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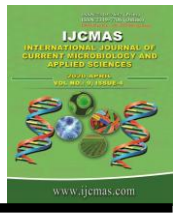

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Effect of Media, Temperature and pH on Growth and Sporulation of *Fusarium oxysporum* F. sp. *udum* under *in vitro* Condition

R. Poorvasandhya*, Bireswar Sinha, Ph. Sobita Devi

Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

**Corresponding author*

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A B S T R A C T

Present research was carried out to study the effect of different culture media, temperature and pH on mycelia growth, sporulation, fresh weight and dry mycelial weight of *Fusarium oxysporum* f. sp. *udum*. Among seven culture media that were tested, the fungus grew the best on PDA and Czapek's Dox agar media. The most suitable pH level for growth of fungus was 6.0 and 7.0 with excellent sporulation. Growth of *Fusarium oxysporum* f. sp. *udum* was maximum at 30˚C after seven days of inoculation, which was reduced drastically below 20˚C and above 30˚C.

Introduction

Pigeon pea (*Cajanus cajan*) is a pre-eminent pulse crop that provides vegetable protein of high quality to human beings and is also feed for animals and used as fire wood. In India, pigeon pea remained at 2nd position in total pulse production with 4.25 Mt of production in an area of 4.43 M ha. In Manipur, red gram is cultivated in the sloppy areas especially in the Sadar hills of Senapati District. Due to water logging conditions, red gram is not grown in the valleys except in some isolated and elevated areas. The productivity of pigeon pea in India is influenced by various biotic and abiotic stresses. It is known to be affected by more than hundreds of pathogens (Nene *et al*., 1989). However, only a few of them causes economic losses.

The diseases of considerable economic importance at present are sterility mosaic, *Fusarium* wilt, *Phytophthora* blight, *Macrophomina* root rot, stem canker and *Alternaria* blight. *Fusarium* wilt is the economically important disease that is most

widely distributed. The causal organism of red gram wilt is *Fusarium udum*. *Fusarium* is a soil-borne facultative parasite that enters the host through fine roots and thereby, colonizes the different parts of the plant (Khune, 1990).

Environmental factors such as temperature, water activity and pH have a great influence on fungal development (Yadav *et al*., 2014). Present work depicts the role of different pH, temperature and media to understand the ecological survival of pathogen which will be helpful in management strategy in the field.

Materials and Methods

Survey and collection of pigeon pea plants showing wilt symptoms is done and isolations are carried out at Department of Plant Pathology, College of Agriculture, CAU, Imphal. Small pieces of discolored vascular tissues from the roots of the infected plants were placed on potato dextrose agar (PDA) media and incubated at 28 ± 1°C in the dark for five days.

Morphologically, the isolate was identified as *Fusarium udum* (Leslie and Summerell, 2006). All the plates were kept at 28 ± 2˚C for 4 days and from these plates pure cultures of *Fusarium udum* were isolated. The pathogen was then sub cultured for the study of the following physiological aspects conducted in the laboratory.

Effect of different culture media

The effect of different culture media *viz.*, Potato Dextrose Agar, Czapek's Dox, Elliot's, Oat Meal, Asthana & Hawker, Asbhy's and Sabouraud's medium on the mycelial growth (radial), sporulation, dry weight and fresh weight of *Fusarium* isolate was studied. The fungus *Fusarium udum* was inoculated with single bit (5 mm) in different culture media and incubated at 28 ±10˚C and the readings were taken after 8 days of inoculation. Sporulation was calculated with the help of hemocytometer.

Effect of different pH

To estimate the growth of the pathogen at various pH levels, *viz*., 4, 5, 6, 7, 8, 9, 10, 11, 12, PDA was taken as the basal medium and the pH was adjusted by adding appropriate amount of HCl and NaOH. For each pH value, there were three replications. The plates and flasks containing sterilized medium was inoculated with 5 mm mycelium disc and incubated at 28 ±10˚C. The linear growth, fresh weight, dry weight and the range of sporulation test ranges on various pH was recorded after 8 days. Sporulation was calculated with the help of haemocytometer.

