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Research Article

Effect of Media and Temperature on Growth and Sporulation of *Fusarium oxysporum* f. sp. *ciceri in vitro*

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ABSTRACT

Chickpea (*Cicer arietinum*) is an important legume crop which is getting affected by high yield losses from wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri*. The present research was carried out to study the effect of different culture media and temperature on mycelia growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. Among eight culture media that were tested and evaluated, the fungus grew the best on Potato Dextrose agar (PDA) and Czapek's Dox agar media. Growth of *F. oxysporum* f. sp. *ciceri* was maximum at 30˚C after seven days of inoculation, which was reduced drastically at below 20˚C and above 30˚C.

Keywords: Chickpea (*Cicer arietinum*), wilt, *Fusarium oxysporum* f. sp. *ciceri*, media, sporulation

Introduction

Chickpea (*Cicer arietinum*) is an important legume crop which is getting affected by high yield losses from wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri*. Chickpea wilt is a major constraint in chickpea production globally. India accounts for 75 percent of the world chickpea production (Singh *et al*., 2006). *F. oxysporum* f. sp. *ciceri* is a soil borne root pathogen colonizing xylem vessels by blocking them resulting in wilting (Bateman *et al*., 1996). Different temperature regimes are required for host pathogen interactions as well as for growth and development of microorganisms. This present work depicts the role of different temperature and media to understand survival of the pathogen in environmental conditions which will further be helpful in management of the wilt disease.

Materials and Methods

Determination of effect of different media on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*

Various media; V-8 Juice Agar [Hi-media], Czapek's Agar [Hi-media], Potato Dextrose Agar (PDA) [Hi-media], Oat Meal Agar [Hi-media],Host Root Extract Agar, Host Root Extract Dextrose Agar, Corn Meal Agar, and Water Agar were prepared and evaluated to ascertain the favourable medium for growth and sporulation of *F. oxysporum* f. sp. *ciceri*.

V-8 Juice Agar [Hi-media]

Readymade mixture of V-8 juice agar **(**44.3g) was suspended in 1000 ml of distilled water. It was boiled till the medium dissolved completely. The prepared medium was sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

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Czapek's Agar [Hi-media]

Readymade mixture of Czapek's agar (49.01g) was suspended in 1000 ml of distilled water. It was boiled till the medium dissolved completely. The prepared medium was sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Potato Dextrose Agar (PDA) [Hi-media]

Readymade mixture of potato dextrose agar (39.0g) was suspended in 1000 ml of distilled water. It was boiled till the medium dissolved completely. The prepared medium was sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Oat Meal Agar [Hi-media]

Readymade mixture of oat meal agar (72.5g) was suspended in 1000 ml of distilled water. It was boiled till the medium dissolved completely. The medium was sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Host Root Extract Agar

100g chickpea root was boiled in 500 ml of distilled water for 30 minutes, later extracted with the help of double layered muslin cloth. Agar was melted in 250 ml of distilled water separately. Both the solutions were mixed thoroughly. Then the volume was made up to one litre and sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Host Root Extract Dextrose Agar

100g chickpea root was boiled in 500 ml of distilled water for 30 minutes. Extract was collected by filtering through muslin cloth. Agar and dextrose were melted in 250 ml distilled water separately. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml and sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Corn Meal Agar

The corn was boiled in 500 ml of distilled water for 30 minutes. Extract was collected with the help of double layered muslin cloth. Agar-agar and dextrose were melted in 250 ml of distilled water separately. Both the solutions were mixed thoroughly. Then the volume was made up to one litre and sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Water Agar

15 gram agar was suspended in 1000 ml of distilled water. It was boiled till agar dissolved completely. We made a solution of 1.5 grams in 100 ml distilled water. The prepared medium was sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

To carry out the experiment, the above mentioned protocol was followed to make 100 ml media each. Thereafter, 20 ml of each of the medium was poured into each or separate Petri plates. Such Petri plates were inoculated with 5 mm disc taken from the periphery of actively growing culture of *F. oxysporum* f. sp. *ciceri* and incubated at 27±1°C. Each treatment was replicated thrice. Radial growth of colony was recorded by measuring the colony diameter in millimeter (mm) at regular intervals. The data obtained was analyzed statistically.

