

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.909.184>

Studies on Effect of Temperature and pH on *Fusarium oxysporum* f.sp. *vasinfectum* causing Okra Wilt

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ABSTRACT

The present study was undertaken to find out the different temperature levels and pH range suitable for growth of twenty five isolates of *Fusarium oxysporum* f. sp. *vasinfectum* collected and isolated from different locations. The temperature of 30°C and 25°C was found best for the growth of all the isolates. At these temperatures, there was significant difference between the isolates in their growth, whereas temperature below 25°C and above 30°C reduced the growth of isolate drastically and least growth was recorded both in solid and liquid media at 15°C. Isolates like MYS-15 and MYS-16 showed mean maximum growth with colony diameter of 58.90 mm and 54.81mm, respectively and found significantly superior over other isolates. Based on the growth on liquid medium with different temperature levels, isolates were categorized in to five groups. Among them none of the isolate fell under group I and group V. Eleven isolates fell under group II. Twelve isolates fell under group III (medium growers) Whereas, remaining two isolates fell under group IV (fast growers) with mean dry mycelial weight ranged from 450.1 mg to 600 mg. Hydrogen ion concentration (pH) 7.0 and 6.0 were found better for all the *F. oxysporum* f. sp. *vasinfectum* isolates with mean maximum growth of 64.37 mm and 57.43 mm colony diameter on solid media and 552.86 and 503.24 mg dry mycelial weight on liquid media respectively.

Keywords

Fusarium oxysporum f. sp. *vasinfectum*, temperature, pH Culture media, Okra, Colony growth

Article Info

Accepted:

12 August 2020

Available Online:

10 September 2020

Introduction

Okra (*Abelmoschus esculentus* L. (Moench)), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. Okra originated from African sub-continent. This crop is suitable for cultivation as a garden crop as well as commercial crop. Okra acts as an important source of vitamins, calcium, potassium and other mineral often lacking in the diet of developing countries

(Anon. 1990). India ranks first in the world with 6350 thousand tonnes of okra produced from over 531 thousand hectare land with a productivity of 11.95 t/ha. In India, okra occupies 5.8 per cent and 3.9 per cent share of major vegetable area and production respectively (Anon, 2013).

Temperature influences the rate of growth, metabolism and morphological characters of fungi. The fungi can thrive under wide range

of temperature and there is minimum, optimum and maximum for each of the species and even for the strain. Clayton (1923) showed the behavior of *F. oxysporum* f. sp. *lycopersici* at different range of temperature and recorded the minimum growth between 9-10°C where the maximum growth was recorded at 37°C and optimum growth at 28°C. Moore (1924) studied the effect of temperature on growth of two strains of *F. coeruleum* and observed that maximum growth at a temperature of 30°C.

In the present study effect of different temperature and pH on different *F. oxysporum* f. sp. *lycopersici* isolates was carried out.

Materials and Methods

The diseased sample showing the wilt symptoms viz., drooping of leaves, loss of turgidity followed by yellowing and peeling of epidermis, softening of roots, watery and browning of vascular bundle collected from various parts of southern Karnataka were used for isolation of pathogen. The standard tissue isolation method was employed under aseptic conditions for isolation and was sub-cultured at regular intervals during the course of investigation to maintain the virulence of the pathogen.

Effect of different temperature

Solid medium

Each isolate was grown on PDA medium. The 25 isolates were also subjected to viz., 10, 15, 20, 25, 30, 35 and 40°C. 15 ml of medium was poured in to each petriplate and allowed for solidification. The pathogen grown on PDA for seven days was cut into 5 mm discs with the help of sterilized cork borer and picked up with the help of sterilized loop and placed on the surface of the medium. Each

treatment was replicated thrice and incubated at respective temperatures for 7 days. The colony diameter was recorded by measuring the radial growth of the mycelium in mm. The difference in rate of growth of the pathogen in different temperatures was recorded and analyzed statistically. Based on the temperature required for the growth *F. oxysporum* f. sp. *vasinfectum*, isolates were categorized into following five groups viz., Very slow (<30 mm), Slow (30.1-45 mm), Medium (45.1-60 mm), Fast (60.1-75 mm) and Very fast (>75mm).

Liquid medium

The procedure was same as solid medium except use of broth. Based on the temperature required for the growth isolates were classified into following five groups viz., Very slow(<150 mg), Slow(150.1-300 mg), Medium(300.1-450 mg), Fast(450.1-600 mg) and Very fast>600.1 mg).