Effect of different temperatures

The various temperatures were arranged in BOD incubator; the fungus *Fusarium udum* was inoculated with single bit (5 mm) in PDA media and incubated at different temperatures 15˚, 20˚, 25˚, 30˚, 35˚ and 40˚C. In each case, four replications were maintained. The radial growth and sporulation was recorded after 7 days. Sporulation was calculated with the help of haemocytometer.

Results and Discussion

Effect of media

For studying the growth of *Fusarium udum*, seven media were used. The results are given in Table 1. Maximum radial growth (8.77 cm) was recorded on PDA. The next best medium was Czapek's agar medium which yielded (7.80 cm) mycelial radial growth followed by Sabouraud's agar medium (7.53 cm). Least radial growth of the mycelium (6.33 cm) of the test fungus was observed in Asbhy's agar medium (Fig. 1).

The test fungus got excellent sporulation in PDA. Maximum fresh weight (5.98 gm) and dry weight (1.40 gm) of *Fusarium udum* was recorded on Potato dextrose broth medium followed by Czapek's Dox medium, which yielded fresh weight (5.17 gm) and dry weight (0.93 gm).

From these results it was found that PDA medium supported the best mycelial growth of the pathogen and gave excellent sporulation followed by Czapek's (dox) agar medium. These findings were more or less similar to the findings of Chakravarty and Gupta (1995), Ingole (1995) and also with Reddy (2002) who reported that PDA supported the best vegetative growth of *F. oxysporum udum*.

However, these findings are also in confirmation with Gangadhara *et al*., (2010) and Jamaria, (1972) who reported that *F. oxysporum* f. sp. *vanillae* isolates showed best growth on Potato dextrose agar media. Khan *et al*., (2011) studied effect of media on *F. oxysporum* f. sp. *ciceri* and found that PDA was the best media for the growth of isolates.

Recently, Singh *et al*., (2016) reported that PDA and Czapek's Dox agar media were the best medium for radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. The present study also indicated that Potato dextrose agar and Czapek's Dox agar media are the best medium for growth of *F. udum*.

Effect of pH

Growth and sporulation of the pathogen *Fusarium oxysporum* f. sp. *udum* at various pH levels showed that it was maximum at pH 6.0 (7.80 cm) after 168 hrs. of inoculation. pH 7.0 (7.57 cm) and pH 8.0 (7.50 cm) were also found favorable (Table 2). Maximum fresh weight (5.56 gm) and dry weight (0.43 gm) of *Fusarium udum* was recorded on pH 6.0 level. Growth of the pathogen is decreased by decreasing or increasing the pH level from the 6.0 level. The foremost acidic and alkaline pH is not suitable for the growth and sporulation of pathogen. These results are found to be similar with Khilare and Ahmed, (2012) and Tyagi and Paudel (2014) who reported that pH level 6.0 is the optimum pH for the growth as well as sporulation of the fungus.

Further, increase in the pH level showed retarding effect on growth and sporulation. Recently, Singh *et al*., (2016) reported that maximum growth of the *Fusarium oxysporum* f. sp. *lentis* is at pH 6.0 (Fig. 2).

Effect of temperature

Among the six different temperatures tested *viz*., 15°, 20°, 25°, 30°, 35° and 40°C, it was seen that there was a quite large variation in the growth of the pathogen after 7 days. The result is presented in Table 3. The maximum mycelial growth was recorded at 30ºC (8.38 cm) followed by 25ºC (6.43 cm) and 20ºC (5.05 cm), while no mycelial growth was recorded at 40ºC. Temperatures from 20º to 30ºC found to be the most favorable for the growth of target pathogen.

The highest growth of pathogen was recorded at 30ºC with higher sporulation. These present findings are in accordance with Reddy (2002) who reported that the growth of 40 isolates of *F. oxysporum udum* differed in their temperature requirement which varied from 20ºC to 30ºC.

Khilare and Ahmed (2012) reported that the most suitable temperature for the growth of *Fusarium oxysporum* f. sp. *ciceri* was 30ºC after seven days of inoculation, which was reduced drastically below 15ºC and above 35ºC (Fig. 3).

