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Short Communication

EFFECT OF DIFFERENT MEDIA AND ENVIRONMENTAL CONDITIONS ON THE GROWTH OF *Fusarium oxysporum* f. sp. *ciceri* CAUSING FUSARIUM WILT OF CHICKPEA

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ABSTRACT

The pathogen *Fusarium oxysporum* f.sp. *ciceri* was tested for variation in growth and cultural characters an five different soiled media, PDA found to be best for the growth of different isolates. The pathogen responded to a wide range of carbon and nitrogen sources with considerable variation in its utilization, glucose and alaine as carbon and nitrogen sources were best for their better growth. The growth of the pathogen was highest at 30^0C. The optimum pH for growth of the pathogen ranged from 65-7.0.

KEY WORDS: *Fusarium oxysporum* f.sp. *ciceri*, PDA, growth, pathogen glucose, aline.

INTRODUCTION

Chickpea (*Cicer arietinum* L) is an important pulse crop which is grown in tropics sub tropics and temperate regions. It is a rich source of proteins (25.3 – 28.9%). The crop is subjected to infection by several fungi, among them *Fusarium* wilt is an important disease incited by *Fusarium oxysporum* schlecht emend. Synd. & Hans. f.sp. *ciceri* causing considerable damage to the crop. This disease was reported for the first time in India by Butler (1918). An understanding of the role of different media and environmental conditions on the infection and survival of this pathogen is necessary to develop cultural disease management practices. The object of the present investigation was to isolate and purify the pathogenic fungi *Fusarium oxysporum* f.sp. *ciceri* causing wilt of chickpea and to know suitable media, carbon, nitrogen, temperature and pH requirement favouring the mycelia growth.

MATERIALS AND METHODS

Plants showing wilting symptoms were collected from the farmers field during the survey. Stem and root region of the infected plants exhibited different symptoms including white profused mycelial growth and also abundant sporulation of the fungus with wilting symptoms were collected and brought to the laboratory. Root and stem were cut into small bits and washed well in running water to remove the adhering soil particles. The cut pieces were surface sterilized by immersing the pieces in 1 per cent mercuric chloride solution for one minute.

The bits were washed thoroughly in sterile distilled water 3 times to remove traces of mercuric chloride and dried between 2 sterilized filter paper and then ascetically transferred to sterile potato dextrose agar (PDA) amended with streptomycin sulphate (0.01 per cent) in petriplates and incubated at 28 ± 1^0C for 7 days. The growing fungi were individually transferred to PDA medium. Pure culture of *Fusarium oxysporum* f.sp. *ciceri* was obtained by using single spore or hyphal tip technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 4^0C for further use. Based on the pathogenesis test, highly virulent isolate of *Fusarium oxysporum* f.sp. *ciceri* was used for further studies.

Variation among five different isolates of *Fusarium oxysporum* f.sp. *ciceri* with respect to growth and cultural characters on five different solid media was carried out. Each medium was used for the five isolates and replicated thrice. Twenty ml each of the following media *viz.*, Potato dextrose agar, Richard's agar, Czapek's agar, Starch agar and Brown's agar were poured into 90 mm diameter sterilized petriplates and allowed to solidify. Five mm discs from 10 days old culture of five different isolates of *Fusarium oxysporum* f.sp. *ciceri* were used for inoculation. The inoculated plates were incubated at 28 ± 1^0C. The colony diameter of all the isolates on different media was measured at 10 days after inoculation.

Studies on the effect of different carbon sources on the growth of the pathogen (Bangalore isolate) were done in the flask containing of 40 ml Richard's solution and substituting the sucrose with different carbon sources like dextrose, Fructose, Glucose and maltose. Five mm discs of *Fusarium oxysporum* f.sp. *ciceri* were inoculated separately into sterilized 100 ml conical flask. After incubation for 10 days at 28 ± 1^0C the mycelial mat of the isolate were harvested and filtered through previously weighed whatman's No. 1 filter paper washed with distilled water and dried at 50^0C in hot air oven for 3 days. The dry mycelia mat weight was recorded separately.

