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INFLUENCE OF PHYSIOLOGICAL FACTORS ON VEGETATIVE GROWTH AND SPORULATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERIS*

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Abstract

All the conditions that affect the ecological survival of pathogens are essential for formulating pathogen management strategies and for multiplication to meet the requirement of further research. *In-vitro* studies revealed that colony growth of *Fusarium oxysporum* f. sp. *ciceris* varied significantly with different culture media, temperatures, nutritional sources and with different pH levels. Moreover, its conidial size and number of macro and micro conidia were also varied with different growth conditions. Richards's agar and potato dextrose agar appear as the best growth media, which produced rapidest colony growth, biggest conidia and highest sporulation as compared to the other culture media. The *Foc* grew between 10-45°C, maximum colony growth occurred at 30°C followed by 25 and 35°C. It produced conidia of significantly higher length and width at 30°C followed by at 25°C. While all other temperature levels produced comparatively smaller conidia. Its highest sporulation took place at 30-35°C and no conidial production occurred below 15°C and above 45°C. Alterations in nutritional sources also caused great influence on the growth and sporulation of the test fungus. Among different nutrients tested, sucrose and dextrose came up as the most suitable carbon sources, while potassium nitrate and peptone as best nitrogen sources. The *Foc* produced maximum dry mycelial weight and sporulation at pH 6-7, whereas the extreme pH levels (8 and 9) produced less numbers of conidia.

Keywords: *Cicer arietinum*, Fusarium Wilt, Physiological Factors, Culture Media, Temperature, Carbon and Nitrogen Sources, pH

Introduction

Chickpea (*Cicer arietinum* L.) is the most important crop belonging to Leguminaceae family. It is well-known due to its high nutritional and medicinal values. Chickpea grains contain different types of nutrients, proteins, carbohydrates and oils (Stallknecht *et al*., 1995; Muehlbauer & Rajesh, 2008). Worldwide chickpea is grown on an area of 11 million hectares with the production of 9 million metric tons. The chickpea cultivation area in Pakistan 2013 was 992 thousand hectares with the production of 751 thousand tones. The per unit yield in Pakistan is very low (7573.19 Hg/Ha) as compared to the high yielding country Israel (61197.67 Hg/Ha) (Anon., 2015). The major reasons for the low productivity may be disease susceptibility, environmental stress, insect pest infestation, poor crop management and low yielding varieties.

The worldwide chickpea production losses were assessed to be 4.8 million tons due to biotic stresses (Ryan, 1997). More than 50 pathogens of chickpea have been identified, including fungi, bacteria, nematodes and viruses (Nene *et al*., 1996). Of these some have the high potential to destroy the crop. Amongst the diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is more serious and widespread throughout the world (Trapero-Casas & Jimenez-Diaz, 1985; Nene & Reddy, 1987; Haware, 1990; Jalali & Chand, 1992). The wilt infection can injure the crop completely and cause 100% yield loss (Halila & Strange, 1996; Navas-Cortes *et al*., 2000), while 10-15% losses in productivity occurred usually (Haware, 1990; Campbell & Madden, 1990). The *F. oxysporum* is a vascular pathogen which spread through seed and soil. The pathogen propagules can survive 3-6 years in soil without any host (Haware *et al.,* 1996; Ayub *et al*., 2003) hence difficult to control once a pathogen established in the soil. The diseases are usually managed through integration of various methods with the aim to suppress the pathogen invasion, multiplication and survival. Plants and pathogens coevolved in nature. Plant growth conditions may be altered to create the worst conditions for the pathogen development but without sacrificing the yield. Documentation of pH, temperature and other physiological requirements of infecting organism is essential to develop an appropriate control strategy (Jaruhar & Prasad, 2011; Farooq *et al.,* 2005; Groenewald *et al*., 2006). On the other hand developments of reliable and suitable methods of pathogen multiplication are prerequisite for advanced research. Such information may be utilized to suppress the seed-borne infection even before the sowing. With this aim, studies on the influence of physiological factors on vegetative growth and sporulation of *Fusarium oxysporum* f. sp. *ciceris* carried out.

Material and Methods

Effect of culture media on colony growth and sporulation: To evaluate the effect of different artificial growth media on the growth of test pathogen, eight different culture media *viz*., Czapek's dox agar (CDA), Richards's agar (RA), glucose peptone agar (GPA), oat meal agar (OMA), potato dextrose agar (PDA), V-8 juice agar (V-8A), Waksman's agar (WA), Sabouraud agar (SA), malt extract agar (MEA), corn meal agar (CMA) and chickpea seed meal agar (CSMA) were tested under control conditions. Each medium was prepared as described in the

literature and sterilized in an autoclave (Dhingra & Sinclair, 1995). Before poring Streptomycin and Penicillin were added in medium to avoid bacterial contamination. Five mm disks were cut with sterilized cork borer from the actively growing culture of the *Foc* and placed in the center of Petri dishes containing different culture media. There were five replications for each treatment and all plates were incubated for 7 days at 30±2°C. The radial colony growth (mm) was recorded daily till the plates of any treatment became full with the growth of test pathogen.

