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Physiological Studies of the *Fusarium oxysporum* f. sp. *lycopersici* causing Tomato Fusarium Wilt

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ABSTRACT: Tomato wilt, incited by *F. oxysporum* f. sp. *lycopersici*is an important disease of tomato vegetable and causes economic yield loss for tomato growers. The challenges posed by Fusarium wilt in tomato production are significant, as the disease can lead to reduced yields and pathogen ability to survive in soil and plant debris for extended periods makes management difficult. In present investigation wilt infected tomato roots were collected from Research Farm of RARI, Durgapura and pathogen was isolated, purified and identified with cultural and morphological behavior as per described by Sacc. Synder and Hansen, further confirmed by Indian Type Culture Collection, New Delhi and confirmed as *Fusarium oxysporum* f. sp. *lycopersici.* To better understand the physiological activities of the pathogen, we investigated the effects of temperature, hydrogen ion concentration, and relative humidity on its growth and sporulation. We found that the maximum mycelial growth (88.51mm) and excellent sporulation were obtained at 25ºC temperature, while the maximum mycelial weight (272 mg) and excellent sporulation were recorded at a pH of 6.5. Additionally, we found that the growth of mycelial (89.10mm) with abundant sporulation was recorded at 90 percent relative humidity.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, Tomato, Physiological activities, Growth and Sporulation.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), a member of the Solanaceae family, is widely recognized as one of the most important and widely cultivated vegetable crops globally (de Melo *et al*. 2015; Adedire *et al*., 2023). With its exceptional processing quality, high nutritional value, and widespread usage in households, tomato has earned the title of being the most sought-after vegetable in the world. It's having third rank to most viable largest vegetable crops after potato and onion and having the first position among processed vegetables (Patel *et al*., 2014). Tomato need moderately cool climatic conditions for well development and it is grown throughout the year. Tomato is intensively cultivated in almost all areas of India. In Rajasthan state, the major tomato growing districts are Jaipur, Sirohi, Udaipur, Tonk, Ajmer, Rajsamand, Dausa, Bundi, Jalore, Paliand Sawai Madhopur.

Cultivation of tomato is swayed by numerous diseases including early blight (*Alternaria solani)*, damping-off (*Pythium* sp. And *Rhizoctonia* sp.), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Verticilium wilt (*Verticillium alboatrum*), late blight (*Phytophthora infestans*), storage rot of tomato (*Cladosporium oxysporium*) and anthracnose (*Colletotrichum phomoides*) *etc*. adversely affects the tomato growth and cause economic yield losses (Akhtar *et al.,* 1994). Among them, wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen (1940) is a major and serious threats in production of tomato and caused considerable yield losses up to 45 per cent under favorable climatic conditions (Ramyabharathi *et al*., 2012).

Losses in Uttar Pradesh have been recorded around 25.14 - 47.94 per cent (Enespa and Dwivedi 2014). Tomato wilt is one of most thoughtful destructive diseases affecting its yield in all over the world (Beckman, 1987). Pathogen *F. oxysporum* f. sp. *lycopersici* is soil borne in nature and remains in infested soil for up and around ten years (Mui-Yun, 2003). Fusarium, a member of the kingdom Fungi, is scientifically classified within the phylum Ascomycota, sub-phylum Pezizomycota, class Sordariomycetes, sub-class Hypocromycetidae, order Hypocreales, family Nectriaceae, and finally genus Fusarium (Fry, 2004; Khuat *et al*., 2022). According to recent research, this fungus is capable of producing three distinct types of asexual spores, including macro conidia, micro conidia, and chlamydospores (Agrios, 2005; Thangaraj*et al*., 2022).

MATERIAL AND METHODS

Experiment was conducted in the Division of Plant Pathology, RARI, Durgapura (SKNAU, Jobner) Jaipur, Rajasthan during, 2020.

Collection of wilted samples. Samples of disease were gathered from the Research Farm at Regional Agricultural Research Institute (RARI) in Durgapura. To conduct a thorough examination of fungus physiological activities, plants exhibiting signs of wilt were collected and brought to laboratory.