In nitrogen study, the different nitrogen sources like were ammonium nitrate, sodium nitrate, Aspargine and Alanine on the growth of the pathogen (Bangalore isolates) were used and incorporated into Richard's solution on the basis

of equivalent weight of potassium nitrate compound served as control.

Effect of temperature on the growth of the pathogen (Bangalore isolate) was studied at different levels of temperature. About 40 ml of PDA medium was poured into sterilized conical flask under asceptic condition. Inoculation was made with five mm disc of *Fusarium oxysporum* f.sp. *ciceri* from 7 days old culture. The inoculated conical flasks were incubated at 15, 20, 25, 30, 35 and 40^{0}C in BOD incubators with three replications. The dry mycelial mat weight was recorded.

Studies on the growth of pathogen (Bangalore isolate) at different pH levels were done by using Richard's solution prepared in 100 ml conical flasks, each containing 40 ml of the medium. The pH of the medium was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0. Flasks were inoculated with 5 mm diameter agar plugs of the isolate and incubated at 28±1^{0}C. The mycelial mat was harvested 10 days after inoculation and dried in oven at 50^{0}C for 3 days. The dry weight of the mycelial mat was then recorded. Each treatment was replicated thrice.

RESULTS AND DISCUSSION

Effect of media

The growth of different isolates tested varied depending upon the type of media used. Among the different culture media tested, all the five isolates produced maximum growth on PDA (85.76 mm) followed by Richard's medium (84.62 mm) and Czapek's medium (72.56mm). The similar observation was made by Jamaria (1972), observed maximum growth of *Fusarium oxysporum* f.sp. *nevum* on PDA, Richard's agar and Czapeak's agar. Khare *et al.,* (1975) reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's agar. The present study indicated that potato dextrose agar was best source for growth of *Fusarium oxysporum* f.sp. *ciceri* (Table 1.)

TABLE 1. Growth of different isolates of *Fusarium oxysporum* f. sp. *ciceri* an different solid media

Media	Isolates (Diameter in mm)				
	Bengaluru	Chitradurga	Hassan	Mysore	Shivamogga
PDA	85.6	83.6	88.6	85.0	86.0
Richard's agar	87.6	83.6	85.0	82.3	84.6
Czapek's Agar	72.3	72.3	74.6	70.6	73.0
Starch agar	60.0	61.6	56.3	52.3	59.0
Brown's agar	56.6	53.3	49.6	51.0	50.0
SEm.,	0.20				
CD @ 5%	0.57				

TABLE 2. Dry mycelial weight of *Fusarium oxysporum* f.sp. *ciceri* on different carbon and nitrogen sources

Carbon source	Dry mycelial weight (mg)	Nitrogen source	Dry mycelial weight (mg)
Sucrose	429.33	Potassium nitrate	504.00
Dextrose	459.00	Sodium nitrate	365.00
Fructose	298.66	Ammonium nitrate	354.00
Glucose	497.33	Alanine	474.00
Maltose	545.00	Asparagines	466.66
Mean	445.86	Mean	432.73
SEM.,	4.89		4.71
CD @ 5%	13.91		13.38

Effect of carbon sources

Carbon is the most important and essential component of fungal cell required for their growth and development. The present investigation revealed the growth of *Fusarium oxysporum* f.sp. *ciceri* on the various carbon sources tested, glucose (483.26 mg) was found to be good source of carbon tested followed by Maltose (476.19 mg) and least growth was noticed in Fructose (333.06 mg) (Table 2). Similar observations were made by Sowmya (1993) that among different carbon sources tested against *Fusarium oxysporum* f.sp. *cubense*, glucose was the best carbon source for isolates I & II .

