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Physiological Studies of *Fusarium oxysporum* F. Sp. *Ciceri*

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ABSTRACT

In vitro studies were conducted on the effect of culture, media, carbon and nitrogen sources, temperature and pH levels on mycelial growth of *F. oxysporum* f.sp. *ciceri*. The fungus grew the best on Czapek dox agar and chickpea seed-meal agar media among eight culture media that were tried. Glucose was found to be the best source of carbon whereas peptone was the best source of nitrogen. Growth of *F. oxysporum* was maximum at 30^0C after seven days of inoculation, which was reduced drastically below 15^0C and above 35^0C. The most suitable pH level for growth of fungus was 7.0 and 6.0.

Key Words: *Fusarium oxysporum; In vitro;* Culture media; pH; Carbon; Nitrogen; Mycelial growth

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes and constitutes about 70% of the pulse crops grown in Pakistan. The national average yield (550 kg ha^{-1}) of chickpea is very low as compared to the other chickpea producing countries of the world (Anonymous, 2003). Although a number of biotic and abiotic factors contribute for low chickpea production but endemic occurrence of wilt disease caused by *Fusarium oxysporum* f.sp. *ciceri* is of significant importance. Chickpea wilt is worldwide in occurrence and has been reported from many countries of the world (Nene *et al.*, 1984).

Since the creation of Pakistan *Fusariun* wilt had always been remained a serious problem in chickpea crop. Although, no precise information on the losses caused by the disease is available, but the yield loss may vary from 10 to 100% depending on the environmental conditions (Grewal & Pal, 1970). Likewise, an estimated annual loss of rupees 12 million was reported due to wilt disease in Pakistan (Sattar *et al.*, 1953). In 1956, the disease appeared in epidemic form and more than 75% crop losses were reported (Akhtar, 1956).

The wilt pathogen is soil-borne and survives through chlamydospores in seed and dead plant debris in soil (Haware *et al*, 1978). Since, the fungus can survive in the soil for several years, it is not possible to control the disease through normal crop rotations. Although a number of chickpea lines have been reported as resistant to wilt from different countries of the world (Nene *et al*., 1981), but their success has been highly localized due to location-specific races of the pathogen (Singh & Reddy, 1991). The seed-borne inoculum can be eradicated by seed-dressing fungicides: Benlate-T: Benomyl 30% + Thiram 30% @ 1.5% (Haware *et al*., 1978).

Resistance to vascular wilt may be expressed before the pathogen gains entry into the xylem of plants or even after that. The very fact that the vascular wilt fungi are confined to the xylem until the later phases of the disease is itself a manifestation of resistance on the part of extra vascular tissues. Since vascular fungi are facultative parasites, they obviously find the xylem environment relatively free of severe host reactions. These peculiar features have to be born in mind to identify the factors of resistance to wilt disease. The present investigation was conducted to study the effect of physiological factors on the mycelial growth of the fungus.

MATERIALS AND METHODS

Studies of the following physiological aspects of *F. oxysporum* f.sp *ciceri* were conducted *in vitro*.

Effect of Culture Media. Following eight culture media were used to find out the most suitable one for the mycelial growth of the fungus. Each culture medium was prepared in 1 liter of water and autoclaved at 120^0C at 15 psi for 20 min. These were cooled to 45^0C and then poured in 9 cm petri dishes for solidification. Chickpea Seed meal Extract Agar (CSMA) Medium (Chickpea seed meal extract 20 g, Dextrose 20 g, Agar 20 g), Potato Dextrose Agar (PDA) Medium (Potato starch 20 g, Dextrose 20 g, Agar 20 g), Cornmeal Agar Medium (Cornmeal 20 g, Dextrose 20 g, Agar 20 g), Malt Extract Agar Medium (Malt extract 20 g, Agar 25 g, Peptone 2 g), Cape dox Agar Medium (Sodium nitrate 2 g, Potassium nitrate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 3 g, Sucrose 30 g, Agar 20 g), Sabouroud's Agar Medium (Dextrose 40 g, Peptone 10 g, Agar 20 g), Waksman's Agar Medium (Agar 26 g, Glucose 10 g, Peptone 5 g, Potassium dihydrogen phosphate 1 g, Magnesium sulphate 0.5 g), Richard's Agar Medium (Agar 20 g, Sucrose 50 g, Potassium nitrate 10 g, Potassium dihydrogen phosphate 5 g, Magnesium sulphate 2.5 g).