Table.1 Effect of different media on growth and sporulation of *F. oxysporum* f. sp. *Udum*

Sl. No.	Media	Radial growth (cm)	Sporulation	Fresh weight (g)	Dry weight (g)
1	Potato Dextrose Media	8.77	++++	5.98	1.40
2	Czapek's Dox Media	7.80	+++	5.17	0.93
3	Elliot's Media	6.40	+	4.27	0.86
4	Asthana & Hawker Media	6.90	-	4.31	0.89
5	Asbhy's Media	6.33	-	3.58	0.81
6	Oat Meal Media	6.87	++	3.95	0.85
7	Sabouraud's	7.53	+++	3.35	0.66
SE(d)±		0.19		0.43	0.12
$CD_{(0.05)}$		0.40		0.93	0.26

*Mean of three replications
++++ Excellent sporulation: More than 100 spores; +++ Good sporulation: 41-80 spores;
++ Fair sporulation: 21-40 spores; + Poor sporulation: less than 20 spores; - No sporulation

Table.2 Effect of different pH on growth and sporulation of *F. oxysporum* f. sp. *Udum*

Sl. No.	pH Level	Radial growth (cm)	Sporulation	Fresh weight (g)	Dry weight (g)
1	4	4.60	-	3.26 (1.94)	0.26 (0.87)
2	5	7.40	++	3.53 (2.01)	0.34 (0.92)
3	6	7.80	++++	5.56 (2.46)	0.43 (0.96)
4	7	7.57	+++	4.80 (2.30)	0.35 (0.92)
5	8	7.50	+++	4.49 (2.23)	0.34 (0.92)
6	9	7.33	+	3.45 (1.99)	0.31 (0.90)
7	10	7.33	-	3.18 (1.92)	0.24 (0.86)
8	11	5.97	-	2.63 (1.77)	0.22 (0.85)
9	12	5.37	-	2.17 (1.63)	0.19 (0.83)
SE(d)±		0.17		0.07	0.02
$CD_{(0.05)}$		0.35		0.15	0.04

*Mean of three replications
Figures in parenthesis are square root transformed values
++++ Excellent sporulation: More than 100 spores; +++ Good sporulation: 41-80 spores;
++ Fair sporulation: 21-40 spores; + Poor sporulation: less than 20 spores; - No sporulation

Table.3 Effect of different temperature on radial growth and sporulation of *F. oxysporum* f. sp. *Udum*

Sl. No.	Temperature	Radial growth (cm)	Sporulation
1	15 ˚C	2.60 (1.76)	+
2	20 ˚C	5.05 (1.62)	++
3	25 ˚C	6.43 (2.63)	+++
4	30 ˚C	8.38 (2.98)	++++
5	35 ˚C	2.13 (2.36)	+
6	40 ˚C	0 (0.71)	-
SE(d)±		0.04	
$CD_{(0.05)}$		0.09	

*Mean of three replications; Figures in parenthesis are square root transformed values
++++ Excellent sporulation: More than 100 spores; +++ Good sporulation: 41-80 spores; ++ Fair sporulation: 21-40 spores; + Poor sporulation: less than 20 spores; - No sporulation.

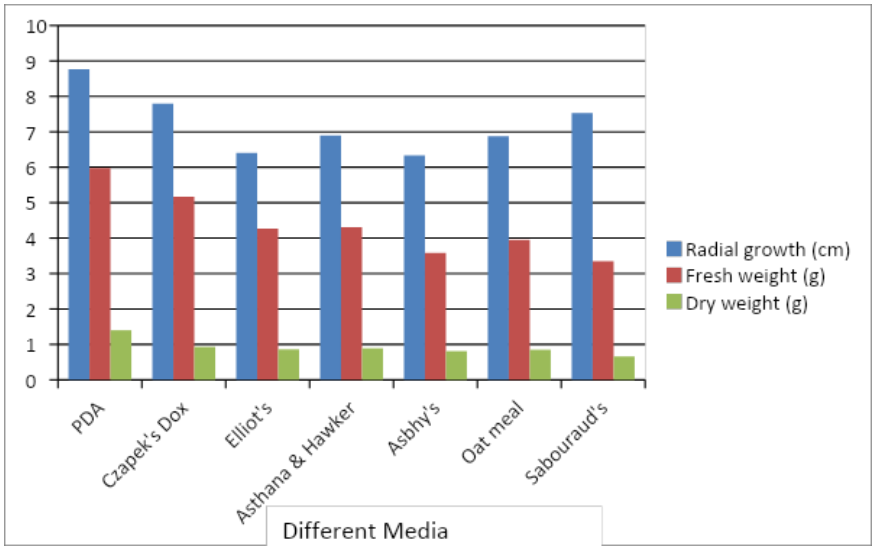

Fig.1 Effect of different media on growth and sporulation of *F. oxysporum* f. sp. *Udum*

