

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.909.175>

Effects of Different Temperature, pH and Relative Humidity on the Growth of *Fusarium oxysporum* f. sp. *ciceri* causing Chickpea Wilt

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ABSTRACT

Keywords

Chickpea, Temperatures, Relative humidity, pH, *Fusarium oxysporum* f.sp. *ciceri*, Mycelial growth and sporulation

Article Info

Accepted:
12 August 2020
Available Online:
10 September 2020

Chickpea (*Cicer arietinum* L.) also known as Bengal gram is one of the most important winter season pulse crop grown in India. It is a member of family *fabaceae* and believed to be originated in South West Asia. An *in vitro* study was carried out on effect of different temperatures, relative humidity and pH on growth of *Fusarium oxysporum* f.sp. *ciceri* under laboratory of Department of Plant Pathology, S.K.N. College of Agriculture, Jobner. Results revealed that among the different temperatures (15, 20, 25, 30 and 35°C) and relative humidity (60, 70, 80, 90 and 100%), maximum mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* was observed at 30°C temperature with 80.95 mm. and at 100 per cent relative humidity with 90.00 mm mycelial growth. Whereas studies on effect of different levels of pH, maximum dry mycelial weight (260 mg) was recorded pH 7.0.

Introduction

Chickpea also known as Bengal gram is one of the most important winter season pulse crop grown in India. It is a member of family *fabaceae* and believed to be originated in South West Asia. Gram considered as the World's second most widely grown legume after beans (*Phaseolus vulgaris*). Its ability to form nitrogen-fixing nodules via interaction with *Rhizobium* adds to its uniqueness which make it's a valuable crop for maintaining soil fertility (Ferguson *et al.*, 2010).

Chickpea is contributing nearly 42 to 47 per cent of total pulse production in India. Nearly 90 per cent of the area and production is from six states *viz.*, Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh (Arunodhayam *et al.*, 2014). In Rajasthan major chickpea growing districts are Bikaner, Churu, Jhunjhunu, Hanumangarh, Ganganagar, Jaipur, Jaisalmer, Sikar and Ajmer. In Rajasthan, chickpea occupied nearly area 1572487 hectares with production of 1670265 tonnes and productivity 1062 kg/ha (Anonymous 2017-18).

The production of chickpea in the Indian sub continent and in other Asian countries is severely affected by many plant pathogenic fungi, bacteria, viruses and nematodes which cause diseases such as Fusarium wilt, dry root rot, Ascochyta blight, collar rot, bacterial blight, filiform virus and root nematode (Nene *et al.*, 1996). Among the diseases, Fusarium wilt causes up to 72.16 per cent yield losses in chickpea (Kumar and Bourai, 2012).

The chickpea wilt plant at seedling stage did not exhibit any rotting on outer surface of the root. However, on splitting vertically from collar region downwards showed brown, black discoloration of internal tissues, while in adult stage the affected plants showed typical wilting *i.e.* drooping of leaflets, rachis and lamina. Affected plants when uprooted and splitted the root of the wilted plants exhibited more pronounced internal brown to black discoloration of the xylem vessels (Patil *et al.*, 2017). Fusarium wilt pathogen favoured 25°C temperature, 100 per cent relative humidity and pH 6.5 for growth and sporulation (Yadav *et al.*, 2014)

Materials and Methods

All the glasswares were thoroughly cleaned and rinsed with distilled water and sterilized in hot air oven at 160°C for 2 hours.

Effect of temperature on mycelial growth

Effect of temperature on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* was studied *in vitro*. Twenty ml of sterilized Potato Dextrose Agar medium was poured in each sterilized Petri- plates. Petri -plates containing Potato Dextrose Agar medium were inoculated with 5 mm disc of 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri* with the help of sterilized cork borer and incubated at different levels of temperature *viz.* 15, 20, 25, 30 and 35°C for 7 days. Observations of mycelial growth and

sporulation were recorded on 7th day of incubation.

Effect of relative humidity on mycelial growth

To study the effect of relative humidity on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*, five different levels of relative humidity *i.e.* 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportions in glass desiccators according to the method suggested by Buxton and Mellanbay (1934).

The composition of the acid solution was used as follows

RH (per cent)	Stock solution (ml)*	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	-	Only distilled water

* 50 per cent v/v solution of concentrate sulphuric acid

Petri -plates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri* with the help of sterilized cork borer. Inoculated Petri- plates without lid were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 30±1°C for 7 days. Observations of mycelial growth and sporulation were recorded on 7th day of incubation.

