	7.50± 0.000 **a**	6.00±0.000 **c**	4.50±0.000 **a**
PSA	9.00±0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	8.00±0.000 **a**
OMA	7.00±0.115 **b**	5.80± 0.100 **b**	7.60±0.100 **a**	5.30±0.100 **b**	8.23±.2185 **b**
CDA	9.00±0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	9.00±0.000 **c**
SNA	9.00± 0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	9.00±0.000 **c**
SDA	9.00± 0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	8.66±.1666 **e**	9.00±0.000 **c**
DA	7.50± 0.000 **c**	9.00±0.000 **d**	9.00±0.000 **c**	5.00±0.000 **a**	9.00±0.000 **c**
YECA	8.00± 0.000 **d**	8.00±0.000 **c**	8.00±0.000 **b**	6.00±0.000 **c**	8.00±0.000 **b**
EYPSSA	8.00±0.000 **d**	9.00±0.000 **d**	9.00±0.000 **c**	8.00±0.000 **d**	9.00±0.000 **c**
PCA	9.00±0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	9.00±0.000 **c**
CMA	9.00± 0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	9.00±0.000 **c**
PSPA	9.00±0.000 **e**	9.00±0.000 **d**	9.00±0.000 **c**	9.00±0.000 **f**	9.00±0.000 **c**
F value	1368.750*	2023.545*	430.091*	865.377*	420.981*

The values given above are three replicates ± standard error. The values followed by different alphabates differ significantly as indicated by ANOVA (α=0.05). *F values significant at 0.000 level.

2 & 3), pigmentation (Table 4), and sector formation. The mycelial growth ranged from whitish pink cottony to violet cottony growth on the media tested. PDA supported whitish pink fluffy mycelial growth, sporulation and pigmentation with restricted and irregular colony diameter ranging from 4.5 cm to 7.5 cm. Almost all the isolates showed more than 7.0 cm diameter colony in seven days in all the media except PDA. The best media that supported the mycelial growth of the pathogen was PSA and CDA (Fig.1 a & b). Chittem and Kulkarni (2008) also reported such variations in eight media and they identified PDA as the best medium for different forma specialis of *F. oxysporum*. Several other workers also stated that PDA is the best media for mycelial growth of *F. oxysporum* (Maheshwari *et al.*, 1999; Saha *et al.*, 2008; Xu *et al.*, 1984). Cumagun *et al.* (2010) recorded luxuriant growth of *F. oxysporum* on SNA and the rate of growth of the isolates of *F. oxysporum* mycelium was found to be variable on different media. Similar results were reported in *F. subglutinans* f. sp. *pini* (Viljoen *et al.*, 1997).

PSA and CDA media were found to support maximum sporulation in all five isolates. Similar results were observed in CDA medium for Vanilla isolate of *F. oxysporum* (Sharma and Pandey, 2010). Naik *et al.* (2010) reported that sucrose is the best carbon source that supported growth and sporulation of *F. oxysporum* infecting Vanilla. During the present investigation, variations in the conidia production were

Table 2 : Mycilial growth produced by *Fusarium oxysporum* on different media

Media	1*Fo*	2*Fo*	3*Fo*	4*Fo*	5*Fo*
PDA	0.7143±0.184 **a**	30.714± 1.898 **ef**	25.285±2.965 **b**	9.4286±1.394 **bc**	7.000±1.112 **abc**
PSA	3.2857±0.778 **b**	24.000±1.309 **de**	114.428±3.344 **e**	14.2857±1.923 **c**	9.142±0.737**c**
OMA	3.1429±0.633 **b**	3.428±0.528 **a**	3.428± 0.719 **a**	4.7143±0.778**ab**	4.285±0.808 **abc**
CDA	1.0000±0.436 **a**	32.428±0.996 **f**	33.571±2.398 **bc**	23.7143± 2.834**d**	17.428±2.418 **d**
SNA	0.4286±0.202 **a**	2.714±0.359 **a**	1.000±0.000 **a**	0.0000± 0.000**a**	2.142±0.769 **a**
SDA	0.0000±0.000 **a**	1.571±0.297 **a**	2.857±0.553 **a**	2.5714±0.428**a**	2.428±0.480 **ab**
DA	6.7143±0.746 **c**	17.714±1.808 **cd**	5.714±0.565 **a**	2.4286±0.368**a**	5.285±0.285 **abc**
YECA	1.5714±0.297 **ab**	11.571±1.525 **bc**	6.857± 0.737 **a**	29.7143±2.043**d**	6.714±0.644 **abc**
EYPSSA	0.0000±0.000 **a**	16.571±1.601 **cd**	38.714±4.246 **c**	6.4286± 0.528**ab**	8.000±0.872 **bc**
PCA	0.0000±0.000 **a**	30.857±2.052 **ef**	5.571±0.996 **a**	3.2857±0.565**ab**	9.142±1.668 **c**
CMA	0.0000±0.000 **a**	8.285±1.822 **ab**	2.000± 0.436 **a**	2.0000±0.436**a**	1.571±0.297 **a**
PSPA	0.0000±0.000 **a**	56.428± 2.877 **g**	69.142±5.565 **d**	29.7143±1.569**d**	21.428±2.114 **d**
F value	26.294*	101.616*	187.516*	66.376*	24.776*