Determination of effect of various temperature regimes on growth and sporulation of *F. oxysporum* f. sp. *Ciceri in vitro*

To determine the minimum, optimum and maximum regime of temperature for mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri*, PDA was poured into the Petri plates and a 5 mm mycelial disc from actively growing culture of the fungus was placed on the surface of solidified medium in laminar air flow. The inoculated plates were incubated at different regimes of temperature *viz*., 20℃, 25, 30, 35 and 40℃ in different BOD incubators which provided the required experimental temperature for the growth of the fungus. Three replications were maintained for each treatment. Radial growth of the colony was recorded by measuring colony diameter in millimeter (mm) at regular intervals of 48 hrs. The data obtained was analysed statistically.

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Results

The results obtained for the effect of eight media *viz*., V-8 juice agar, Czapek's agar, potato dextrose agar, oat meal agar, host root (chickpea) extract agar, host root (chickpea) extract dextrose agar, water agar and corn meal agar on the mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri* are as presented in Table 1 below.

Table 1: Effect of different culture media on radial growth (mm) and sporulation of wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri*)

Medium Name	Colony diameter in mm*	Sporulation**
T_1= V-8 juice agar	78.33	+++
T_2= Czapek's agar	87.00	+++
T_3= Potato Dextrose agar	89.00	++++
T_4= Oat meal agar	65.19	++
T_5= Host root (chickpea) extract agar	47.00	-
T_6=Host root (chickpea) extract dextrose agar	38.00	-
T_7= Corn meal	31.67	-
T_8= Water agar	60.33	++
S.Em.(±)	0.84	
C.D. at 0.05%	2.52	

*Mean of three replications

**Categories of sporulation: Excellent (++++) = 61 & above, Good (+++) = 41-60, Fair (++) = 21-40, Poor (+) = Less than 20, No (-) = Nil.

The result of the effect of different culture media on radial growth (mm) of wilt of chickpea pathogen *Fusarium oxysporum* f. sp. *Ciceri* is as shown in Figure 1 below.

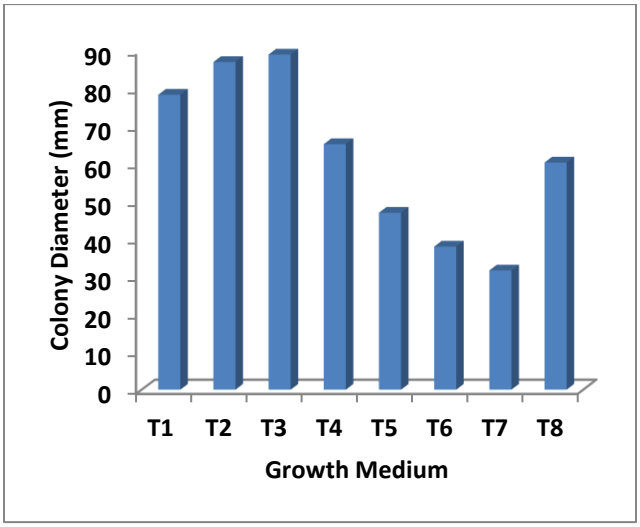

Fig. 1: Effect of different culture media on radial growth (mm) of wilt of chickpea pathogen *Fusarium oxysporum* f. sp. *ciceri*

Legend: **T_1**= V-8 juice agar, **T_2**= Czapek's agar, **T_3**= Potato Dextrose agar, **T_4**= Oat meal agar, **T_5**= Host root (chickpea) extract agar, **T_6**=Host root (chickpea) extract dextrose agar, **T_7**= Corn meal, **T_8**= Water agar.