Effect of pH on mycelial growth

Stock solutions

Solution A: 0.1 M solution of citric acid (192.1 mol. wt.) in one liter of distilled water 19.21 gm of citric acid was dissolved.

Solution B: 0.2 M solution of dibasic sodium hydrogen phosphate (293.9 mol. Wt.) in one liter of distilled water 58.78 gm of dibasic sodium hydrogen phosphate was dissolved.

The effect of pH on the growth of *Fusarium oxysporum* f. sp. *ciceri* was determined by adjusting the pH of Potato Dextrose broth at 5.0, 6.0, 7.0, 8.0 and 9.0 by using citrate phosphate buffer (Singh *et al.*, 2005) before sterilization with the help of pH meter. Aliquante of 20 ml medium were dispensed in 100 ml conical flask and autoclaved at 1.045kg/cm² for 20 minutes.

Inoculations were made with 5 mm disc of mycelia obtained from 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri*. An observation of dry mycelial weight was recorded on 14th day of incubation at 30±1^oC.

Results and Discussion

Effect of temperature on mycelial growth

All microorganisms are growing in certain ranges of temperature within which a minimum, optimum and maximum temperature could be located. It is evident from the data presented in Table 1 that the fungus grow at all the ranges of temperature from 15 to 35°C, but it differed significantly at all temperature ranges under study at 7 days of inoculation. Maximum mycelial growth of the fungus was observed at 30°C temperature (80.95mm) and followed by 25°C temperature (62.02mm). Minimum mycelial growth of the fungus was observed at 15°C temperature (22.30mm) (Fig.1 and Plate-1).

Table.1 Effect of temperatures on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* on 7th day of incubation

S. No.	Temperature (°C)	Mycelial growth* (mm)
1	15	22.30
2	20	50.32
3	25	62.02
4	30	80.95
5	35	59.30
	SEm ±	0.73
	CD (p = 0.05)	2.33

*Average of three replications

Table.2 Effect of relative humidity on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* on 7th day of incubation at 30 ± 1°C

S.No.	Relative humidity (%)	Mycelial growth* (mm)
1	60	56.66
2	70	64.00
3	80	73.32
4	90	81.00
5	100	90.00
	SEm±	0.70
	CD (p = 0.05)	2.24

*Average of three replications

Table.3 Effect of pH on dry mycelial weight of *Fusarium oxysporum* f.sp. *ciceri* on 14th day of incubation at 30 ± 1°C

S. No.	pH	Dry mycelial weight * (mg)
1	5.0	198
2	6.0	220
3	7.0	260
4	8.0	129
5	9.0	79
	SEm±	2.20
	CD (p = 0.05)	7.04

*Average of three replications

Fig.1 Effect of temperatures on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* on 7th day of incubation

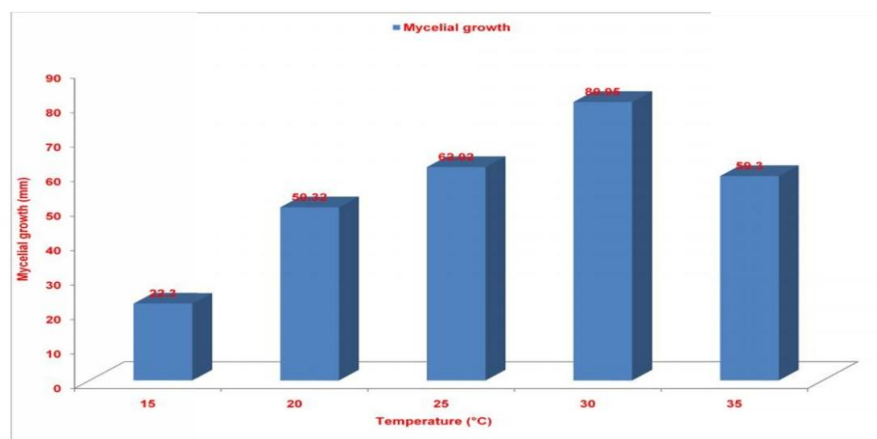


Fig.2 Effect of relative humidity on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* on 7th day of incubation at 30 ± 1°C