The values given above are three replicates ± standard error. The values followed by different alphabets differ significantly as indicated by ANOVA (α=0.05). *F values significant at 0.000 level.

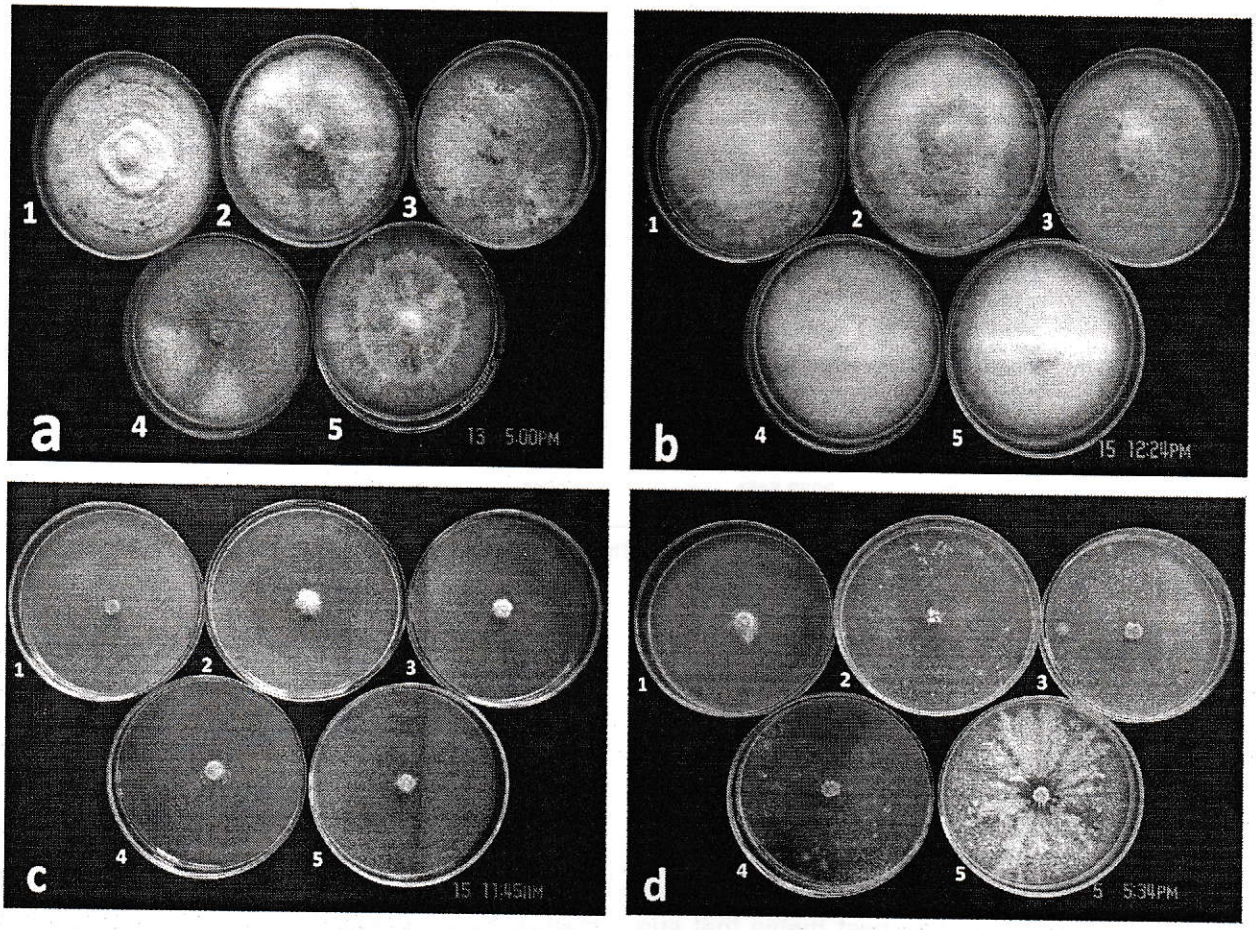

Fig.1: Growth of *Fusarium oxysporum* on a) Potato Sucrose Agar, b) Czapek Dox Agar c) Spezieller Nahrstoffarmer Agar d) Corn Meal Agar

observed in PDA by different isolates. Highest micro and macro conidia production was recorded in the isolate 3 *Fo* on PDA which contained glucose as the main carbon source. Very scanty growth and very poor sporulation was recorded on SNA and CMA in the present study (Fig.1 c & d).

Nanda and Prasad (1974) reported that *F. oxysporum* infecting castor exhibited typical white fluffy mycelial growth on PDA which turned pinkish when exposed to day light. Similar observation was made during the present study on PDA and OMA

Table 3 : Number of macroconidia produced by *Fusarium oxysporum* on different media

Media	1*Fo*	2*Fo*	3*Fo*	4*Fo*	5*Fo*
PDA	0.000± 0.000 **a**	1.000 ±0.000 **abc**	0.000 ±0.000 **a**	1.142 ±0.142 **ab**	0.000 ±0.000 **a**
PSA	0.000 ±0.000 **a**	0.142 ± 0.142 **a**	0.000 ±0.000 **a**	6.000 ±0.617 **b**	0.000 ±0.000 a