Effect of Different Carbon and Nitrogen Sources. Czapeck Dox agar medium (in one liter of water) was used as the medium for studying the effect of carbon and nitrogen sources.

Nitrogen sources. Three nitrogen compounds viz; Potassium nitrates 10 g, Sodium nitrate 8.5 g and Peptone 2.5 g were amended in Czapeck Dox agar medium.

Carbon sources. Three carbon compounds viz; glucose 13.5 g, sucrose 12.5 g and starch 12.5 g were tried individually as a constitute of carbon source in Czapeck Dox agar medium.

Effect of Temperature. The fungus *F. oxysporum* was inoculated in Czapeck Dox medium using five petri dishes for each temperature, which was applied at 5, 10, 15, 20, 25, 30 and 35^{0}C.

Effec t of Different pH Levels. The test fungus was inoculated on Czapeck Dox agar medium whose pH was adjusted to 5.0, 6.0, 7.0, 8.0 and 9.0, respectively.

All these experiments were conducted in five replicates. Plates were inoculated by placing 4 mm agar medium plugs containing active mycelium of the fungus and were placed in the centre of the petri dishes. Plates were incubated at 30^{0}C (except for the study of temperatures). Observations on linear growth were recorded after seven days of inoculation.

RESULTS AND DISCUSSION

Effect of culture media. The results of the experiment revealed that the Czapeck Dox agar and CSMA media were the best for the radial growth of *F. oxysporum* as this fungus gave maximum growth of 85 and 80 mm, respectively, after seven days of inoculation followed by Cornmeal agar and Malt extract agar media which showed growth of 70 and 65 mm, respectively (Fig. 1). Khanzada *et al.* (2003) also reported similar results with *M. phaseolina.* Different synthetic and non synthetic cultural media have profound influence on cultural and morphological characteristics of fungus (Shaikh, 1974). Haware *et al.* (1986) had modified Cape do agar medium by adding PCNB, streptomycin and malachite green. This medium is highly effective for the growth of *F. oxysporum*.

Effect of different carbon and nitrogen sources. The results of this experiment indicated that all the carbon sources were suitable for the fungus growth. However, glucose was found to be the best carbon source for this purpose (Fig. 2). The fungus may convert certain forms of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al*., 1970).

As is evident from Fig. 3, Peptone was found to be best source of nitrogen for *F. oxysporum* and the maximum growth of fungus 90 mm was attained. It was followed by Potassium nitrate. On potassium nitrate (KNO_3), the growth of fungus was 80 mm after seven days of inoculation. Similar observations were made by Hussain *et al*. (2003) for the mycelial study of *Sclerotium rolfsii* Sacc. Tariq *et al*. (1993) obtained the maximum growth of *Botrytis glaiolorum* when glucose and potassium nitrate were used as carbon and nitrogen sources, respectively.

Fig. 1. Effect of different culture media on the mycelial growth of *F. oxysporum* f.sp. *cicri*

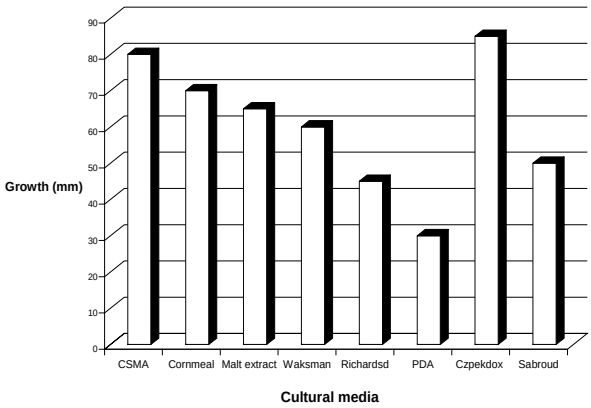


Fig. 2. Effect of carbon sources on the mycelial growth of. *F. oxysporum* f.sp. *cicri*