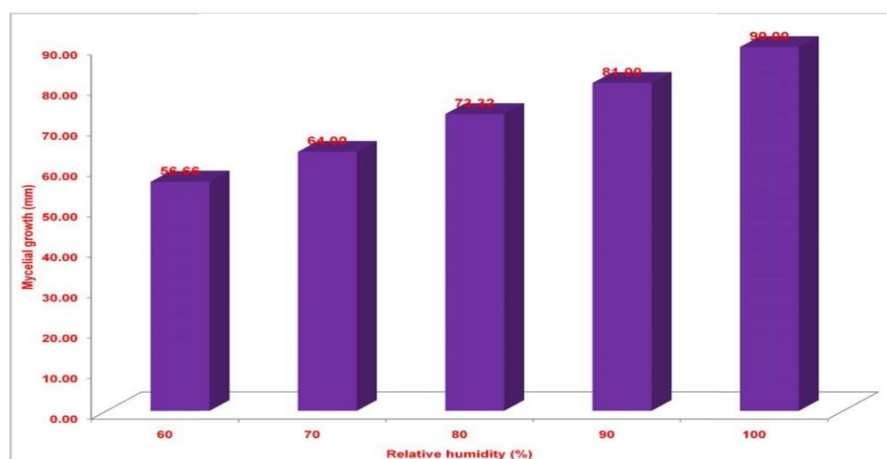


Fig.3 Effect of pH on dry mycelial weight of *Fusarium oxysporum* f.sp. *ciceri* on 14th day of incubation at 30 ±1°C

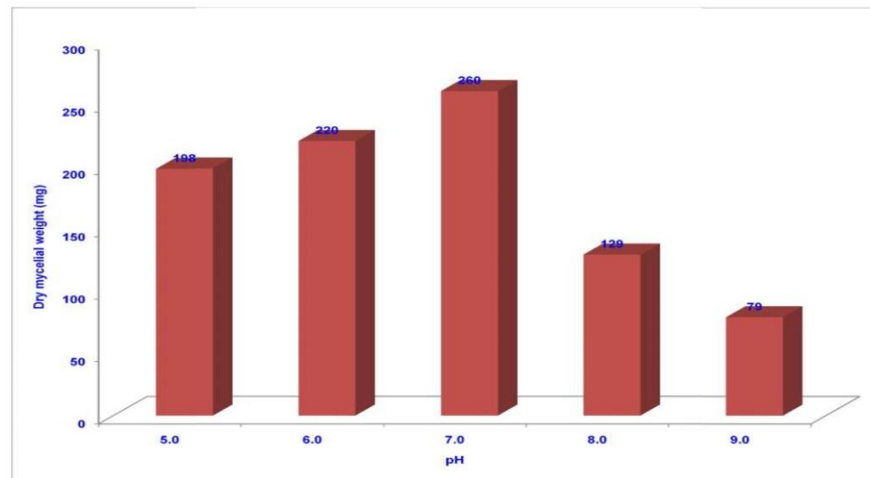


Plate.1 Effect of temperatures on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* on 7th day of incubation

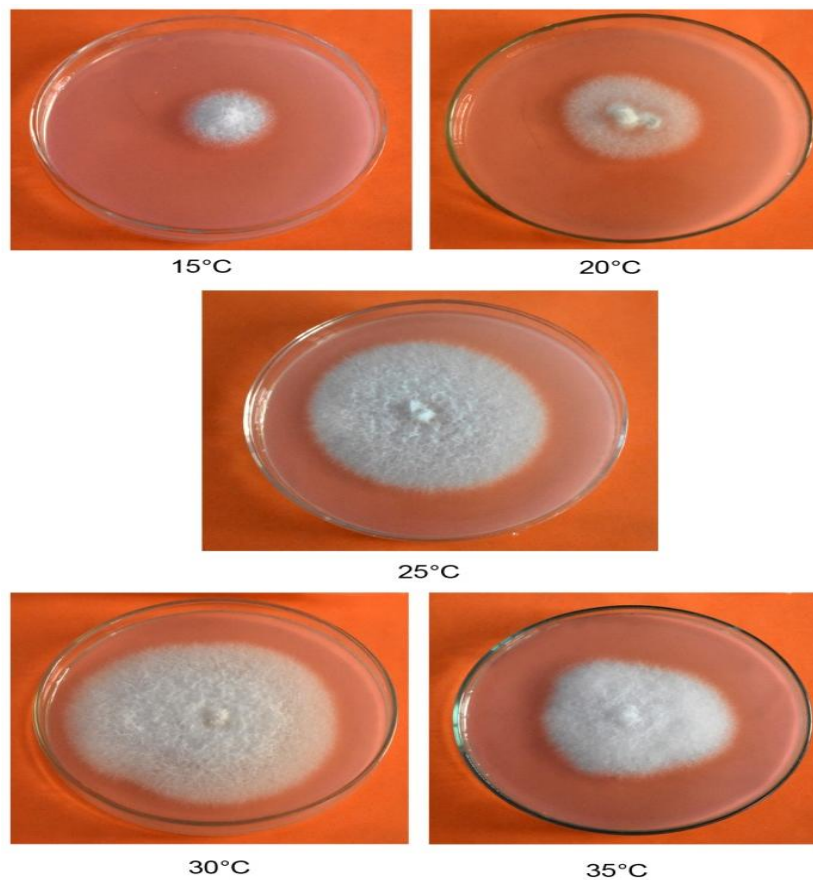


Plate.2 Effect of different levels of relative humidity on growth of *Fusarium oxysporum* f.sp. *ciceri* on Potato Dextrose Agar medium