OMA	1.857 ±0.000 **b**	3.000 ± 0.487 **d**	2.571 ±0.428 **e**	1.142 ±0.142 **ab**	1.000 ±0.000 **b**
CDA	0.142 ±0.000 **a**	2.142 ± 0.404 **cd**	0.857 ±0.142 **bc**	1.428 ±0.428 **ab**	0.285 ±0.184 **a**
SNA	0.000 ±0.000 **a**	0.571 ± 0.202 **ab**	0.000 ±0.000 **a**	12.857±3.807 **c**	0.000 ±0.000 a
SDA	0.000 ±0.000 **a**	1.000 ± 0.000 **abc**	0.142±0.142 **ab**	0.285 ±0.184 **ab**	0.000 ±0.000 **a**
DA	0.000±0.142 **a**	1.428 ±0.202 **abc**	1.714±0.184 **d**	0.571 ±0.297 **ab**	0.000 ±0.000 **a**
YECA	0.000 ±0.202 **a**	1.000 ± 0.000 **abc**	1.000 ±0.000 **cd**	13.571±1.231 **c**	0.000 ±0.000 **a**
EYPSSA	0.000 ±0.000 **a**	1.571 ± 0.202 **bc**	0.000 ±0.000 **a**	5.857 ±0.594 **b**	0.000 ±0.000 **a**
PCA	0.000 ±0.000 **a**	1.428 ± 0.202 **abc**	0.000 ±0.000 **a**	0.000 ±0.000 **a**	0.428±0.297 **a**
CMA	0.000 ±0.000 **a**	1.714 ±0.285 **bcd**	0.000 ±0.000 **a**	1.142 ±0.142 **ab**	0.000 ±0.000 **a**
PSPA	0.000 ±0.000 **a**	3.000 ± 0.534 **d**	1.142 ±0.142 **cd**	2.142 ±0.260 **ab**	0.000 ±0.000 **a**
F value	18.626*	9.655*	31.344*	16.082*	9.091*

The values given above are three replicates ± standard error. The values followed by different alphabets differ significantly as indicated by ANOVA (α=0.05). *F values significant at 0.000 level.