Effect of nitrogen

Nitrogen is an important component required for protein synthesis and other vital functions. The nitrogen requirement by pathogen was studied by using different nitrogen sources and the results are presented in Table 2. The study revealed that the maximum growth of the pathogen was observed in alanine (485.87 mg). It was found to be good source of nitrogen tested followed by Aspargine (483.79 mg) Table 2. The results are in agreement with the reports of Bhatnagar *et.al.,* (1968) in case of *Fusarium oxysporum* f.sp. *aurentifoliae* which showed good growth on D-leucine and aspargine. Patel (1990) reported that aspargine supported maximum mycelial growth of *Fusarium solani.*

Effect of temperature

Temperature in the most important physical factor influencing growth of *Fusarium oxysporum* f.sp. *ciceri*. The different levels of temperature *viz.*, 15^{0}C, 20^{0}C, 25^{0}C, 30^{0}C, 35^{0}C and 40^{0}C were studied and results are presented in Table 3. The results of the study indicated that, among the temperature levels 30^{0}C was the common optimum temperature for the growth of *Fusarium oxysporum* f.sp. *ciceri* (329.39 mg) which was followed by 25^{0}C (306.66 mg). Temperatures below 25^{0}C and above 30^{0}C reduced the growth of fungus drastically. Temperature is one of the important factor governing distribution growth, reproduction and survival of the

fungus. The results of the studies are in conformity with report of Ward (1930) that strains of *Fusarium oxysporum* f.sp. *cubense* showed vigorous growth at 30^0C while growth was less vigorous at 20^0C. Similarly Ajid *et al.* (2005) and El-sayed *et al.* (2008) indicated that fungal growth of *Fusarium* Spp. was best between 25^0C-30^0C.

Effect of pH on growth

The hydrogen ion concentration is one of the most important factors influencing growth of the fungi. The requirement of different levels of pH on growth of *Fusarium oxysporum* f.sp. *ciceri*. was studied on potato dextrose broth. The maximum growth of 484.60 mg of Mycelial mat was recorded at pH 7.0 and was significantly superior to other pH levels followed by pH 6.5 (476.13 mg) and at pH 6.0 (473.53 mg). Least growth was observed in pH 9.0 (243.60 mg). The significant growth of fungus was observed at pH levels from 5.5 - 8.0. Above and below these pH levels there was a sudden decline in the growth. Least growth of fungus was observed at pH levels of 5.0 and 9.0 (Table 3.).The results of the present study revealed that between pH 6.5 and pH 7.0 was found to be optimum for the growth of pathogen. The results of the study are in conformity with the report of Sowmy (1993) that four isolates of banana wilt pathogen *Fusarium oxysporum* f.sp. *cubense* produced maximum growth at pH 7.0. El-sayad *et.al.*, (2008) reported that *Fusarium* species grew Well at a pH range of 6.5 to 7.0.

TABLE 3. Dry mycelial wilt of *Fusarium oxysporum* f.sp. *ciceri* on different temperature and pH regimes.

Temperature (^{0}C)	Dry mycelial weight (mg)	pH levels	Dry mycelial weight (mg)
15	164.33	5.0	325.00
20	236.33	5.5	379.00
25	347.33	6.0	450.00
30	362.00	6.5	497.33
35	285.00	7.0	583.33
40	192.00	7.5	477.33
		8.0	325.66
		9.0	226.66
SEM.,	4.54		5.26
CD @ 5%	12.85		14.79

CONCLUSION

Among all isolates tested on different media for variability of *Fusarium oxysporum* f.sp. *ciceri* all the five isolates produced maximum growth on PDA (85.76 mm) followed by Richard's medium (84.62 mm). Among the carbon sources tested against *Fusarium oxysporum* f.sp. *ciceri* glucose was found to be the best for the growth (483.26 mg) followed by maltose (476.19 mg). Among the nitrogen sources tested, Alanine was found to be the best (485.87) followed by Aspargine (483.79 mg).

The studies conducted to find out the role of different temperature levels on growth *Fusarium oxysporum* f.sp. *ciceri* revealed that maximum mycelial growth was observed at a temperature of 30^oC (329.29 mg) followed by 25^oC (306.66 mg). The least growth was occurred at 15^oC (175.13 mg). The fungus reached minimum growth at pH 7 (484.60 mg) followed by pH 6.5 (476.13 mg).

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