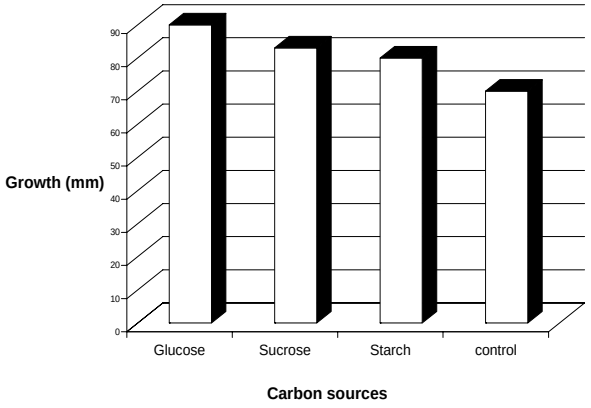
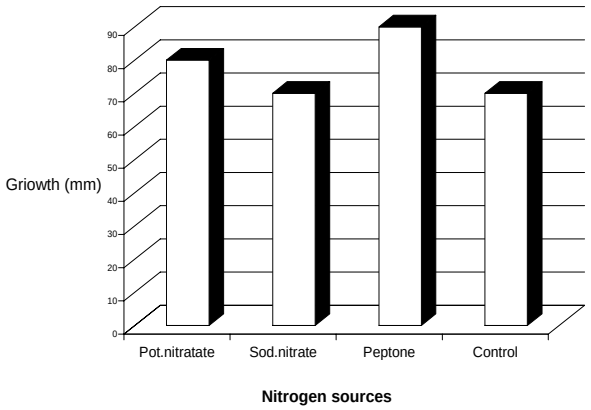


Fig. 3. Effect of nitrogen sources on the mycelial growth of *F. oxysporum* f.sp. *cicri*



Results of our study indicated that the role of C: N ratio is very important. The fungus readily colonizes organic substances in the soil. Increased inoculum potential and disease severity are positively correlated with the food base of organic substances. Crop debris that serves as a food base can also serve as an infection bridge. The fungus becomes active primarily near the soil surface and hyphea of the fungus penetrate into the roots and cause severe blockage in the xylem vessels which results in the wilting of plants.

Effect of temperature. As evident from Fig. 4, the fungus grew at the temperature range of 10–35^{0}C. However, growth of the fungus was drastically reduced below 15^{0}C and started to decline above 35^{0}C, as these temperatures did not favour for growth of the fungus. It was observed that at 25^{0}C and 30^{0}C, the fungus attained the maximum growth 76.8 and 85.4 mm while at 25^{0}C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5^{0}C. Gupta *et al.* (1986) reported similar findings regarding temperature requirements to this fungus. Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30^{0}C (Chauhan, 1965).

Fig. 4. Effect of different pH levels on the mycelial growth of *F. oxysporum* f.sp. *cicri*

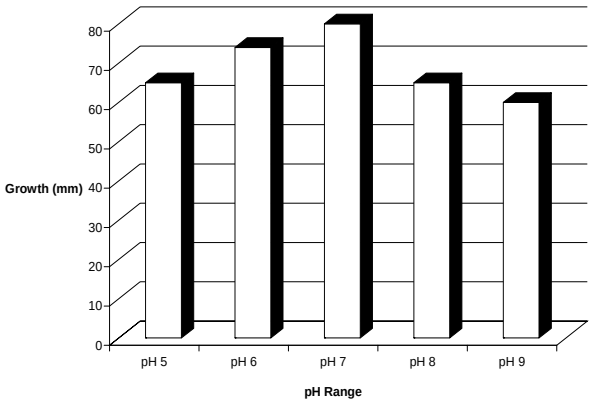
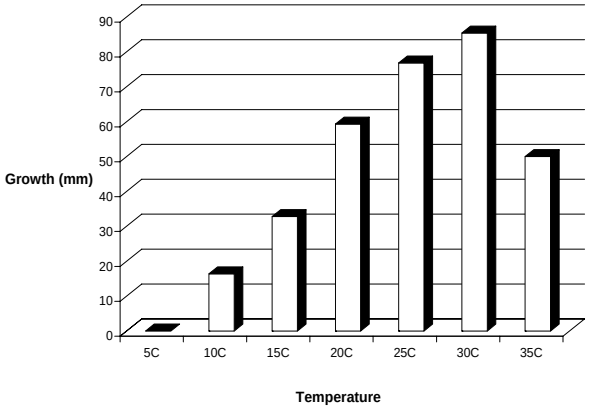


Fig. 5. Effect of temperature on the mycelial growth of *F. oxysporum* f.sp. *cicri*



Effect of different pH levels. Growth of the fungus was obtained at all the pH levels tested but it was maximum at pH 7 where it was 80 mm after seven days of inoculation (Fig. 5). pH 6 (74 mm) and pH 8 (65 mm) were also favourable. Growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. The results of the present study are in agreement with those achieved by Hayes (1978). This fungus can tolerate a wide range of pH 5.0–6.5 (Shaikh, 1974).

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