Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 2005-2011

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7692 **Special Issue-6 pp. 2005-2011**
Journal homepage: http://www.ijcmas.com

Original Research Article

Effect of Different Media, pH and Temperature on Growth and Sporulation of *Fusarium udum* Causing Wilt of Pigeonpea

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Keywords

Media, pH, temperature, F. udum, Wilt, Pigeonpea

A B S T R A C T

Laboratory studies were conducted to study the effect of different culture media, pH and temperature on mycelia growth, sporulation and dry mycelia weight of *Fusarium udum*. The fungus grew the best on PDA and Richard's agar media among seven culture media were tested. The most suitable pH level for growth of fungus was 6.0 and 6.5 with excellent sporulation. Growth of *F. udum* was maximum at 30^0C after seven days of inoculation, which was reduced drastically below 10^0C and above 35^0C.

Introduction

Pigeon pea is an important pulse crop of India. It is a demanding and always in need food crop in India. India holds a major contribution of 90% of total world production. India engages an area of 3.63 million ha with a production of 2.76 million tonnes and the productivity of 751 kg/ha (Singh and Singh, 2014). The crop is prone to many serious diseases caused by fugal, bacterial viral and nematode. High sensitivity of the crop to the attack of diseases appears to be the main reason for low yields. Pigeonpea is known to be affected by more than hundred pathogens (Nene *et al.*, 1989). However, only a few of them cause 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.

Wilt caused by *Fusarium udum* Butler is the most important disease caused to this crop (Kannaiyan *et al.*, 1984). *Fusarium udum* is a soil born pathogen and also the pigeonpea specific wilt pathogen (Kannaiyan *et al.*, 1985). It causes significant loss to the crop as well as economic loss to the farmers. The pathogen has an ability to survive in the infected crop debris for 3–4 years and cause serious economic loss, sometimes upto 100% in the susceptible cultivars (Kiprop *et al.*, 2002). The economic loss caused by Fusarium wilt in Pigeonpea is 470,000 tonnes in India (Joshi *et al.*, 2001). Environmental factors such as temperature, water activity and pH have a great influence on fungal development (Yadav *et al.*, 2014). Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum

size have great influence on the growth of pathogen (Tyagi and Paudel, 2014; Dubey, 2016). Present work depicts the role of different pH and media to understand ecological survival of pathogen which will be helpful in management strategy in the field.

Materials and Methods

Isolate of *Fusarium udm* was recovered from diseased pigeonpea plants from research farm of Jwaharlal Nehru Krishi Vishwa Vidyalaya- Jabalpur. Small pieces of discolored vascular tissue from roots of diseased plants were placed on potato dextrose agar (PDA) and incubated at 28 ± 1°C in the dark for four days. Isolate was identified as *Fusarium udum* by morphological criteria (Leslie and Summerell, 2006). A single macroconidial culture was prepared from isolate. Studies of the following physiological aspects of *Fusarium udum* isolates were conducted in laboratory.

Effect of culture media

Following seven 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 121.6ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 90 mm Petri dishes for solidification.

1. Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g).
2. Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-agar 20g).
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. Asthana and Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen Phosphate 1.75g, Magnesium sulphate 0.75g, Agar- agar 20g).
5. Ashby's agar medium (Mannitol 20g, Di potassium phosphate 0.2g, Magnesium sulphate 0.2g, Sodium chloride 0.2g, Potassium sulphate 0.1g, Calcium carbonate 5g, Agar-agar 15g, final pH (at 25°C) 7.4±0.2).
6. Browns agar (BA) medium (Dextrose 2g, Tri basic potassium phosphate 1.25g, Magnesium sulphate 0.75g, Agar-agar 20g).
7. Coon's agar (CA) medium (Sucrose 7.2 g, Dextrose 3.60g, Magnesium sulphate 1.23g, Potassium nitrate 2.02g, Potassium di-phosphate2.72g, Agar- agar 15g).

Effect of pH

There were eight different pH level ranging from 5.0 to 8.5 with a difference of 0.5 were prepared by using pH meter and by using either N/10 HCl or NaOH before autoclaving the PDA medium. For each pH value, three replications were maintained. The Petriplates containing sterilized medium was inoculated with 5mm mycelium disc and incubated at 28 $\pm$ 1ºC. At the interval of 24hrs, the linear growth was measured till 7 days. The range of sporulation test ranges on various pH was recorded after 7 days. Sporulation was calculated with the help of haemocytometer.

Effect of temperature

The experiments were conducted to find out, the most suitable temperature for mycelial growth and sporulation of *F. udum*. The sterilized poured petriplates with PDA were

inoculated with 5 mm disc of the test pathogen of seven days old culture. The petriplates were incubated at 10, 15, 20, 25, 30, 35 and 40ºC temperature. Three replications were maintained for each treatment and observation for mycelial growth was recorded after seven days. Sporulation was recorded at seven days after inoculation with the help of haemocytometer.