These culture media were also evaluated to see their effects on the population and size of conidia produced by the test pathogen. For this purpose, after 7 days of growth 10 m1 of distilled water was added in each culture plate and conidia were harvested with the help sterilized spatula and spore suspension was collected in a glass beaker. Numbers of macro and micro-conidia per ml in each treatment was determined with the help of heamocytometer (Somasegaran & Hoben, 1985). The size (length and width) of macro and micro conidia were also measured under a compound microscope with the help of the ocular micrometer for each treatment.

Effect of different temperatures on colony growth and sporulation: The fungus was grown at ten variable temperatures *viz*., 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C to find the optimum temperature for its colony growth. A 5 mm disc was cut from one week old culture of *F. oxysporum* and placed in the center of the petri plate having 20 ml of sterilized PDA medium. Inoculated plates were incubated for 6-7 days at each temperature. The observations on the radial colony growth of the pathogen in millimeter were recorded after each 24 hours, till the plates of any treatment became full with the growth of test pathogen. Five Petri dishes were kept for each temperature level. The effect of different temperatures (5-50°C) on the size and number of micro and macro conidia was also determined as described above.

Effect of different carbon or nitrogen sources on colony growth: To evaluate the effect of different carbon sources on the colony growth of the *Foc*, the Czapek's dox agar (CzDA) without sucrose was used as a basic growth medium. Different carbon sources *viz*., sucrose, fructose, lactose, mannitol, maltose, dextrose and starch @ 1%, 2% and 3% were added in basic medium before the sterilization. While, potassium nitrate, ammonium chloride, ammonium nitrate, magnesium nitrate, ammonium sulfate, sodium nitrate, calcium nitrate, peptone and urea @ 0.1%, 0.3% and 0.5% were added in basic medium (Czapek's dox agar without sodium nitrate) before the sterilization. Before pouring, penicillin @100, 000 units L^{-1} and streptomycin @ 0.2g L^{-1} were mixed into the medium to avoid bacterial contamination. The media were poured in 9cm diameter Petri plates @ 20 ml/plate. After solidification of medium, 5mm diameter disc of *Foc* was placed at the center of each Petri plate. The plates were incubated at 28±2°C and diameters of colony growth (mm) were recorded after each 24 hours.

Effects of selective carbon and nitrogen (C+N) sources on colony growth: On the basis of the above studies, the combined effect of resulting most suitable carbon and nitrogen source on the growth and sporulation of *Foc* was also studied. For this purpose, sucrose @ 2% (selected carbon source) and potassium nitrate @ 5% (selected nitrogen source) were added to the CzDA medium (without sodium nitrate). A 5mm disc from 7 days old culture of test pathogen was placed in the center of each Petri plate containing amended media. The plates were incubated at 28±2°C and the colony diameter was recorded after every 24 hours.

Effect of various pH levels on the growth and sporulation: Diverse pH levels *viz*., 5.0, 6.0, 7.0, 8.0 and 9.0 were maintained to check their effects on the growth of test pathogen. The pH of potato dextrose broth (PDB) was adjusted to a required level by adding 1 M NaOH or 1 M HCl before autoclaving. Conical flasks containing 100 ml sterilized medium were inoculated with 4 disks (5mm each) of fresh culture of the *Foc*. The inoculated flasks were incubated for 12 days at 30°C. For determining dry mycelial weight, the growing mycelial mats were filtered through pre-weighted Whatman's filter paper and placed in a hot air oven at 70°C for 24 hours. After drying the dry mycelial weight was determined by weighting and subtracting the initial weight of the filter paper. The number of macro and micro-conidia of the fungus at various pH levels was recorded as described previously.

Results

Effect of culture media: Among used media, significantly highest colony growth of the *Foc* was occurred on Richards's agar medium followed by potato dextrose agar (PDA). While the least growth of test fungus observed in Czapek's dox agar medium. All other remaining media produced moderate colony growth of the test pathogen (Fig. 1).

The length and width of the *Foc* conidia greatly varied on different culture media. Significantly longest conidia were produced on Richard's agar followed by PDA and chickpea seed meal agar. The lengthwise smallest conidia were observed on V-8 juice agar and malt extract agar followed by corn meal agar and oat meal agar. The conidia of maximum width was produced on PDA and Richard's agar followed by Waksman's agar and chickpea seed meal agar, whereas the minimum width of conidia was recorded on oat meal agar, V-8, malt extract agar and corn meal agar followed by Sabouraud agar. Generally, length found to be corresponding to their width (Figs. 2-3). The highest number of macro and micro-conidia of *Foc* were recorded on Richards's agar medium followed by PDA and Waksman agar medium. The lowest numbers of macro and micro conidia were found on glucose peptone agar and corn meal agar medium (Fig. 4).