Isolation of pathogen. To carry out a precise experiment, necessary glassware was cleaned and sterilized, and all steps were performed in aseptic conditions. Diseased tomato roots were first washed, cut into small pieces, and treated with a 0.1% mercuric chloride solution before being thoroughly rinsed with distilled water. The pieces were then dried using sterilized blotting paper and placed in Petri plates filled with 2% PDA. The pathogen was purified by transferring the colony's tip mycelium to a sterilized PDA-filled Petri plate. The purified culture was characterized based on morphological and cultural characteristics using modern methods, and results were confirmed by Indian Type Cu1ture Collection as *F. oxysporum* f. sp. *lycopersici*. To study growth and sporulation of the fungus under various physiological conditions, radial growth was measured for regular colonies by using a linear scale in two perpendicular directions, and for irregular colonies, widest and narrowest diameters were recorded and an average was taken. All radial gr0wth measurements were taken after 168 hours of incubation. Frequency of conidial sporu1ation was evaluated by counting the number of conidia per microscopic field, using a stage micrometer and ca1ibrated ocular micrometer at 40X magnification. Slides of the purified culture were prepared using lactophenol from a 7-day-old culture to examine morpho1ogical characters. The number of conidia per microscopic field was recorded, and observations were made regarding radial growth and sporulation.

Effect of different levels of temperature. It has been extensively documented that temperature plays a crucial role in impacting metabolic processes of pathogens. To study this, 20 ml of potato dextrose agar (PDA) was added to sterilized Petri dishes, which were then inoculated with a 5 mm disc from a 7-day-old PDA culture. The inoculated dishes were incubated at five different temperatures (15, 20, 25, 30, and 35°C) and replicated four times. Observations on mycelial growth and sporulation were made after 7 days of incubation.

Effect of hydrogen ion concentration (pH). Growth of *F. oxysporum* f. sp. *lycopersici* was evaluated with regards to pH levels by adjusting the pH of Potato Dextrose broth with citrate phosphate buffer. The pH levels tested were 6.0, 6.5, 7.0, 7.5, and 8.0, and were confirmed with use of a pH meter. A total of 20 ml of adjusted medium was dispensed into 100 ml conical flasks and sterilized through autoclaving at 1.045 kg/cm^2 for 20 minutes. The inoculations were carried out using a 5 mm disc of mycelia from a 7-day-old culture of *F. oxysporum* f. sp. *lycopersici*.

Stock solutions. A solution of citric acid (0.1 M) was prepared by dissolving 19.21 g of citric acid (mol. wt. 192.1) in one liter of distilled water. Solution B was made by mixing 0.2 M of dibasic sodium hydrogen phosphate (mol. wt. 293.9) in one liter of distilled water, which required dissolving 58.78 g of dibasic sodium hydrogen phosphate. These solutions were used to adjust the pH of the potato dextrose broth for the growth experiment of *F. oxysporum* f. sp. *lycopersici*.

Table 1: Stock solutions for different levels of pH.

Solution A (ml)	Solution B (ml)	pH
73.7	126.3	6.0
54.5	145.5	6.5
35.3	164.7	7.0
12.7	187.3	7.5
5.5	194.5	8.0

The dry weight of mycelium was determined after 14 days of incubation at a temperature of 25±1ºC. To measure the dry weight, 20 ml of Potato Dextrose Broth (PDB) was sterilized in 150 ml conical flasks at 1.045 kg/cm^2 for 20 minutes. The flasks were then inoculated with a 5 mm diameter agar block taken from a 7-day-old culture of *F. oxysporum* f. sp. *lycopersici*. After 7 days of incubation at 25±1ºC, the cultures were filtered through pre-weighed Whatman No. 42 filter paper and dried at 85ºC for 24 hours to obtain the dry weight of the mycelium. The weight of the dry fungal mycelium was calculated by subtracting the weight of the filter paper from the total weight of the filter paper and mycelium (Arey, 2010).

Weight of mycelium = (Weight of filter paper + Weight of mycelium) – (Weight of filter paper)

Effect of different levels of relative humidity (RH). The influence of relative humidity (RH) on the mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici* was evaluated by maintaining five different

RH levels (60%, 70%, 80%, 90%, and 100%) in glass desiccators. The concentrations of concentrated sulfuric acid and sterilized distilled water were adjusted according to the method proposed by Buxton and Mellanby (1934) to maintain the desired levels of RH. The acid solution used had the following composition.