Effect of different pH levels

Solid medium

F. oxysporum f. sp. *vasinfectum* isolates were grown on PDA medium with different pH levels viz., 5.0, 6.0, 7.0, and 8.0. The pH levels were adjusted by adding 1N alkali (NaOH) or acid (HCl). 15 ml of medium was poured in to each petriplate and allowed for solidification. The pathogen grown on PDA for seven days was cut into 5 mm discs with the help of sterilized cork borer and picked up with the help of sterilized loop and placed on the surface of the medium. Each treatment was replicated thrice and incubated at a temperature of $28 \pm 1^\circ\text{C}$ for 7 days. The colony diameter was recorded by measuring the radial growth of the mycelium in mm. The difference in rate of growth of the pathogen in different pH were recorded and analyzed statistically.

Based on the pH required for the growth isolates, were classified into following five groups *viz.*, Very slow (<30 mm), Slow (30.1-45 mm), Medium (45.1-60 mm), Fast (60.1-75 mm) and Very fast (>75.1mm).

Liquid medium

The procedure was same as solid medium except use of broth. Based on the pH requirement isolates were classified into following five groups *viz.*, Very slow (<150 mg), Slow(150.1-300 mg),Medium(300.1-450 mg), Fast(450.1-600 mg) and Very fast>600.1 mg).Statistical analysis was carried out as per the procedures given by Gomez and Gomez (1984).

Results and Discussion

Effect of temperature on the growth on PDA solid medium

Temperature influences the growth rate, metabolism and morphological characters of the fungi. Variations in growth among the isolates at different temperatures regimes were found significant. MYS-15 and MYS-16 showed mean maximum growth with colony diameter of 58.90 mm and 54.81 mm, respectively and found significantly superior over other isolates. Two isolates *viz.*, MYS-15 AND MYS-16 recorded significantly higher growth at 30°C (90.00 mm) followed by RMNR-10 and CRNR-19 (75.60 mm and 74.12 mm, respectively). However, least growth (40.43 mm) was noticed in MDY-12 isolate. All the isolates differed significantly in their relative growth indicating that each isolate had an ability to withstand specific temperature.

Based on the growth on solid medium with different temperature levels, isolates were categorized into five groups (Table 1). Among them none of the isolate fell under

group I, group IV and Group V. Whereas, 15 isolates fell under group II showed slow growth with mean colony diameter ranged from 30.1 to 45 mm. Ten isolates were fell under group III (medium growers) with mean colony diameter of more than 60 mm.

Effect of temperature on the growth on PDA liquid medium

The variations in growth among the isolates at different temperatures regimes were found significant MYS-15 and MYS-16 showed mean maximum dry mycelial weight of 474.20 mg and 465.53 mg respectively at all temperatures evaluated and found significantly superior over other isolates. Temperature of 25°C supported the maximum growth (648.67 mg) in MYS-15, followed by MYS-16 with mean dry mycelial growth of 639.33 mg, whereas least growth (325.33 mg) was noticed in TMK-6.

Based on the growth on liquid medium with different temperature levels, all the isolates were categorized in to five groups (Table 2). Among them none of the isolate fell under group I and group V. Eleven isolates fell under group II where as Twelve isolates fell under group III with mean dry mycelial weight ranged from 300.1 mg to 450 mg. Remaining two isolates fell under group IV (fast growers) with mean dry mycelial weight ranged from 450.1 mg to 600 mg.

Effect of pH on the growth on PDA solid medium

The different levels of pH *viz.*, 5.0, 6.0, 7.0 and 8.0 were studied on PDA agar medium and presented in Table 18. Differences due to isolate in respect of pH levels found to be significant.

The isolate MYS-15 and MYS-16 produced mean maximum growth of 75.83 mm and

73.76 mm colony diameter. However, isolates TMK-7 recorded poor mean colony growth (33.00 mm) in comparison to the rest of the isolates. pH 7.0 and pH 6.0 were found better for all the isolates with mean maximum growth of 64.37 mm and 57.43 mm colony diameter, respectively. It could be clearly seen that there was a wide variation in growth in respect to pH indicating the variability among the isolates (Fig. 1).

Based on the growth on solid medium with different pH levels, none of the isolate fell under group-I (very slow growers). Two isolates fell under group-II (slow growers) and Eighteen isolates fell under group-III. Three isolates viz., BNGU-2, RMNR-10, CHD-22 were fell under group-IV (Fast growers) with mean colony diameter of more than 60.1 mm to 75 mm. remaining two isolates were fell under group-V (very fast growers).