Effect of temperatures: The growth responses of *Foc* to variable temperatures ranging from 5-50°C were studied and it was observed that it can grow between 10-45°C, whereas no growth was observed at extreme temperatures of 5 and 50°C. The growth start from 10°C, gradually increased with increasing temperature, reached to peak at 30°C and then gradually decline. The data indicate that *Foc* produced maximum colony growth on 30°C followed by 25 and 35°C (Fig. 5).

The incubation temperatures greatly influenced the conidial size. The conidia of higher length and width

observed at 30°C followed by 25°C. While all other temperature levels produced comparatively smaller conidia (Figs. 6-7). The maximum number of both types of conidia was produced at 30°C followed by 35°C. No conidial production occurred at below 15°C and above 45°C (Fig. 8).

Effect of carbon sources: The addition of various carbon sources in basic medium (Czapek's dox agar without sucrose) significantly enhanced the colony growth of test pathogen. Among different carbon sources, maximum colony growth of *Foc* observed in medium containing dextrose and sucrose. The least colony growth observed in control (basic medium), followed by medium with mannitol. A non-significant difference in colony growth was found within the higher and medium concentrations of most of the carbon sources used (Fig. 9).

Effect of nitrogen sources: In case of different nitrogen sources, the maximum colony growth of *Foc* recorded with potassium nitrate followed by peptone. While all other nitrogen sources showed a moderate effect on the colony growth of the test fungus. In each individual treatment, the higher concentrations of particular nitrogen source (0.5%) produced more growth than medium (0.3%) and lower (0.1%) concentrations (Fig. 10).

Combined effect of selected carbon and nitrogen sources: On the basis of the results of the above experiments, sucrose 2% and potassium nitrate 0.3% were selected as best carbon and nitrogen source, respectively. The addition of these two in the basic medium produced more rapid colony growth as compared to the growth produced in medium, either with sucrose or potassium nitrate alone (Fig. 11).

Effect of different pH levels: The dry mycelium weight of the *Foc* varied with the different pH levels. The test fungus produced maximum dry mycelial weight in PDB having the pH of 6 or 7 followed by pH 5 and 4 (Fig. 12). Similar trend also observed for sporulation of test fungus. The highest number of macro and micro-conidia was observed at pH-6 followed by 7, whereas the extreme pH levels (8 and 9) produced comparatively less numbers of conidia (Fig. 13).

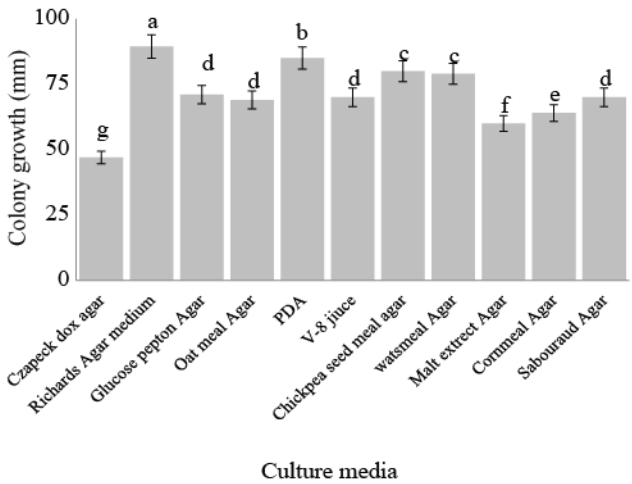

Fig. 1. Effect of different cultural media on redial colony growth of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

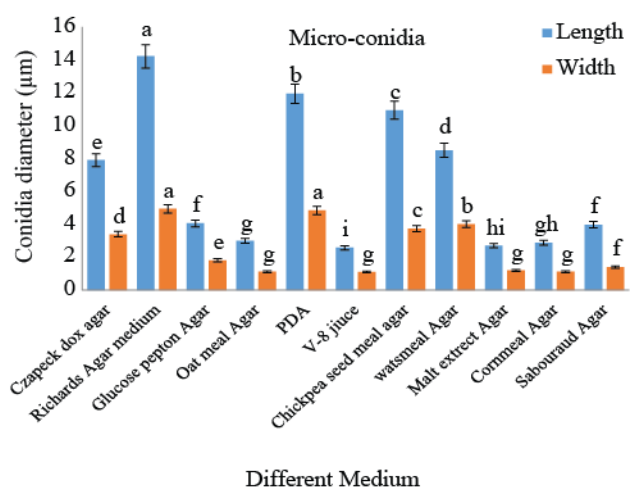

Fig. 2. Effect of different cultural media on micro-conidial size of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