Effect of different temperature levels on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*

Different temperature regimes are required for host pathogen interactions as well as for growth and development of microorganisms. The results obtained and recorded for the effect of different temperature levels *viz.*, 20°C, 25°C, 30°C, 35°C and 40°C on mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri* in order to find optimum temperature for mycelial growth and sporulation of the fungus are described in Table 2 below:

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Table 2: Effect of different temperature level on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*

Temperature	Colony diameter in mm*	Sporulation**
T_1= 20 ºC	55.33	++
T_2= 25 ºC	64.88	+++
T_3= 30 ºC	71.88	++++
T_4= 35ºC	33.17	+
T_5= 40 ºC	0.00	-
S.Em.(±)	0.54	
C.D. at 0.05%	1.62	

*Mean of three replications

**Categories of sporulation: Excellent (++++) = 61 & above, Good (+++) = 41-60, Fair (++) = 21-40, Poor (+) = Less than 20, No (-) = Nil.

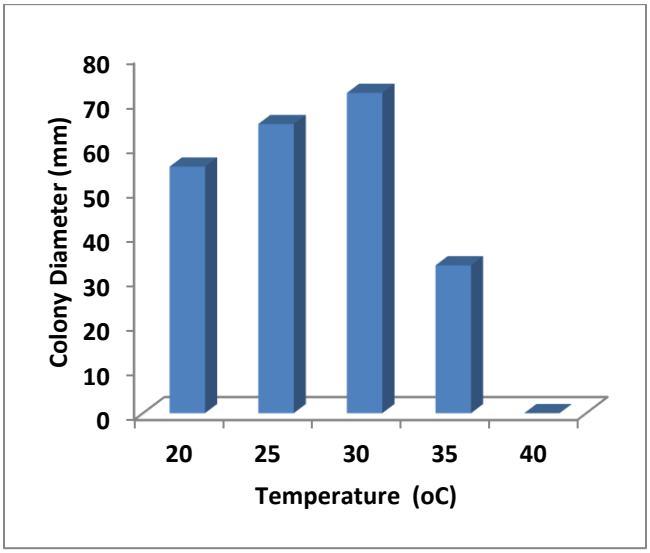

Fig. 2: Effect of different temperature level on growth (mm) of *Fusarium oxysporum* f. sp. *ciceri*

Discussion

The present study has revealed the effect of various culture media on mycelia growth of *Fusarium oxysporum* f. sp. *ciceri* when inoculated on different culture media. The maximum colony diameter (89.33 mm) was recorded on potato dextrose agar (PDA) medium after 168hrs of inoculation, which was significantly superior over all the other tested media. This showed that maximum growth of *F. oxysporum* f. sp. *ciceri* was supported by PDA followed by Czapek's agar (86.00 mm), V-8 juice agar (79.33 mm) and oat meal agar (90.67 mm), water agar (63.33 mm), host root (chickpea) extract agar (50.00 mm) and host root (chickpea) extract dextrose agar (44.33 mm). Significantly least colony diameter (66.67 mm) was recorded on corn meal agar. It was concluded that potato dextrose agar and Czapek's agar were significantly best culture media for growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. Osman *et al.* (1992) found that potato extract agar was the best medium for mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri.* It was followed by Czapek's agar which yielded 86.00 mm colony diameter and good sporulation. Khillare *et al.* (2007) also reported that Czapek's dox agar medium was an ideal medium for growth and sporulation of *F. oxysporum* f. sp. *ciceri.*

The results of this study also revealed the effect of different temperature on radial growth. Results

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obtained showed that, after 168 hrs of inoculation, significantly higher mycelial growth (71.00 mm) was observed at 30℃ as compared to 25℃ (65.00 mm) and 20℃ (55.00 mm). A significant reduction in mycelial growth of *F. oxysporum* f. sp. *ciceri* was observed at 35℃ and 40℃ which recorded a colony diameter of 33 mm and 00.00 mm, respectively. Our findings corroborated with the findings of Landa *et al.* (2001) who reported similar findings regarding the temperature requirements for the growth of *F. oxysporum* f. sp. *ciceris*. Reddy (2002) reported that growth of 40 isolates of *Fusarium udum* required different temperature levels (from 20°C to 35°C). Results are in confirmation with Khillare and Ahmed (2012) who reported that maximum growth of *Fusarium oxysporum* f. sp. *ciceri* was obtained at 30℃.

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