Table.1 Classification of *F. oxysporum* f. sp. *vasinfectum* isolates based on effect of different temperatures in the colony growth on PDA solid medium

Group	Growth Rate	Growth of isolates (mm)	No. of isolates	Name of the isolates
I	Very slow	<30	0	-
II	Slow	30.1-45	15	BNGU-1, BNGU-2, BNGR-3, TMK-4, TMK-5, TMK-6, TMK-7, RMNR-8, MDY-11, MDY-12, MDY-13, HSN-20, HSN-21, CHD-22 and CBP-24
III	Medium	45.1-60	10	RMNR-9, RMNR-10, MDY-14, MYS-15, MYS-16, MYS-17, CRNR-18, CRNR-19, CHD-23 and CBP-25
IV	Fast	60.1-75	0	-
V	Very fast	>75	0	-

Table.2 Classification of *F. oxysporum* f. sp. *vasinfectum* isolates based on effect of different temperatures on mycelial weight on PDA liquid medium

Groups	Growth rate	Growth of isolates (mg)	No. of isolates	Name of the isolates
I	Very slow	<150	0	-
II	Slow	150.1-300	11	TMK-6, TMK-7, RMNR-8, RMNR-9, RMNR-10, MDY-11, MDY-12, MDY-13, MDY-14, CRNR-18 and CRNR-19
III	Medium	300.1-450	12	BNGU-1, BNGU-2, BNGR-3, TMK-4, TMK-5, MYS-17, HSN-20, HSN-21, CHD-22, CHD-23, CBP-24 and CBP-25
IV	Fast	450.1-600	02	MYS-15 and MYS-16
V	Very fast	>600.1	0	-

Fig 1: Influence of pH levels on the growth of *F. o. f. sp. vasinfectum* isolates on PDA solid medium

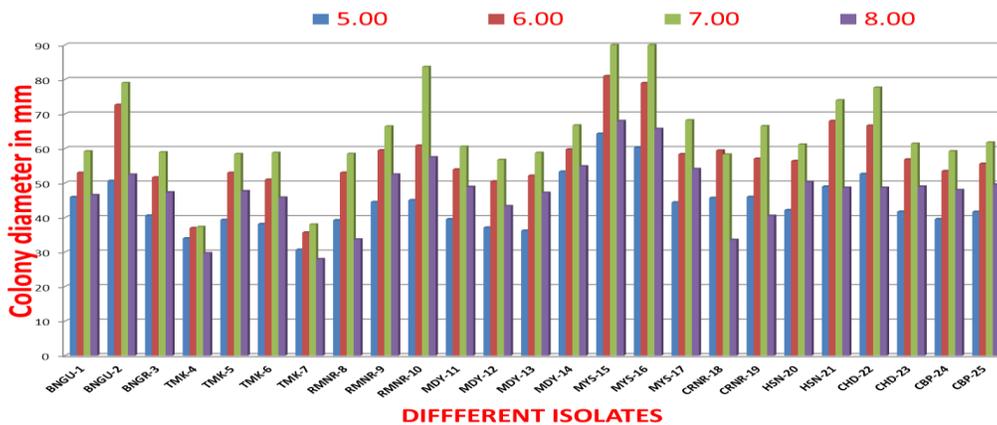
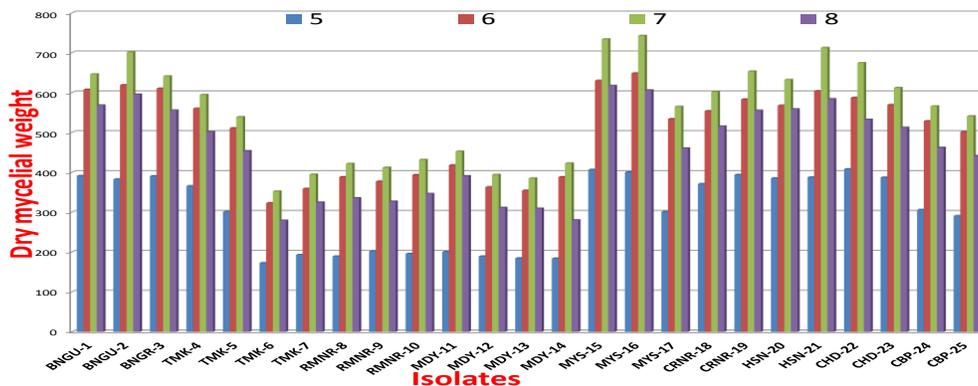


Fig 2: Effect of pH levels on the growth of *F. o. f. sp. vasinfectum* isolates on PDA liquid medium



pH studies on PDA liquid medium

The variation in growth among the isolates at different pH levels was found significant. Isolates MYS-15 and MYS-16 showed mean maximum dry mycelial weight of 597.00 mg and 599.42 mg respectively. All the isolates differed significantly in their relative dry mycelial growth indicating that each isolate had an ability to withstand specific pH (Fig. 2).