Table. 4: Five isolates showing variation in pigmentation on Oat meal agar and Potato sucrose agar

Isolates	Oat meal agar		Potato dextrose agar	
	Mycelial growth	Pigmentation	Mycelial growth	Pigmentation
1*Fo*	Violet white cottony	Violet	Whitish pink cottony Irregular margin	Dark pink
2*Fo*	Pale violet white cottony	Pale violet	Whitish pink cottony Irregular margin	Pale pink
3*Fo*	Violetish white cottony	Violet	Whitish pink cottony Irregular margin	Pinkish white
4*Fo*	Dark violet white cottony	Violet	Whitish cottony Irregular margin	Dark brown mycelium and media also turned brown
5*Fo*	Dark pinkish cottony	Dark pink	White cottony Irregular margin	Pale yellow pigmentation in mycelia

(Fig. 2a & b). However, the development of colour was irrespective of the exposure to day light. In terms of pigmentation the colonies originating from the plug exhibited an initial light pink colour on PDA, which later turned to varying colours on reverse side of Petriplates. The results of the present investigations are in accordance with the work of Siddique *et al.,* (2010) and Chittem and Kulkarni (2008). According to Leslie and Summerell (2006) pigmentation of *Fusarium* species in culture depends on the species. In the present work, colour variation of the colony was observed within the same species and ranged from light pink to dark pink and light brown to dark brown. For both pigmentation and good mycelial growth OMA is recommended. Apart from pigment formation, fungal

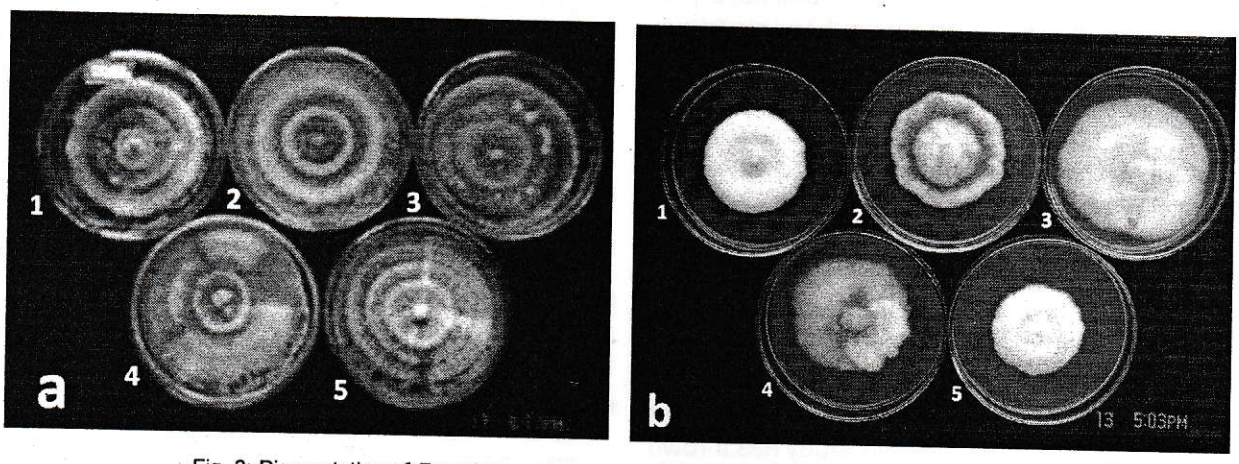

Fig. 2: Pigmentation of *Fusarium oxysporum* on a) Oat Meal Agar b) Potato Dextrose Agar

colony showed concentric rings in OMA.

Sector formations were seen to greater extent on PSA and OMA. These results were similar to those of Ho and Varghese (1988) who observed the same phenomenon in *F. oxysporum* isolated from oil palm. Sector formation depicts extent variations which can occur in a fungal strain while growing itself. The fungal systematics is still mainly based on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes. Moreover, the variations in cultural and morphological and physiological features are one of the main criteria used widely for their identification and taxonomic placement (St-Germain and Summerbell, 1996). Present work has clearly shown that the differences in the pattern of growth are also due to change in the constituents of a culture medium. Thus the investigation of a given fungal strain on different media will enable us to identify this and it appears to be a basic requirement to understand the morphological and cultural variations.