Table 2: Stock solutions for different levels of Relative Humidity.

RH (%)	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	0.00	Only distilled water

* 50 per cent v/v solution of concentrate H_2SO_4

An investigation into the growth of *F. oxysporum* f. sp. *lycopersici* was conducted by using Potato Dextrose Agar (PDA) medium in Petri dishes. The culture was obtained from a 7-day old source, and 5mm discs were aseptically taken from it using a sterilized cork borer. These discs were then placed on the PDA medium in each Petri dish. Inoculated Petri plates were then incubated in a controlled environment at 25±1°C with a relative humidity maintained through a mixture of H_2SO_4 and distilled water in glass desiccators. Observations on mycelial growth were recorded after 7 days of incubation, with 4 replicates performed to ensure accuracy of results.

RESULT AND DISCUSSION

Physical parameters (Temperature, pH and RH) are important factors for regulating reproduction and vegetative growth of any living organism in controlled as well as in natural conditions. Present research was carried out to determine most favorable environmental conditions for growth and reproduction of *F. oxysporum* f. sp. *lycopersici.*

Effect of different temperature levels on mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici.* The mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici* was studied by incubating Petri dishes at different temperatures *viz*., 15, 20, 25, 30 and 35°Cand it was evident from observations presented in Table 3 and Plate 1. *F. oxysporum* f. sp. *lycopersici* grow at all ranges of temperature from 15 to 35°C but all were significantly differing from each other at 7th days of incubation. Maximum growth of mycelial (88.51mm) with excellent sporulation of tested fungus was obtained at 25ºC strongly followed by 30ºC temperature (79.74mm). Minimum mycelial growth (58.89 mm) with poor sporulation was recorded at 15°C. There was a significant difference among different temperatures *i.e.*, 15°C (58.89 mm), 20°C (69.56 mm) followed by 35°C (67.44 mm). Observed results indicated that a minor increase or decrease in temperature from 25ºC, growth and sporulation of fungus undesirably affected.

Table 3: Effect of different levels of temperature on growth of mycelial and sporulation of *F. oxysporum* f. sp. *lycopersici* at 7th day after incubation.

Sr. No.	Temp. (°C)	Growth of Mycelium (mm)*	Sporulation **
1.	15	58.89	+
2.	20	69.56	++
3.	25	88.51	++++
4.	30	79.74	+++
5.	35	67.44	++
SEm±	-	0.56	
CD (5%)	-	1.69	

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

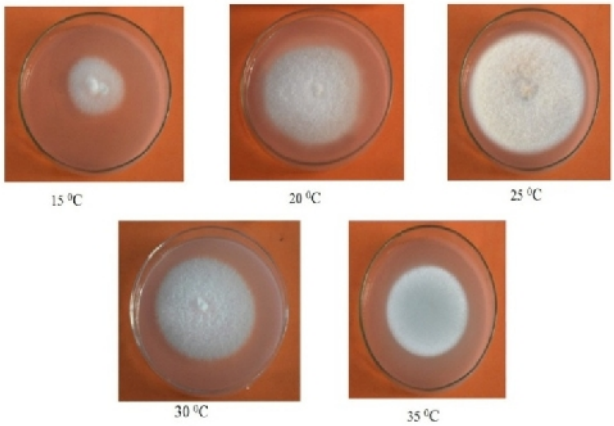

Plate 1. Mycelial growth of *F. oxysporum* f. sp. *lycopersici* at different levels of temperature.

