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EFFECT OF DIFFERENT MEDIA, TEMPERATURE AND HYDROGEN-ION ON THE GROWTH AND SPORULATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* CAUSING CHICKPEA

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ABSTRACT

An experiment was conducted at division of Plant Pathology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur (S.K.N.A.U, Jobner). *In vitro* studies were conducted to know the effect of different solid and liquid media, temperature and hydrogen-ion concentrations on sporulation and growth of *Fusarium oxysporum* f.sp. *ciceri*. The fungus grew the best on Potato Dextrose Agar media (90.00 mm) after 7 days of incubation at 25 ± 1°C and significantly superior in comparison to other media tested. Among the different liquid media tested, maximum dry mycelium weight was recorded in Potato Dextrose Broth (472.75 mg) superior to all other liquid media tested. The most suitable pH level and temperature for growth of fungus was 6.0 (90.00 mm) and growth of *F. oxysporum* was maximum at 30^{0}C.

Figure : 00 References : 15 Tables : 05
KEY WORDS : *Fusarium oxysporum* f.sp. *ciceri,* Hydrogen-ion, Liquid media, pH, Solid media, Temperature.

Introduction

Chickpea (*Cicer arietinum* L.) remarkably predominates among other pulse crops in terms of both area and production and the crop is widely growing in 'India as well as other tropical, sub tropical and temperate regions of the worlds. It is a self pollinated diploid (2n=16) annual grain legume or pulse crop. It is the third most important grain legume crop in the world after common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) The term "pulse" is Latin origin, meaning "thick soup[11]". Pulse have huge benefits to human health, the United Nations has announced 2016 as the International Pulse Year. Plant diseases play an important role in the destruction of crops. Phytopathogens cause important loses. Phytopathogenic fungi such as *Fusarium, Alternaria, Colletitrichum, Rhizoctonia, Sclerotium* etc. has spread during the last few years[15]. *Fusarium* wilt [*Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo and K. Sato)] is one of the major yield limiting factors of chickpea[2,4]. Management of Fusarium wilt can be achieved by use of resistant cultivars and adjustment of sowing dates, chemical and biological treatments. However, several factors influence the efficacy of these management practices, including pathogenic variability in the fungus populations as well as abiotic factors such as temperature and moisture. *F. oxysporum* f. sp. *ciceris* exhibits significant pathogenic variability[9]. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* holds a cardinal place causing annual losses ranging from 10% to 100% under conditions favourable for the disease[1,12,13]. Present work depicts the role of different media, temperature and hydrogen–ion concentration to understand ecological survival of pathogen which will be helpful in management strategy and laboratory evaluation.

Material and Methods

Isolation and Purification of the pathogen

The root of each collected plant sample was washed thoroughly in running tap water to remove the adhering soil. These were then cut into small pieces with the help of a sterilized scalpel, washed in sterilized water, surface sterilized by dipping in 0.01 per cent mercuric chloride ($HgCl_2$) for 1-2 minutes rinsed thrice in sterilized distilled water and transferred on Potato Dextrose Agar (PDA) medium in Petri plates. The plates were incubated

TABLE- 1 : Grades of sporulation

Grades of Sporulation		
Category	**No. of spores per microscopic field**	**Description**
Absent	0	-
Scanty	below 5	+
Moderate	6-15	++
Good	16-30	+++
Abundant	above 30	++++

at 25±1°C for growth.

In order to obtain the pure cultures, single spore culture technique for *Fusarium oxysporum* f.sp. *ciceri* was used. The spore suspension of the *Fusarium* isolates was prepared in sterilized distilled water so as to obtain 10-12 spores per microscopic field (10x). The suspension was spread over the surface of sterilized 2 per cent plain agar medium in Petri plates and incubated at 25°C. After ten hours, the single germinating spore was observed under low power objective and cut through dummy objective. Such pieces containing germinating spores were transferred separately on potato dextrose agar slants with the help of an inoculating needle and incubated at 25°C for seven days. Single spore were placed under stereo binocular microscope and transferred on PDA. These cultures were observed under microscope.

Effect of culture media

Following ten culture media were used to find out the most suitable one for the mycelial growth and sporulation. Each culture medium was prepared in 1 liter of water and autoclaved at 120ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 8 cm Petri dishes for solidification. Composition of solid and liquid media

TABLE-2 : Effect of different solid media on growth and sporulation of *F. oxysporum* f.sp*. ciceri*

S. No.	Media	Average Colony diameter (mm)*	Sporulation**
1.	Potato dextrose agar (PDA)	90.00(71.57)	++++
2.	Czapek's (dox) agar	77.35(61.59)	+++
3.	Asthana and Hawkers's agar	63.83(53.03)	+++
4.	Richards' agar	27.50(31.62)	++
5.	Brown's agar	23.68(29.11)	+
	S.Em.±	**0.71**	
	C.D. at 5%	2.18	
	C.V.%	**2.54**	

*Mean of four replications; Figures in parentheses are angular transformed values.