Results and Discussion

A total of seven media were used for studying the growth of *Fusarium udum*. The results are given in Table 1. Maximum colony diameter (82.0 mm) was recorded on PDA. The next best medium was Richard's agar medium which yielded 79.33 mm colony diameter followed by Czapek's agar medium (56.83 mm). Least colony diameter (45.0 mm) of the test fungus was observed in Coon's agar medium (Plate-1). The test fungus sporulated in all medium tried but excellent sporulation were observed in PDA and Richard's agar medium. Maximum dry weight (375.0 mg) of *Fusarium udum* was recorded on Richard's broth medium followed by Potato dextrose broth medium, which yielded 348.30 mg dry mycelial weight (Plate-2). The test fungus sporulates in all tested medium but excellent sporulation was not observed in any medium. Good sporulation was recorded in Richard's broth. These results were in confirmation with Ingole (1995) reported that PDA and Richard's agar supported best mycelial growth of *F. udum*. Reddy (2002) observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar. Gangadhara, *et al.,* (2010) reported that *F. oxysporum* f. sp. *vanillae* isolates showed best growth on Richard's agar and Potato dextrose agar media. Khan *et al.,* (2011) studied effect of media on *F. oxysporum* f.sp. *ciceri* and found that PDA was best for the growth of isolates. Recently, Singh *et al.,* (2016) studied effect of different solid media and liquid media on radial growth and sporulation of *Fusarium oxysporum* f.sp. *lentis*. Potato dextrose agar and Richard's agar were the best medium for radial growth and sporutation of *Fusarium oxysporum* f.sp. *lentis*. The present study also indicated that Potato dextrose agar and Richard's agar are best medium for growth of *F. udum*.

Growth and sporulation of the test fungus was obtained at all the pH levels tested but it was maximum at pH 6.0 (84.33mm) after 168 hrs of inoculation. pH 6.5 (78.33 mm) and pH 7.0 (75.16 mm) were also found favorable (Table 2). Growth of the test fungus decreased by increasing or decreasing the pH level from the 6.0 level. The foremost acidic and alkaline pH is not suitable for the growth and sporulation of pathogen. The results of the present study are in agreement with those achieved by Khan *et al.,* (2011) who also reported optimum pH for growth of *Fusarium oxysporum* f.sp.*ciceri* ranged from 6.5 to 7.0. Khilare and Ahmed, (2012) reported most suitable pH level for growth of *Fusarium oxysporum* f.sp. *ciceri* was 6.0 and 6.5. Tyagi and Paudel (2014) reported that pH level 6.0 is the optimum pH for the growth as well as sporulation of the fungus. Further increases in the pH level showed retarding effect on growth and sporulation. Recently, Singh *et al.,* (2016) reported maximum growth of the *Fusarium oxyspon* f.sp. *lentis* at pH 6.5. There results very much support the present result in which the most suitable pH level for growth of the test fungus was 6.0 and 6.5.

Table.1 Effect of solid and liquid media on radial growth and sporulation of *Fusarium udum*

S. No.	Name of the medium	Solid Media		Liquid Media	
		Colony diameter (mm) after 168 hrs*	Sporulation	Dry mycelial weight (mg) after 21 day*	Sporulation
1	Potato dextrose agar	82.0	++++	348.3	++
2	Richard's agar	79.3	++++	375.0	+++
3	Czapek's Dox agar	56.8	+++	302.1	++
4	Asthana and Hawker's agar	49.6	++	202.2	++
5	Browns medium	47.0	++	166.1	++
6	Ashby's agar	46.3	+	125.6	+
7	Coon's medium	45.0	+	75.0	+
	CD (0.05)	2.331		1.951	

*****Average of 3 replications

Table.2 Effect of various pH on radial growth and sporulation of *Fusarium udum*

S. No.	pH	Colony diameter (mm) after 168 hrs*	Sporulation
1	5.0	63.00	++
2	5.5	70.33	+++
3	6.0	84.33	++++
4	6.5	78.33	++++
5	7.0	75.16	+++
6	7.5	51.00	++
7	8.0	45.00	++
8	8.5	20.00	--
CD (0.05)		1.773	

*Average of 3 replications

Table.3 Effect of different temperature on radial growth and sporulation of *F. udum*

S. No.	Temp. ^{o}C	Colony diameter (mm) after 168 hrs*	Sporulation
1	10	11.66	--
2	15	27.33	+
3	20	49.67	++
4	25	66.33	+++
5	30	89.23	++++
6	35	21.83	+
7	40	0	--
	CD (0.05)	2.441	

*Average of 3 replications

Plate.1 Effect of solid media on radial growth and sporulation of *Fusarium udum*