Effect of different levels of pH on sporulation and weight of MYCELIA. The hydrogen ion concentration has an impact on the regulation of physiological activities of pathogens, as demonstrated by the growth

rate of the *F. oxysporum* f. sp. *lycopersici* pathogen at different pH levels (6.0, 6.5, 7.0, 7.5, and 8.0). The dry mycelial weight of tested fungus was found to be significantly present at all five different pH levels in broth medium, but maximum weight (272 mg) and highest sporulation were seen at pH 6.5 after 7 days of incubation, closely followed by pH 7.0 (221.00 mg) and pH 6.0 (200.75 mg), which were considered to be most favorable for maximizing the dry mycelial weight. On other hand, the minimum weight was recorded at pH 8.0 (130.25 mg) and pH 7.5 (168.75), both of which were significantly lower than other tested pH ranges. As pH level increased from 7.0, growth and dry mycelial weight of fungus decreased. Maximum sporulation was also seen at pH 6.5 and 7.0, while lowest sporulation was observed at pH 8.0.

Table 4: Effect of pH on weight of mycelial and sporulation of *F. oxysporum* f. sp. *lycopersici* on PDA broth medium.

Sr. No.	pH	Dry mycelial weight (mg)*	Sporulation **
1.	6.0	200.75	+++
2.	6.5	272.00	++++
3.	7.0	221.00	+++
4.	7.5	168.75	++
5.	8.0	130.25	+
SEm+	-	1.72	
CD (5%)	-	5.24	

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

Effect of different levels of RH on mycelial growth and sporulation. At different levels of relative humidity, the fungus directly showed significant difference. It was observed that all the five humidity levels (60, 70, 80, 90 and 100 %) induced the growth of *F. oxysporum* f. sp. *lycopercisi*. Significantly best mycelial growth (89.10 mm) with abundant sporulation was recorded at 90 per cent RH followed by mycelial growth (86.14 mm) at 100 % RH level. A significant decrease in mycelium growth was observed at 80 and 70 per cent humidity level (83.09 and 75.28, respectively). Minimum mycelium growth (70.55 mm) was observed at 60 per cent relative humidity level. At 90 and 100 % levels of humidity excellent sporulation was recorded. Observations were revealed that 90-100 % humidity levels favored maximum growth and excellent sporulation of *F. oxysporum* f. sp. *lycopersici*.

Table 5: Effect of RH on growth of mycelial and sporulation of *F. oxysporum* f. sp. *lycopersici* at 7th day of incubation.

Sr. No.	RH (%)	Growth of mycelial (mm)*	Sporulation **
1.	60	70.55	+
2.	70	75.28	++
3.	80	83.09	+++
4.	90	89.10	++++
5.	100	86.14	++++
SEm+	-	0.90	
CD (5%)	-	2.75	

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

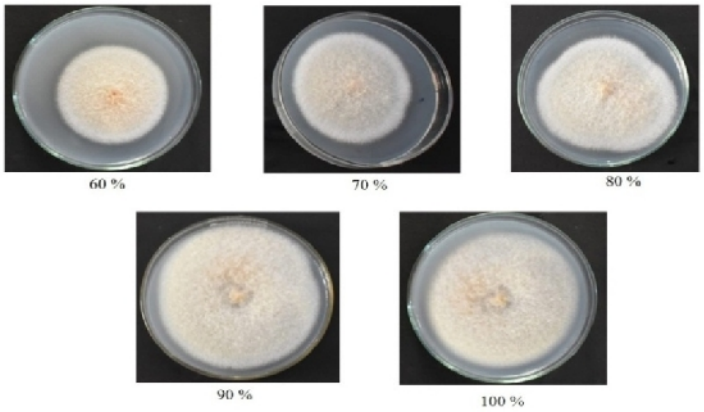

Plate 2. Growth of *F. oxysporum* f. sp. *lycopersici* at different levels of relative humidity (RH).

Present research was carried out to determine the most favorable environmental conditions for growth and reproduction of the *F. oxysporum* f. sp. *lycopersici*. The pathogen grows at different ranges of temperature at 15

to 35°C. From the present investigations results revealed that all temperature ranges were significantly differed to each other. Maximum mycelial growth and sporulation was recorded at 25ºC to 30ºC and minimum growth was recorded at 15 ºC at 7th days of incubation. A study by Fayzalla *et al*. (2008) found that the ideal temperature range for the mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici* was between 25 to 30°C. This was supported by research of Imran Khan *et al.* (2011); Pempee *et al*. (2020), who found that fungus experienced maximum colony growth and sporulation at 30°C, resulting in a mycelial growth of 80.95 mm and 90.00 mm at 100% relative humidity, respectively. At pH 7.00, the fungus showed maximum dry mycelial weight of 260 mg with abundant sporulation. Additionally, Scott *et al*. (2010) reported that highest growth of *F. oxysporum* f. sp. *lactucae* was observed at a temperature near 25°C.