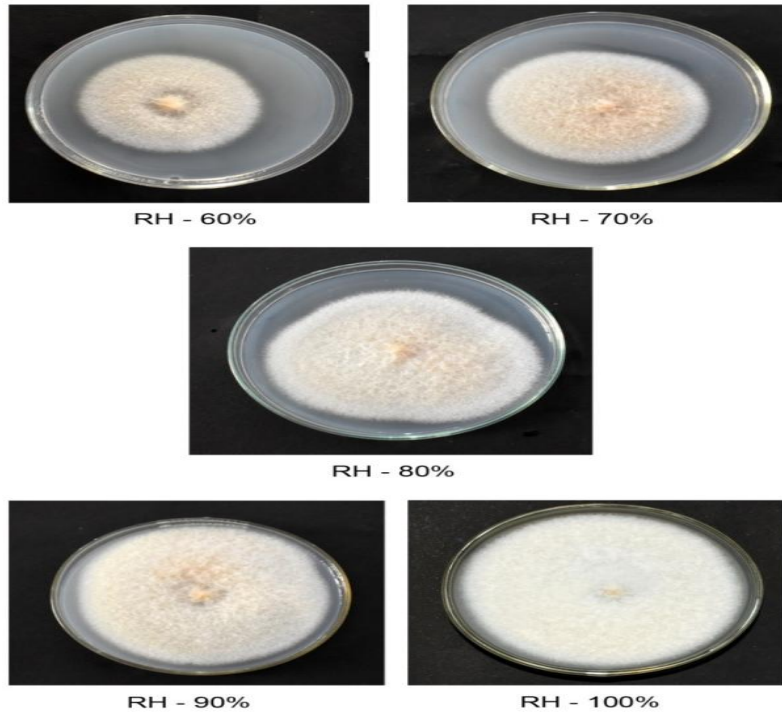
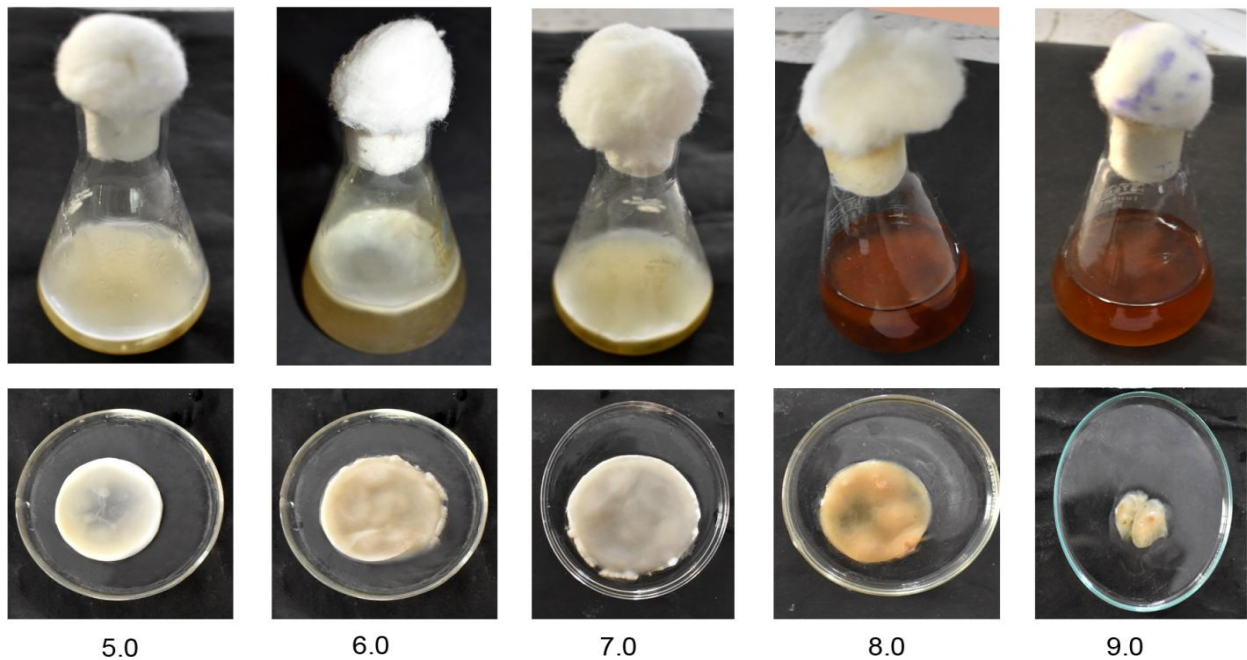