Based on the growth on liquid medium with different pH levels, isolates were categorized into five groups. Among them none of the isolate fell under group-I (very slow growers). Isolate TMK-5 fell under group-II and Eight

isolates fell under group-III (medium growers) Sixteen isolates fell under group-IV (fast growers) with mean dry mycelial weight ranged from 450.1 mg to 600 mg. Whereas, none of the isolates fell under group-V (very fast growers).

The fungi have minimum temperature below which they cannot grow and above which they are inactivated. Temperature is one of the important factors governing distribution, growth, reproduction and survival of the fungus. It also plays an important role in infection and disease development. An effort was made to know the optimum temperature for the growth. The temperature of 30°C and 25°C was found best for the growth of the *F.*

oxysporum f. sp. *vasinfectum* isolates. At these temperatures there was significant difference between the isolates in their growth, whereas temperature below 25°C and above 30°C reduced the growth of isolate drastically and least growth was recorded both in solid and liquid media at 15°C. Isolates such as MYS-15 and MYS-16 showed mean maximum growth with colony diameter of 58.90 mm and 54.81mm, respectively and found significantly superior over other isolates. The results of the present study indicated that the temperature of 30°C favoured maximum growth of the isolate both in solid (70.51 mm) and liquid (530.71 mg) medium used. The present study established that temperature of 28°C supported maximum growth which could be suggested as optimum temperature for growth of *F. oxysporum* f. sp. *vasinfectum*. This optimum temperature can be used in future for laboratory studies. The results of the study are in conformity with reports of Ajid *et al.*, (2005) and Khilare and Rafi Ahmed (2012), they also observed that *Fusarium* showed vigorous growth at 30° C while growth was less vigorous at 15°C.

Ragazzi (1992) also collected 5 isolates of *F. oxysporum* f. sp. *vasinfectum* from different Provinces in Angola and tested for their growth at different temperature levels. He found that the optimum temperature for mycelial growth and sporulation was 25°C and optimum pH was 7.0 for growth and sporulation of *Fusarium* isolates.

Similarly, Chi and Hassan (1964) reported that *F. solani* isolates grew well at higher temperature of 28°C. The fungus grew at the temperature range of 15– 30°C. However, growth of the fungus was drastically reduced below 15°C and started to decline above 30°C and become zero at 45°C, as these temperatures did not favour for growth of the fungus. Results of present findings are also in confirmation with Sataraddi (1998) and Sharma *et al.*, (2005),

The metabolic event like transformation of a substrate into product is carried out with the help of biological catalyst enzymes. Various fungal enzymes have been seen to be active at a particular pH range. The pH of the medium has a profound effect on the rate and amount of growth and other life process of the fungus

Based on the observations of present study on hydrogen ion concentration (pH), pH 7.0 and pH 6.0 were found better for all the *F. oxysporum* f. sp. *vasinfectum* isolates with mean maximum growth of 64.37 mm and 57.43 mm colony diameter on solid media and 552.86 and 503.24 mg dry mycelial weight on liquid media respectively. The hydrogen ion concentration of the medium and growth of the isolate are interdependent. Every organism requires optimum pH for its better growth and sporulation. Growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. The pH of the medium has profound effect on the rate and amount of growth and many other life process of the fungus. The results of the present study revealed that pH between 6.0-7.0 were found to be optimum for the growth of pathogen. The same results were also shown by Khilare and Rafi Ahmed (2012) and they recorded maximum growth at a pH 6.5 and 7.00 on PDA. The present results are also confirmed with other findings of earlier workers. Sataraddi (1998), Biswas *et al.*, (2003) and Mahesh (2008).

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How to cite this article:

Hanumanthe Gowda, B., P. R. Ramesh and Prashanth, J. M. 2020. Studies on Effect of Temperature and ph on *Fusarium oxysporum* f.sp. *vasinfectum* causing Okra Wilt. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 1448-1454. doi: <https://doi.org/10.20546/ijcmas.2020.909.184>