**Categories of sporulation + Scanty ++ Moderate +++ Good ++++ Abundant

TABLE- 3 : Effect of different liquid media on growth and sporulation of *F.oxysporum* f. sp*. ciceri*

S. No.	Media	Average Drymycelial weight (mg)*	Sporulation**
1.	Potato dextrose broth	472.75	++++
2.	Czapek's (dox) broth	395.75	+++
3.	Asthana and Hawkers's broth	318.75	+++
4.	Richards' broth	217.50	++
5.	Brown's broth	193.25	+
	S.Em.±	**6.76**	
	C.D. at 5%	20.57	
	C.V. %	**4.23**	

*Mean of four replications; **Categories of sporulation
+ Scanty ++ Moderate+++ Good ++++ Abundant

as following

Solid media:

1. Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g)
2. Richards' Medium (Potassium nitrate10 g, Potassium dihydrogen phosphate 5 g, Magnesium sulphate 2.50 g, Ferric chloride 0.02 g, Sucrose 50 g, Agar-agar 20 g)
3. Czapeks Dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g, Sucrose 30g, Agar-agar 20g).
4. Brown's Medium (Dextrose 2 g, Magnesium sulphate 0.75 g, Tribasic potassium phosphate 1.25 g, Asparagine 2 g, Agar-agar 20 g)
5. Asthana & Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen phosphate 1.75g, Magnesium sulphate 0.75g, Agaragar 20g).

Liquid media: Potato Dextrose Broth, Richards' broth, Czapek's broth, Brown's broth and Asthana & Hawker's broth media. Composition of liquid media was similar to solid media without using agar –agar because this is a solidifying agent. The *F. oxysporum* f. sp *ciceri* was inoculated at the center of the agar plates. The plates were incubated for a week in order to see the growth. The observations on colony diameter and growth characters were recorded on different media. Sporulation was recorded from the four replications. Culture block of 2 mm diameter mycelial disc from 7 days old culture from different media were suspended in 20 ml distilled sterilized water, homogenized, filtered through sterilized muslin cloth and a drop from such filtrate was examined under microscope. The number of conidia per microscopic field under low power magnification (10 X) was recorded from five such microscopic fields randomly in each case.

Effect of different temperatures and hydrogen-ion concentrations

Various temperatures were arranged in BOD incubator. The *F. oxysporum* f. sp *ciceri* was inoculated on Potato Dextrose Agar plates. These Petri plates were incubated at various temperatures *viz.* 20, 25, 30, 35 and 40°C. The effect of H-ion concentrations was studied by growing fungus at different pH levels *viz.,* 6.0, 6.5, 7.0, 7.5 and 8.0 on Potato Dextrose liquid and solid media. The pH levels were adjusted by using 0.1 N HCl or 0.1 N NaOH solution with the help of pH meter. Petri plates were incubated at 25 ± 1°C temperature in BOD incubator. The *F. oxysporum* f. sp *ciceri* was inoculated at the center of the agar plates. The plates were incubated for a week in order to see the growth. Observations on radial growth, dry weight of mycelium and sporulation of fungus were recorded.

TABLE-4 :Effect of different temperatures on growth and sporulation of *F. oxysporum* f. sp. *ciceri*

S. No.	Temperature	Average Colony diameter (mm)*	Sporulation**
1.	20	65.93(54.29)	++
2.	25	75.15(60.11)	+++
3.	30	89.00(70.65)	++++
4.	35	54.00(47.30)	++
5.	40	29.03(32.60)	+
	S.Em.±	**0.68**	
	C.D. at 5%	2.06	
	C.V.%	**2.16**	

*Mean of four replications; Figures in parentheses are angular transformed values.
**Categories of sporulation
+ Scanty ++ Moderate +++ Good ++++ Abundant

Measurement of growth and sporulation: Radial growth was determined by measuring the colony diameter (mm) in two directions. An observation on sporulation was recorded from four repetitions in each physiological study. The disc of 2 mm diameter of *F. oxysporum* f.sp. *ciceri* culture was taken after the incubation period and were suspended in 20 ml sterilized distilled water homogenized with the help of homogenizer and filtered through muslin cloth. A drop from such filtrate was examined under microscope. The number of spores per microscopic field under low power objective (10X) was recorded from five randomly selected microscopic fields in each case and grades of sporulation were given as under.

Result and Discussion

Effect of different medium

Solid media: The results revealed that there was a considerable variability in growth and sporulation on different solid media tested. The maximum growth of the pathogen was obtained on Potato Dextrose Agar medium (90.00 mm) after 7 days of incubation at 25 ± 1°C and significantly superior in comparison to other media tested. Maximum growth of *F. udum* on Richard's agar and potato dextrose agar was observed[3]. Subsequently suitable media in order of merit were Czapek's (dox) agar (77.35 mm), Asthana & Hawker's agar (63.83 mm) and Richards' agar (27.50 mm). Least growth was obtained on Brown's agar medium (23.68 mm). Potato Dextrose agar medium supported abundant sporulation. Czapek's (dox) agar medium and Asthana & Hawker's agar produced good sporulation, while moderate sporulation was observed on Richards' agar. Scanty sporulation was observed in Brown's agar medium (Table-2). These results are consistent with the findings of others scientists[8] who reported that the fungus grow best on Czapek dox agar and PDA media among six culture media were tested. The Growth of *F. oxysporum* was maximum at 30°C. Several succeful endvoors had been made in physiological studies[6,10,13].