Pathogenicity tests showed that the isolate 5 *Fo* was highly pathogenic when compared to all the other isolates, where complete drooping and death of the seedling is seen within six days of inocula-

tion. Healthy tobacco seedling and different stages of wilting caused by different isolates were graded as least (3 *Fo*), minimum (1*Fo*), moderate (2 *Fo*), maximum (4 *Fo*) and high virulent strains (5 *Fo*). The disease symptom started on 4th day after inoculation and in case of 4*Fo* and 5*Fo* (Table 3). Of all the isolates 5 *Fo* took only 5 days for 100% disease incidence. However, 1 *Fo*, 2 *Fo*, and 4 *Fo* isolates showed nearly 100% damage on the 7th day of inoculation. The isolate 3 Fo showed 90% loss of the seedling on the 7th day. Thus the variability with respect to pathogenicity is observed only during initiation stages. Later all the isolates showed high disease incidence as the days progressed. Though variability of pathogenic intensity of different isolates was recorded by Rupe (1989), the importance of the data related to the days taken by the isolates to initiate disease was not stressed. Disease symptom will start very early in one field due to one strain. Under the same environmental condition, another strain in different crop field may take more days to initiate disease. This is because in a small geographical area more than one *Fusarium* strain may be present. The knowledge of variations in the days required for the disease initiation can be exploited to evolve better control measures. Thus this study has thrown light for the first time on the reason for evolving a better control measure in all the crop fields as soon as the disease incidence is seen in one crop field.

Table 5. Percentage of disease incidence

Days	4th day	5th day	6th day	7th day
Control	Nil	Nil	Nil	Nil
1 *Fo*	Nil	30	80	100
2 *Fo*	Nil	60	100	100
3 *Fo*	Nil	30	80	90
4 *Fo*	20%	70	100	100
5 *Fo*	60%	100	100	100

ACKNOWLEDGEMENTS

Authors are grateful to Yuvaraja's College (Autonomous), University of Mysore, Mysore and Central Tobacco Research Institute, Research Station, Hunsur, for providing lab facilities to carry out this work.

REFERENCES

Agrios, G. N. 2005. *Plant Pathology*. Elsevier Academic Press, USA.608 pp.

Booth, C. 1975. The status of *Fusarium* taxonomy. *Annual Review of Phytopathology,* Pusa, India. 547pp.

Chittem, K. and Kulkarni, S. 2008. Effect of Media on the Growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum*f. sp. *dianthi. Karnataka Journal of Agricultural Science.***21**:303-304.

Cumagun,C. J. R., Aguirre, J. A. and Relevante, C. A. 2010. Pathogenicity and Aggressiveness of *Fusarium oxysporum* Schl. in Bottle Gourd and Bitter Gourd. *Plant Protection Science*. **46**:51-58.

Devaki, N.S. 1991. *Studies on Pythium and Phytophthora on Tobacco in Karnataka,* Ph. D. Thesis, 165pp.

Dudley, N. S., James, R. L., Sniezko, R. A. and Yeh, A. 2007.Pathogenicity of four Fusarium species on Acacia koa seedlings. *Forest Health Protection.***1**:1-9.

Garner, W. W. 1951. *The Production of Tobacco.* The Blakiston Company, New York, USA, 520pp.

Gopalachari, N.C. 1984. *Tobacco*. Indian Council of Agricultural Research, New Delhi, 327pp.

Haware, M. P., Jimenez-Diaz, R. M., Amin, K. S., Phillips, J. C. and Halila, H. 1990. Integrated management of wilt and root rots of chickpea 129-137. In: *Chickpea in the Nineties*: Proceedings of the second international work shop on chickpea improvement, Patancheru, India.

Haware, M. P. Nene, Y. L. and Rajeshware, R. 1978. Eradication of *Fusarium oxysporum* f.sp. *ciceri* transmitted in chickpea seed. *Phytopathology.* **68**:1364-1367.

Haware, M. P. and Nene, Y. L. 1980. Influence of wilt at different stages on the yield loss in chickpea. *Trop. Grain Legume Bullet.* **19**:38-40.

Ho, Y. W. and Varghese, G. 1988. Cultural variants of Fusaria from Oil Palm habitats. *Pertanika.* **11**:317-321.

Jahagirdar, S., Siddaramaiah, A. L. and Ramaswamy, G. R. 2001.Influence of biocontrol agents and MPG-3 on *Fusarium oxysporum* fsp *cubense*, incitant of Panama disease of banana. *Plant Disease Research.* **16**: 68-72.

Leslie, J. F. and Summerell, B. A. 2006. *The Fusarium Laboratory Manual.* Blackwell Publishing Professional, USA. 388 pp.

Lucas, G. B. 1975. *Diseases of Tobacco* (third edition). Biological Consulting Associates, Raleigh, N.C. 621 pp.