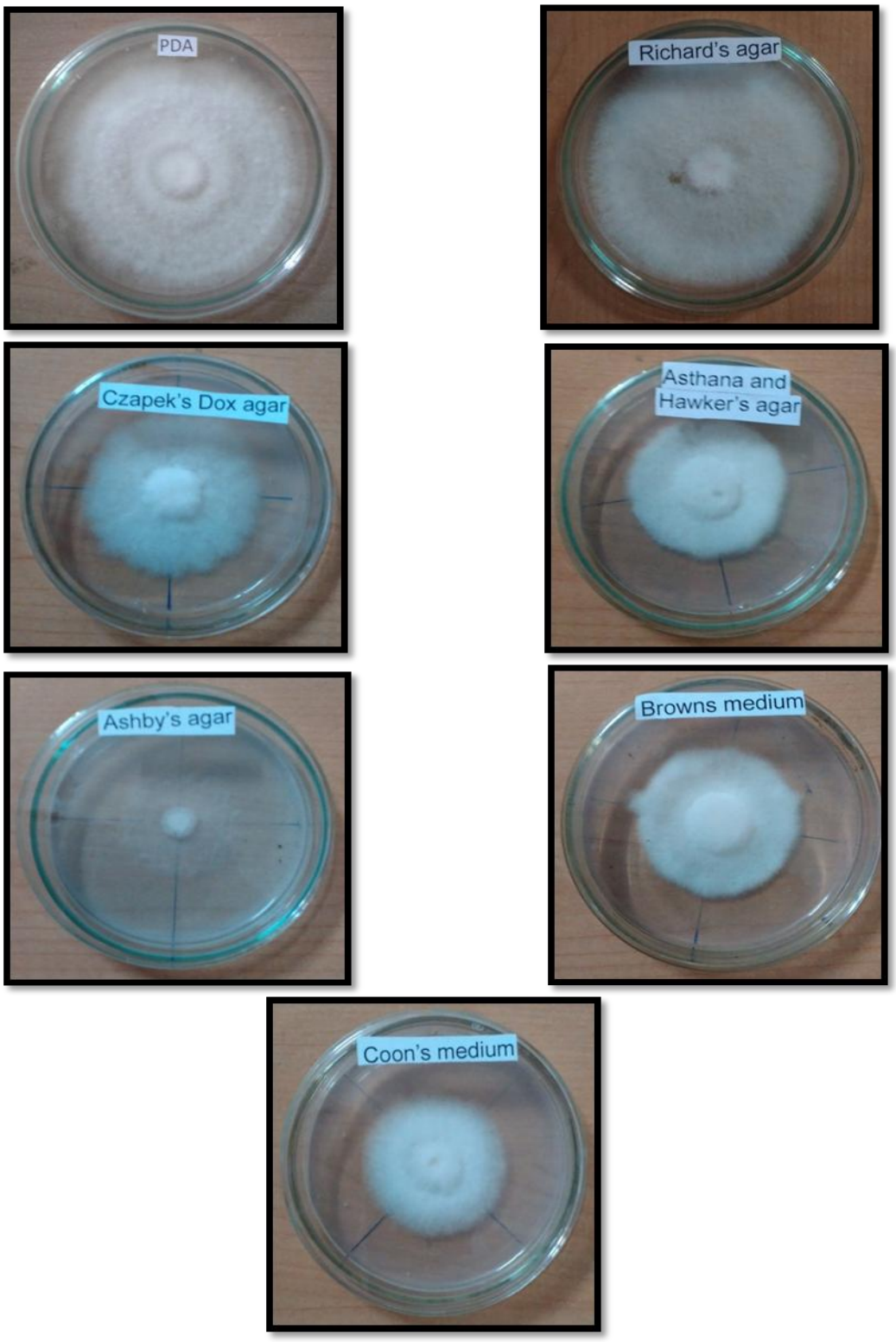

Plate.2 Effect of liquid media on dry mycelia weight of *Fusarium udum*

Growth of *F. udum* was studied from 10 to 40ºC temperature and result is presented in Table 3. It was seen that there was quite a large variation in the growth of these isolate at different temperature after 7 days. The maximum mycelial growth was recorded at 30ºC (88.23mm) followed by 25ºC (66.83mm), 20ºC (49.67mm) and 35ºC (21.00mm), while no mycelial growth was recorded at 10 and 40ºC. Temperatures from 25 to 35ºC were most favorable for the growth of target pathogen. The highest growth of pathogen was recorded at 30ºC with higher sporulation. Reddy (2002) reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 20ºC to 35ºC. Scott, *et al.*, (2010) studied effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*), caused by *F. oxysporum* f. sp. *lactucae*, were observed to increase in radial growth from 10ºC up to an apparent maximum near 25ºC. Khilare and Ahmed (2012) reported most suitable temperatures level for growth of *Fusarium oxysporum* f.sp. *ciceri* was 30ºC after seven days of inoculation, which was reduced drastically below 15ºC and above 35ºC. Recently, Desai *et al.*, (2016) reported maximum growth of the *Fusarium udum* at 28ºC. There results very much support the present studies in which most suitable temperatures level for growth of the test fungus was 25 and 30ºC.

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