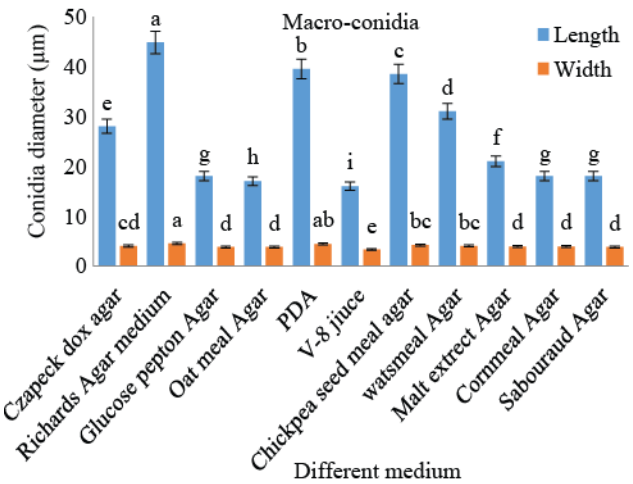

Fig. 3. Effect of different cultural media on macro-conidial size of *Fusarium oxysporum* f.sp. *ciceris*.

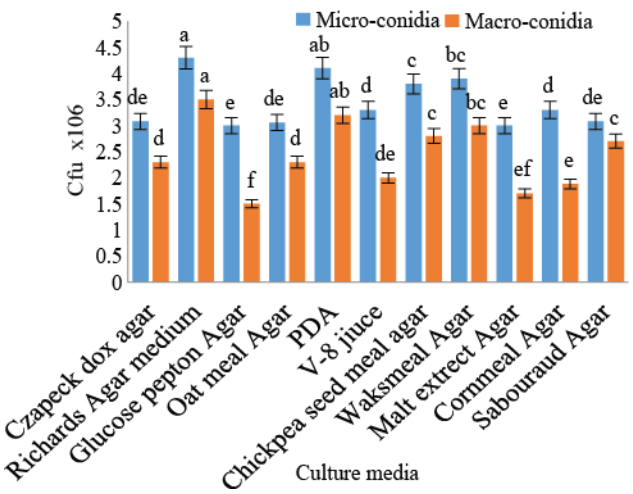

Fig. 4. Effect of different cultural media on number of micro and macro-conidia of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

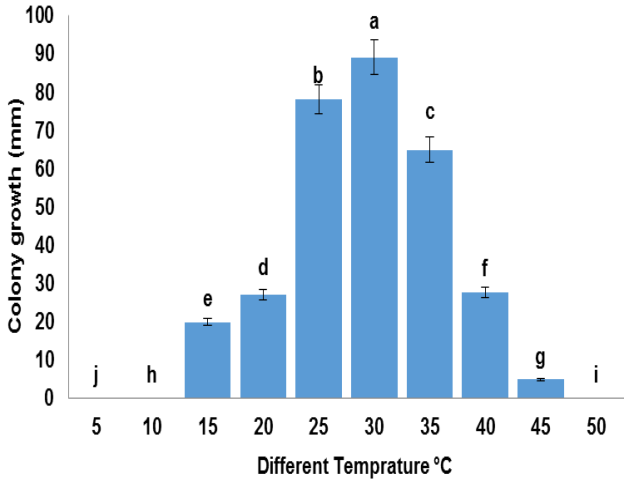

Fig. 5. Effect of different temperatures on redial colony growth of *Fusarium oxysporum* f.sp. *ciceris***.** Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

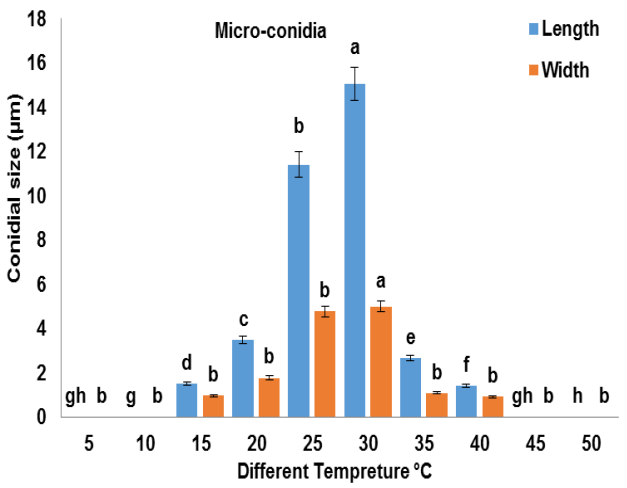

Fig. 6. Effects of different temperatures on micro-conidial size of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