Liquid media: Among the different liquid media tested, maximum dry mycelial weight was recorded in Potato Dextrose Broth (472.75 mg) and significantly superior over all other media tested followed by Czapek's broth with dry mycellial weight (395.75 mg) and Asthana& Hawker's broth (318.75 mg). Moderate growth was observed in Richards' broth having dry mycellial weight (217.50 mg). Minimum mycelial growth was observed in Brown's broth (193.25 mg). In sporulation studies Potato Dextrose broth supported abundant sporulation whereas Czapek's (dox) broth and Asthana & Hawker's broth medium produced good sporulation. Moderate sporulation was observed on Richards' broth and scanty sporulation was observed in Brown's broth medium (Table -3). Effect of media on *F. oxysporum* f.sp. *ciceri* and found that PDA is best for the growth of different isolates[7].

TABLE -5 :Effect of different hydrogen-ion concentrations (solid and liquid media) on growth and sporulation of *F. oxysporum* f.sp. *ciceri*

S.No	pH	Solid media (mm)*		Liquid media (mg)*	
		Average Colony Diameter	Sporulation***	Average Dry mycelial weight	Sporulation***
1.	6.0	90.00(71.57)	++++	461.25	++++
2.	6.5	80.38(63.72)	++++	422.75	++++
3.	7.0	68.45(55.83)	+++	325.00	+++
4.	7.5	27.30(31.48)	++	218.75	++
5.	8.0	23.80(29.19)	+	190.25	++
	S.Em.±	**0.78**		**4.31**	
	C.D. at 5%	**2.38**		**13.12**	
	C.V. %	**2.71**		**2.66**	

*Mean of four replications; Figures in parentheses are angular transformed values.
**Categories of sporulation
+ Scanty ++ Moderate +++ Good ++++ Abundant

The present study indicated that potato dextrose agar and Czapek's Dox agar were best medium for growth of *F. oxysporum* f.sp. *ciceri*.

Effect of different temperature on PDA media

Fusarium oxysporum f. sp. *ciceri* showed maximum growth and sporulation at 30°C followed by 25°C and minimum growth and sporulation was recorded at 40°C temperature. The results revealed that maximum growth (89.00 mm) and sporulation were obtained at 30°C followed by 25°C temperature (75.15mm). Minimum growth (29.03 mm) and sporulation was observed at 40°C temperature. The effects of temperature of *F. oxysporum* f. sp. *ciceris* was studied[10]. They found the disease development was greater at 25°C compared with 20 and 30°C. Effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*), caused by *F. oxysporum* f. sp. *lactucae*, were observed to increase from 10°C up to an apparent maximum near 25°C[14]. The aim of this work was to study the effect of temperatures ensure the elimination of *F. oxysporum* f.sp. *ciceri*. Results are in confirmation withother worker[7] showed the *F. oxysporum* f.sp. *ciceri* grew highest at 30°C.The results clearly indicates that a slight increase or decrease in the temperature from 30°C the growth and sporulation of fungus adversely affected (Table- 4).

Effect of different hydrogen-ion concentrations on Potato Dextrose solid and liquid media

The results showed that the fungus grew on wide range of pH from 6.0 to 8.0, however maximum colony diameter (90.00 mm) was obtained at pH 6.0.This was followed by 6.5 pH (80.38 mm) and 7.0 pH (68.45mm). Least colony diameter was recorded (23.80 mm) at 8.0 pH (Table-5). Effect of pH levels on growth of *F. oxysporum* f. sp. *vanillae* isolates was studied[5]. The fungus showed best growth pH at 5.0, Least growth of all the isolates was recorded at 9.0 pH. Optimum pH for growth of *F. oxysporum* f. sp. *ciceri* ranged from 6.5 to 7.0 was reported[6].

In case of liquid medium, the results showed that significantly maximum dry mycelial weight (461.25 mg) was observed at 6.0 pH followed by 6.5 pH with mycelia weight (422.75 mg). least mycelial dry weight (190.25 mg) was obtained at pH level 8.0 (Table- 5). Similarly worker[8] who reported that the fungus grow best on Czapek dox agar and PDA media. The most suitable pH level for growth of fungus was 6.0 and 6.5. Moderate to abundant sporulation was recorded on a wide range of pH. However, abundant to good sporulation

was noted at 6.0 and 6.5 pH. As the pH increased above 6.5 level the sporulation gradually decreased.

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