Luxuriant mycelial sporulation and growth of *F. oxysporum* f. sp. *lycopersici* occurred on pH 6.5. The obtained results are similar to studies by earlier workers and in conformity with results stated by Swati and Rajan, (2014). They reported that highest sporulation of *Fusarium oxysporum* was detected at pH 6.5 (5.06 spore/100 ml macro conidia & 122.4 spore/100 ml micro conidia) and minimum macro and micro conidia sporulation was detected at pH 2.0 (0.47 and 2.42 macro and micro conidial spore/100 ml, respectively). With pH of (5.5 and 6.5) that is slightly acidic medium was most favorable for vegetative growth of fungus (Fayzalla *et al.,* 2008; Patil *et al.,* 2015; Pempee *et al.*, 2020). However, it is known that temperature affects various physiological and biochemical processes in fungi, including respiration, enzyme activity, and membrane fluidity (Saeed *et al*., 2022; Saied *et al*., 2021).

Excellent sporulation with luxuriant radial growth of the fungus was recorded at 90 to 100 % RH among different levels of RH *viz.*, 60, 70, 80, 90 and 100 per cent. Maximum radial growth with abounded sporulation was observed at 90 per cent (89.10 mm) and closely followed at 100 per cent (86.14 mm) RH at 7th days of incubation at 25°C. These results were supported with observation of Benaouali *et al*. (2014) reported that different isolates of *F. oxysporum* f. sp *radicis lycopersici* best grown at 23°C and 28°C temperature with74 to 80% RH. One recent study that supports the idea of optimal humidity levels for fungal growth is by Li *et al*. (2021), who investigated the effect of different humidity levels on the growth of the fungus *Fusarium oxysporum* f. sp. *cubense*, which causes banana wilt disease. The authors found that the highest radial growth and sporulation occurred at a humidity level of 90%, with decreased growth and sporulation observed at both lower and higher humidity levels.

Similarly, in a study by Wang *et al*. (2021) on the fungus *Verticillium dahliae*, which causes vascular wilt disease in a wide range of crops, optimal growth was observed at a humidity level of 85%. The authors noted that higher humidity levels (90-95%) led to increased growth in the early stages of fungal development but ultimately resulted in decreased growth and sporulation. Another study by Sujayasree *et al*. (2022) investigated the effect of temperature and humidity on the growth of the fungus *Botrytis cinerea*, which causes gray mold disease in various crops. The authors found that the highest radial growth occurred at a temperature of 25°C and a humidity level of 95%, with decreased growth observed at both lower and higher humidity levels.

SUMMARY AND CONCLUSION

This study examined sporulation and growth of *F. oxysporum* f. sp. *lycopersici* under different temperature, pH, and relative humidity conditions. Results showed that the fungus was able to grow at a temperature range of 15-35°C, with maximum growth and sporulation observed at 25-30°C. The fungus showed maximum dry mycelial weight at pH 7.0 and maximum sporulation at pH 6.5. The highest growth and abundant sporulation were seen at 90-100% relative humidity. The results were in agreement with previous studies that also found that the ideal conditions for growth and reproduction of *F. oxysporum* f. sp. *lycopersici* were between 25-30°C and slightly acidic pH levels, with high relative humidity. Overall, this study sheds light on the physiological behavior of *Fusarium oxysporum* f. sp. *lycopersici*, providing insights that may be useful in developing more effective control measures.

FUTURE SCOPE

After know out favorable physiological factors of FOL, Authors are suggesting controlling the pathogen with avoiding the favorable condition of growth and multiplication of pathogen. Authors may suggest to altering the pathogen behavior in natural filed condition for further study. However, further research is needed to fully understand the complex mechanisms underlying this devastating disease and develop strategies to mitigate its impact on tomato production.

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Conflict of Interest. None.

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