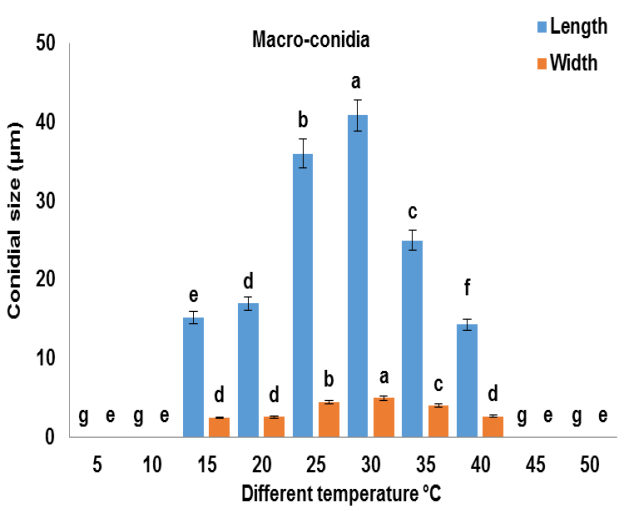

Fig. 7. Effect of different temperatures on macro-conidial size of Fusarium *oxysporum* f.sp. *ciceris***.** Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

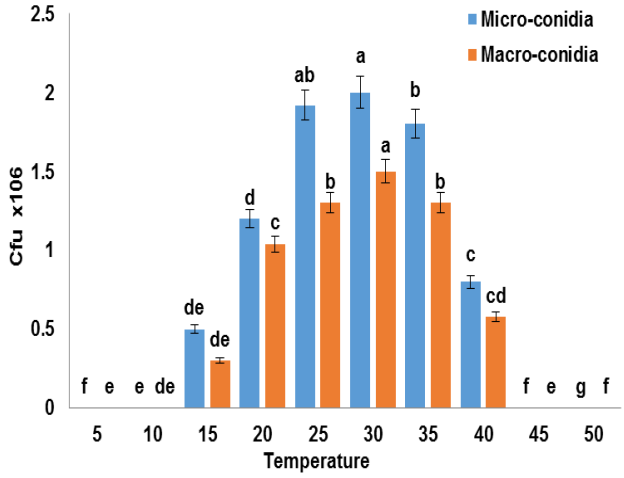

Fig. 8. Effect of different temperatures on number of micro and macro-conidia of Fusarium *oxysporum* f.sp. *ciceris***.** Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

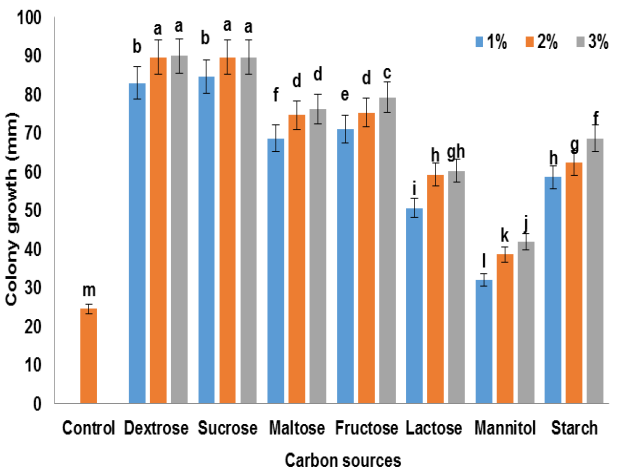

Fig. 9. Effect of different carbon sources on colony growth of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

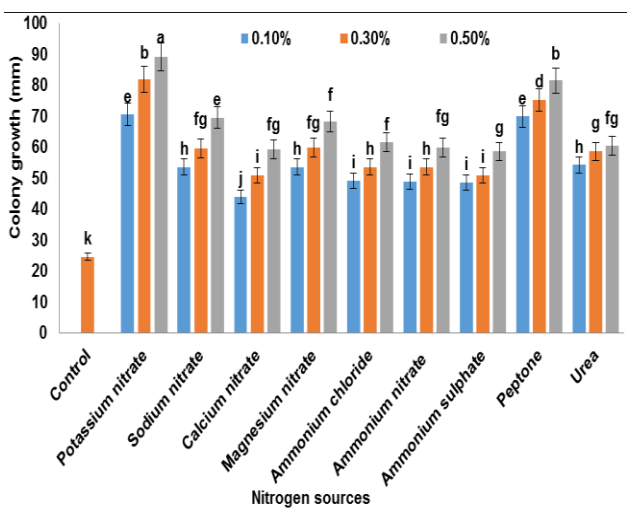

Fig. 10. Effect of different nitrogen sources on colony growth of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

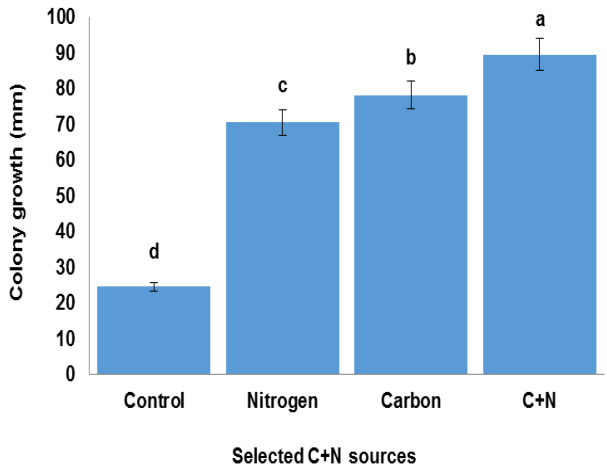

Fig. 11. Effect of selected carbon (sucrose 2%) and nitrogen (potassium nitrate 0.3%) sources on colony growth of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