Maheshwari, S. K. Singh, D. V. and Sahu, A. K. 1999. Effect of several nutrient media, pH and carbon sources on growth and sporulation of *Alternaria alternata. J. Mycopathol. Res.* **37**:21-23.

Naik, G. N., Nagaraja, R., Basavaraja, M. K. and Naik, K. R. 2010. Variability studies of *Fusarium oxysporum* f. sp. *vanilla* isolates. *International Journal of Science and Nature.* 1: 12-16.

Nanda, S. and Prasad, N. 1974. Wilt of castor a new record. *Journal of Mycology and Plant Pathology.* **4**:103-105.

Navas-Cortes, J. A., Hau, B. and Jimenez-Diaz, R. M. 2000. Yield loss in chickpea in relation to development to Fusarium wilt epidemics. *Phytopathology.* **90**:1269-1278.

Nene,Y. L. and Thapliyal, P. N. 1979. *Fungicides in Plant Disease Control.* Oxford and IBH Publishing Co. Pvt. Limited, New Delhi. 507pp.

Pathak, V. N. 1984. *Laboratory Manul of Plant Pathology*. Second edition. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. 11-12 pp.

Prameela, M., Rajeshwari, B., Prasad, R. D. and Reddy, D. R. R. 2005. Bioefficacy of antagonists against *Fusarium oxysporum* f. sp. *carthami* isolates inciting safflower wilt. *Journal of Mycology and Plant Pathology.* **35**: 272-274.

Rupe, J. C. 1989. Frequency of *Fusarium solani* recoverd from soybeans with sudden death syndrome. *Plant Disease.* **73**: 581-584.

Saha, A., Mandal, P. Dasgupta, S. and Saha, D. 2008. Influence of culture media and environmental factors on mycelial growth

and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. *Journal of Environmental Biology.* **29**:407-410.

Sharma, G. and Pandey, R. R. 2010. Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. *Journal of Yeast and Fungal Research.* **1**:157-164.

Shenoi, M. M., Sreenivas, S. S., and Subrahmanya, K. N. 2002. *Preliminary investigation on Fusarium wilt disease in FCV tobacco crop of Karnataka light soils.* Proceedings of 54th Annual Meeting and National Symposium on Crop Protection and WTO-An Indian Perspective (22nd-25th January, 2002).Venue: Indian Phytopathological Society, New Delhi, India. Organized by: Central Plantation Crops Research Institute (Indian Council of Agricultural Research), Kasaragod, Kerala.

Shenoi, M.M., and Nagarajan, K. 2000. *Diseases of FCV tobacco and their management in Karnataka light soil region.* Central Tobacco Research Institute, Rajamundry, (Indian Council of Agricultural Research), CTRI Research Station, Hunsur, Karnataka, India, 31pp.

Shenoi, M.M., Sreenivas, S.S., and Subrahmanya, K.N. 2004. Studies on Fusarium wilt in FCV tobacco crop of Karnataka light soils. *Tobacco Research.* **30** : 27-32.

Siddique, S., Yusuf, U. K. and Zainudin, N. A. I. M. 2010. Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatum. Journal of Biotechnology.* **9**:4081-4090.

St-Germain, G. and Summerbell, R. 1996. *Identifying Filamentous Fungi – A Clinical Laboratory Handbook,* 1st Ed. Star Publishing Co., Belmont, California. 314pp.

Tawfik, A. A. and Allam, A. D. A. 2004. Improving cumin production under soil infestation with Fusarium wilt pathogen: I-Screening of biocontrol agents. *Association of Universal Bulletin Environmental Research.* **7**:35-44.

Viljoen, A., Marasas, W. F. O., Wingfield, M. J. and Viljoen, C. D. 1997. Characterization of *Fusarium subglutinans* f.sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Research.* **101**:437-445.

Xu, S. O., Yuan, S. Z. and Chen, X. C. 1984. Studies on pathogenic fungus (*Alternaria tennui* Nees) of poplar leaf blight. *Journal of North East Forestry Institute.* **12**:56-64.

Zain, M. E., Razak, A. A., El-Sheikh, H. H., Soliman, H. G. and Khalil, A. M. 2009. Influence of growth medium on diagnostic characters of *Aspergillus and Penicillium* species. *African journal of Microbiology Research.* **3**: 280-266.