Plate.3 Effect of pH on dry mycelial weight of *Fusarium oxysporum* f.sp. *ciceri* on Potato Dextrose Broth medium



Effect of relative humidity on mycelial growth

To evaluate the effect of atmospheric moisture on mycelial growth and, the fungus was exposed directly to different level of relative humidity *viz.*, 60, 70, 80, 90 and 100 per cent and incubated at $30 \pm 1^\circ\text{C}$ temperature for 7 days. Maximum mycelial growth of fungus was observed 90.00 mm at 100 per cent relative humidity and closely followed 81.00 mm at 90 per cent relative humidity. A significantly decrease in mycelial growth of fungus was observed 73.32 mm at 80 per cent and 64.00 mm at 70 per cent relative humidity. Minimum mycelial growth of fungus was observed 56.66 mm at 60 per cent relative humidity (Table 2, Fig. 2 and Plate-2).

Effect of pH on mycelia growth

To determine the optimum pH level for mycelial growth of *Fusarium oxysporum* f.sp. *ciceri*, five different pH levels ranging from 5.0 to 9.0 were studied on Potato dextrose broth medium at 14th day of incubation. Maximum dry mycelial weight (260 mg) was observed at pH 7.0 and followed by at pH 6.0 (220 mg). Minimum dry mycelial weight (79 mg) was observed at pH 9.0. The dry mycelial weight obtained from Potato Dextrose Broth medium of pH 7.0 and 6.0 were significantly more than any other pH range tested (Table 3, Fig. 3 and Plate-3).

All microorganisms are growing in certain ranges of temperature the fungus grow at all the ranges of temperature from 15 to 35°C , but it differed significantly at all temperature ranges under study at 7th day of incubation. Maximum mycelial growth of the fungus was observed 80.95 mm at 30°C temperature and closely related to the 62.02 mm at 25°C temperature. To evaluate the effect of pH on mycelial growth, the fungus was exposed

directly to different level of pH *viz.*, 5.0, 6.0, 7.0, 8.0 and 9.0 in Potato Dextrose Broth and incubated at $30 \pm 1^\circ\text{C}$ temperature for 7 days. Maximum dry mycelial weight of fungus was observed 260 mg at pH 7.0 and followed by 220 mg at pH 6.0. Similar observations were also made by Khan *et al.*, (2011) who found that among different temperature levels 30°C was the common optimum temperature for growth of *Fusarium oxysporum* f.sp. *ciceri* followed by 25°C and maximum growth of mycelial mat of *Fusarium oxysporum* f. sp. *ciceri* was recorded at pH 7.0 and was significantly superior to other pH levels followed by pH 6.5 and at pH 6.0. To evaluate the effect of different levels of relative humidity *viz.*, 60, 70, 80, 90 and 100 percent and incubated at $30 \pm 1^\circ\text{C}$ temperature for 7 days. Maximum mycelial growth of fungus was observed 90.00 mm at 100 percent relative humidity and followed by 81.00 mm at 90 percent. Our results are in accordance with the findings of Yadav *et al.*, (2014) who observed the effect of relative humidity on growth and sporulation of *Fusarium oxysporum* and found that the most favourable at 100 per cent relative humidity. Pokhar and Thakore (2003) who evaluated that relative humidity from 80-100 per cent favored the incidence of *Fusarium* rot of sponge gourd fruits.

In conclusion the studies on effect of physiological parameters *in vitro* conditions recorded maximum mycelial growth of the fungus was observed at 30°C temperature, 100 per cent relative humidity and pH 7.0.

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How to cite this article:

Pempee, Mahabeer Singh, Satyadev Prajapati and Poonam Kumari. 2020. Effects of Different Temperature, pH and Relative Humidity on the Growth of *Fusarium oxysporum* f. sp. *ciceri* causing Chickpea Wilt. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 1381-1388.
doi: <https://doi.org/10.20546/ijcmas.2020.909.175>