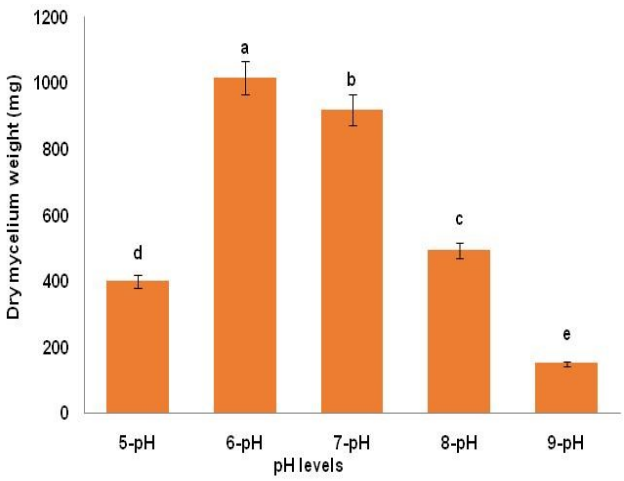

Fig. 12. Effect of different pH levels on dry mycelium weight of *Fusarium oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

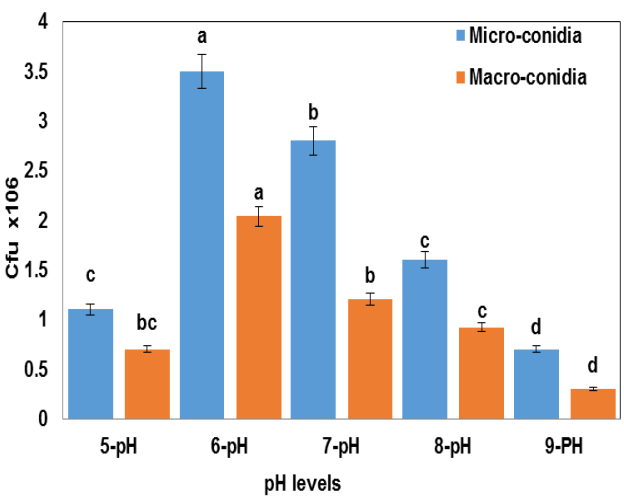

Fig. 13. Effect of different pH levels on micro and macro-conidia of Fusarium *oxysporum* f.sp. *ciceris*. Bars labeled with the same letter indicating that the mean values are statistically not different ($p < 0.05$).

Discussion

Factors that affect the cultivation and sporulation of pathogenic fungi are numerous, including temperature, light, aeration, nutrient in the growth medium, pH etc. (Dhingra & Sinclair, 1995). The present work indicated that Richards's agar and potato dextrose agar media were the suitable medium for the mycelial growth, conidial size and numbers of macro and micro-conidia of *Foc*. It has also observed by other workers that *Foc* produced maximum vegetative growth and sporulation on oat meal agar, Richard's agar media, PDA and Czapeck's agar medium (Hafiz, 1986; Dikkar & Deshmukh, 2003), while Khan *et al*., (2012) obtained best growth on PDA medium. Anjaneya Reddy (2002) recorded the highest mycelium growth of *F. udum* on Richard's agar and potato dextrose agar. Many scientists have worked on optimum conditions required for survival and sporulation (Volz & Beneke, 1969; Saxena *et al*., 2001; Xiao & Sitton, 2004; Kim *et al*., 2005; Saha *et al*., 2008; Gao & Liu, 2010). As the vegetative and reproductive requirement of fungi varied, a "two-stage" cultivation method of Sun *et al.,* (2009) may be utilized. In this method, the fungus grows vegetatively on one medium and transferred to a second medium for sporulation.

The incubation temperatures caused remarkable effects on the vegetative growth and sporulation of fungal species. The minimum, optimum and maximum temperature varies with the species. Like other group of fungal pathogens, *Foc* has also minimum, optimum and maximum temperatures for vegetative growth and sporulation. The present study showed that the *Foc* can grow between 10-45°C, although the best temperature for growth and sporulation was 25-30°C. It produces no spores below 15°C and above 45°C. They may vary from isolate to isolate belonging to diverse ecological zones. Hafiz (1986) found 25-30°C, 10°C and 35-40°C as optimum, minimum and maximum temperature, respectively. Other scientists also found that 25°C was the best temperature for its growth (Chauhan, 1963; Singh & Dahiya, 1973; Desai *et al.,* 1994). However, Chi and Hansen (1964) found 28°C and Khan *et al*. (2012) found 30°C best for *Foc* growth. It also grew well between 15-35°C and produced maximum colony growth and mycelial dry weight at 30°C (Sharmin *et al.,* 2012).