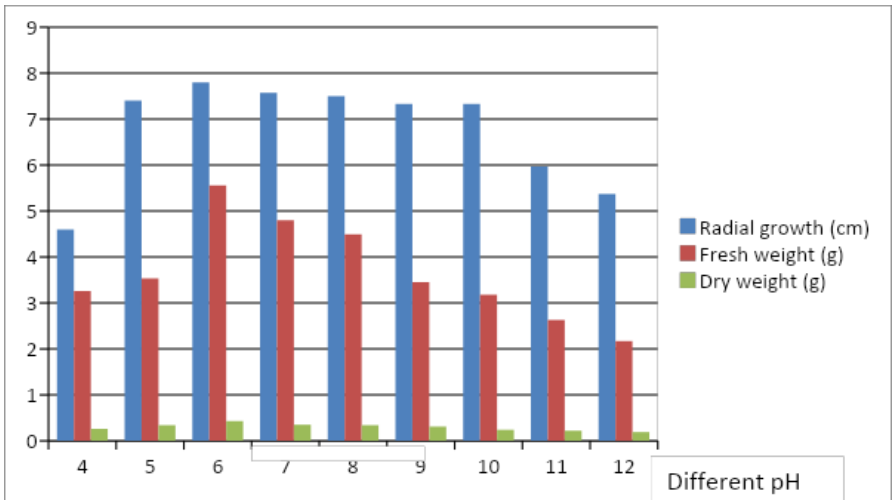

Fig.2 Effect of different pH on growth and sporulation of *F. oxysporum* f. sp. *Udum*

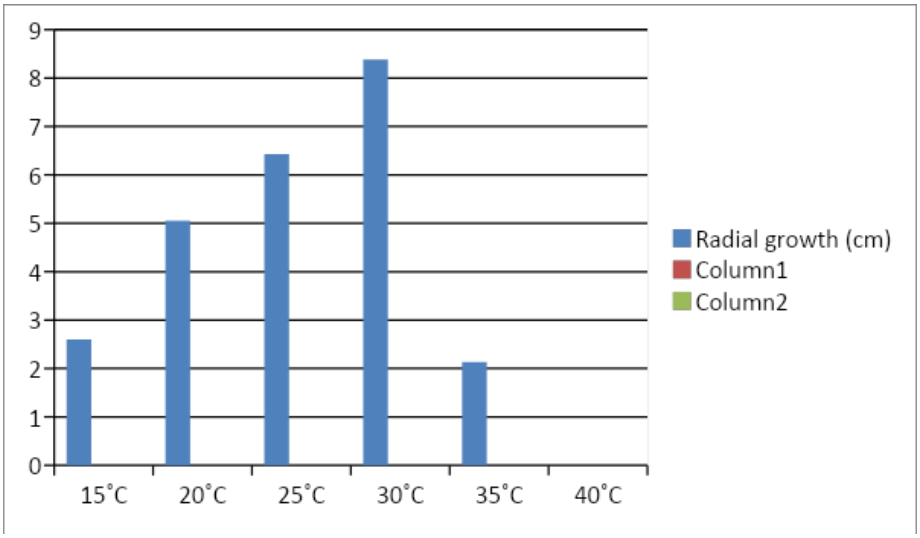

Fig.3 Effect of different temperature on growth and sporulation of *F. oxysporum* f. sp. *Udum*

Recently, Desai *et al*., (2016) reported that the maximum growth of the *Fusarium udum* was at 28ºC. There results very much support the present studies in which the most suitable temperatures level for the growth of *Fusarium* isolate was 25º and 30ºC. These results will help in further studies of pathogen and its identification.

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