The types and quantity of the nutrients utilized by the fungi also play a vital role in their growth and sporulation. During present studies, dextrose and sucrose appeared as best carbon sources; while potassium nitrate and peptone as best nitrogen sources for the growth of *Foc.* These findings are in close agreement with those reported by Sharmin *et al.* (2012) found peptone and sucrose as most suitable nitrogen and carbon source for *Foc.* Other workers also found mannitol, dextrose and sucrose as best carbon sources (Paulkar *et al.,* 2002) and potassium nitrate as nitrogen source for *Foc* (Paulkar *et al.,* 2002; Dikkar & Deshmukh, 2003). On the other hand, Khan *et al*., (2012) found glucose and alanine as the most suitable carbon and nitrogen sources for *Foc* growth. Sucrose was also considered the best carbon source for *Fusarium solani* (Ramteke & Kamble, 2011).

Like other groups of soil borne fungi, the *Foc* has also its own preferences of pH. The present investigation revealed that pH 6 or 7 were the most suitable for the vegetative growth as well as for sporulation of *Foc.* The present

findings are in confirmation to those reported by Sharmin *et al.* (2012) found that the *Foc* produced maximum dry mycelial weight at pH 6.5. Other workers like Khan *et al*. (2012) observed pH 6.5-7.0 was the best for maximum growth of *Foc.*

References

Anjaneya Reddy, B. 2002. Variability of *Fusarium udum* and evaluation of pigeonpea (*Cajanus cajan* (L).Mills) genotypes. M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, India.

Anonymous. 2015. FAOSTAT, *FAO Statistical Pocket Book 2015*. Available at: http://www.fao.org/documents/card/en/c/383d384a-28e6-47b3-a1a2-2496a9e017b2/

Ayub, M. A., S. M. Khan, R. Ahmad and K. Iftikhar. 2003. Screening of chickpea germplasm for the sources of resistance against chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*). *Pak. J. Phytopath.,* 15: 25-27.

Campbell, C.L. and L.V. Madden. 1990. *Introduction to Plant Disease Epidemiology.* Wiley-Interscience, NY.

Chauhan, S.K. 1963. Incidence of Fusarium wilt of gram in relation to soil moisture. *Agra Univ. J. Res.,* 12: 271-274.

Chi, C.C. and E.W. Hansen. 1964. Relation of temperature, pH and nutrition to growth and sporulation of *Fusarium* spp. from red clover. *Phytopathology*, 54: 1053-1058.

Desai, S., Y.L. Nene and A.G.R. Reddy. 1994. Races of *F. oxysporum* causing wilt of chickpea growth variability. *Indian J. Mycol. Pl. Path*., 24: 120-20.

Dhingra, O.D. and J.B. Sinclair. 1995. *Basic Plant Pathology Methods*. CRS Press, Inc. Boca Raton, Florida, USA.

Dikkar, M. and V.V. Deshmukh. 2003. Nutrional requirement of Fusarium wilt of chickpea. *Crop Res*., 25(1): 197-199.

Farooq, S., S.M. Iqbal and C.A. Rauf. 2005. Physiological studies of *Fusarium oxysporum* f. sp. *ciceris. Int. J. Agri. Biol*., 7(2): 275-277.

Gao, L. and X. Liu. 2010. Effects of carbon concentrations and carbon to nitrogen ratios on sporulation of two biological control fungi as determined by different culture methods. *Mycopathologia*, 169(6): 475-81.

Groenewald, S., N. van den Berg, W.F.O. Marasas and A. Viljoen. 2006. Biological, physiological and pathogenic variation in a genetically homogenous population of *Fusarium oxysporum* f. sp. *cubense. Australasian Plant Pathology,* 35(4): 401-409.

Hafiz, A. 1986. *Plant Diseases*. Pakistan Agricultural Research Council Islamabad, Pakistan.

Halila, M.H. and R.N. Strange. 1996. Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f. sp. *ciceris* race. *Phytopathol. Mediterr*., 35: 67-74.

Haware, M.P. 1990. Fusarium wilt and other important diseases of chickpea in the Mediterranean area. *Options M´editerran´eennes. S´erie S´eminaires,* 9: 63-166.

Haware, M.P., Y.L. Nene and M. Natarajan. 1996. The survival of *F. oxysporum* f. sp. *ciceris* in the soil in the absence of chickpea. *J. Phytopathologia Mediterranea*., 35: 9-12.

Jalali, B.L. and H. Chand. 1992. Chickpea wilt. In: Singh, U.S., H.S. Chaube, J. Kumar and A.N. Mukhopadhyay (Eds.), *Plant Diseases of International Importance. Vol. 1. Diseases of Cereals and Pulses*. Prentice Hall, Englewood Cliff, New Jersey, USA, pp. 429-444.

Jaruhar, H.B. and A. Prasad. 2011. Effect of different pH levels on the growth of sporulation of *Fusarium oxysporum* Schlecht f. sp. *lintis* (Vasudeva and Srinavasin) the causal organism of wilt disease of lentil. *The Bioscan,* 6(1): 289-291.

Khan, H.S.I., M. Saifulla, A.S.N. Nawaz, P.R. Somashekharappa and Rafiulla. 2012. Physiological and nutritional studies of *Fusarium oxysporum* f. sp. *ciceris* causing Fusarium wilt of chickpea. *Environment & Ecology*, 30 (3): 573-577.

Kim, Y.K., C.L. Xiao and J.D. Rogers. 2005. Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyriputrescens*. *Mycologia*, 97: 25-32.

Muehlbauer, F.J. and P.N. Rajesh. 2008. Chickpea, a common source of protein and starch in the semi-arid tropics. *Genomics of Tropical Crop Plants*, 1: 171-186.

Navas-Cortes, J.A., A.R. Alcala-Jimenez, B. Hau and R.M. Jimenez-Diaz. 2000. Influence of inoculum density of races 0 and 5 of *F. oxysporum* f. sp. *ciceris* on development of Fusarium wilt in chickpea cultivars. *Eur. J. Pl. Pathology,* 106: 135-146.

Nene, Y.L. and M.V. Reddy. 1987. Chickpea diseases and their control. In: Saxena, M.C. and K.B. Singh (Eds.), *The Chickpea*. CABI Publishing, CAB Int. Wallingford, UK, pp. 233-270.

Nene, Y.L., V.K. Sheila and S.B. Sharma. 1996. *A World List of Chickpea and Pigeonopea Pathogens*. (5th Ed). ICRISAT, Patancheru, India.

Paulkar, P.K.B., T. Raut and K.B. Kale. 2002. Variability in chickpea wilts isolates. *Karnatka J. Agric. Sci*., 15: 389-390.

Ramteke, P.K. and S.S. Kamble. 2011. Physiological studies in *Fusarium solani* causing rhizome rot of ginger (*Zingiber officinale* Rocs). *The Bioscan*, 6(2): 195-197.

Ryan, J.G. 1997. A global perspective on pigeon pea and chickpea sustainable production systems-present status and future potential. In: Asthana, A.P. and M. Ali (Eds.), *Recent Advances in Pulses Research*. Indian Society of Pulses Research & Development, Kanpur, India. pp. 1-31.

Saha, A., P. Mandal, S. Dasgupta and D. Saha. 2008. Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. *J. Environ. Biol*., 29(3): 407-410.

Saxena, R.K., L. Sangetha, A. Vohra, R. Gupta and R. Gulati. 2001. Induction and mass sporulation in lignin degrading fungus *Ceriporiopsis subvermispora* for its potential usage in pulp and paper industry. *Curr. Sci*., 81: 591-594.

Sharmin, S., K.A. Bhuiyan, M.R. Uddin and M.B. Anwar. 2012. Influence of some growth factors on *in-vitro* growth of *Fusarium oxysporum* f. sp. *phaseoli* causing seedling mortality of bush bean. *Bangladesh J. Plant Pathol.* , 28(1&2): 9-14.

Singh, K.B., and B.S. Dahiya. 1973. Breeding for wilt resistance in chickpea. (Abstr.). In: *Symposium on Wilt Problems and Breeding for Wilt Resistance in Bengal Gram*. *Indian Agric. Res. Inst*., New Delhi, India. pp. 13-14.

Somasegaran, P. and H.J. Hoben. 1985. *Methods in Legume-Rhizobium Technology*. University of Hawai'i NifTAL Project and MIRCEN, Department of Agronomy and Soil Science, Hawai'i Institute of Tropical Agriculture and Human Resources, College of Tropical Agriculture and Human Resources, Paia, Maui, Hawai.

Stallknecht, G., K.M. Gilberston, G.R. Carloson, J.L. Eckhoff, G.D. Kushnak, J.R. Sims, M.P. Wescott and D.M. Wichman. 1995. Production of chick peas in Montana. *Agri. Res.,* .12: 46-50.

Sun, M.H., L. Gao, X.Z. Liu and J.L. Wang. 2009. Fungal sporulation in two-stage cultivation. *Mycosystema*, 28(1): 64-72.

Trapero-Casas, A. and R.M. Jiménez-Díaz. 1985. Wilt and root rot diseases of chickpea in Southern Spain. *Phytopathology*, 75: 1146-1151.

Volz, P.A. and E.S. Beneke. 1969. Nutritional regulation of basidiocarp formation and mycelial growth of Agaricales. *Mycopathol. Mycol. Appl*., 37(3): 225-53.

Xiao, C.L. and J.W. Sitton. 2004. Effects of culture media and environmental factors on mycelial growth and pycnidial production of *Potebniamyces pyri*. *Mycol. Res*., 108: 926-932.

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