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Influence of physiological factors on mycelial growth and sporulation of *Fusarium udum*

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Abstract
Pigeonpea wilting caused by *Fusarium udum* has emerged as one of the biotic threats to profitable pigeon pea cultivation. An *in vitro* study was conducted on the effects of different media, pH values, and temperature on mycelial growth, sporulation, and mycelial dry weight of *Fusarium udum*. The test organism grew best on PDA and Richards agar out of his seven media tested. The optimal pH for growth of the test pathogens was 6.0 and 6.5, which showed excellent sporulation. Growth of *F. Udum* peaked at 30 °C after 7 days of inoculation and decreased sharply below 10 °C and above 35 °C

Keywords: *Fusarium udum*, Mycelial growth, Physiological parameters, Sporulation

Introduction
Pigeon Pea (*Cajanus cajan*) is an important Indian legume. The crop is susceptible to many serious diseases caused by fungi, bacteria, viruses and nematodes. The plant's high susceptibility to disease attack seems to be the main reason for the low yield. Pigeonpea is known to be affected by over 100 pathogens (Nene *et al*., 1989) [13]. However, few cause financial loss. Diseases of great economic importance today are sterility mosaic, Fusarium wilt, Phytophthora rot, Macrophomina root rot, Stem canker and Alternative blight. Wilt caused by *Fusarium udum* Butler is the most important disease caused by this crop (Kannaiyan *et al*., 1984) [7]. *Fusarium udum* is a soil-borne pathogen and a wilt fungus endemic to pigeon peas (Kannaiyan *et al*., 1985) [8]. It causes great loss to crops and economic loss to farmers. Pathogens are capable of surviving in infected plant debris for 3–4 years and causing severe economic losses, which can be up to 100 per cent in susceptible cultivars (Kiprop *et al*., 2002) [10]. The economic damage caused by Fusarium wilting of pigeonpeas is 470,000 tons in India (Joshi *et al*., 2001) [5]. Environmental factors such as temperature, water activity and pH have a significant impact on fungal development (Yadav *et al*., 2014) [19]. Differences in the nature of the carbon and nitrogen sources, along with changes in pH, temperature, incubation time, shaking, and inoculum size, have significant effects on pathogen growth (Tyagi and Paudel, 2014, Dubey, 2016) [18, 2]. Current work demonstrates the role of different pH values, temperature and media in understanding the ecological survival of pathogens, and is useful for management strategies in this area.

Material 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) [11]. A single macroconidial culture was prepared from isolate. Studies of the following physiological aspects of *Fusarium udum* isolates were conducted in laboratory.

Influence 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 121.6 ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 90 mm Petri dishes for solidification. Asthana & Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen Phosphate 1.75g, Magnesium sulphate 0.75g, Agar-agar 20g), Coon's agar (CA) medium (Sucrose 7.2 g, Dextrose 3.60g, Magnesium sulphate 1.23g, Potassium nitrate 2.02g, Potassium di-phosphate 2.72g, Agar- agar 15g),

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), Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g), Browns agar (BA) medium (Dextrose 2g, Tri basic potassium phosphate 1.25g, Magnesium sulphate 0.75g, Agar-agar 20g), 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) and 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).

Influence of pH: There were eight different pH level ranging from 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 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 ± 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 seven days. Sporulation was calculated with the help of haemocytometer.

Influence 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 to 40 ºC 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

Influence of medium

A total of seven media were used to study the growth of *Fusarium udum*. Results are shown in Table 1. Maximum colony diameter (82.0 mm) was recorded on PDA. The second best medium was Richard's agar, yielding a colony diameter of 79.33 mm, followed by Czapek's agar (56.83 mm). The smallest colony diameter (45.0 mm) of the test pathogen was observed on Kuhn's agar medium. The test fungi sporulated on all media tested, but excellent sporulation was observed on PDA and Richard's agar. The maximum dry weight of *Fusarium udum* (375.0 mg) was recorded on Richard broth medium followed by potato dextrose broth medium, yielding a dry mycelium weight of 348.30 mg. The test fungus sporulated on all media tested, but excellent sporulation was not observed on any media. Good sporulation was observed in Richard's broth. These results were confirmed by his Ingole (1995) [4], who confirmed that his PDA and Richard Agar were his *F. udum*. Reddy (2002) [15] observed maximum growth of *F. udum* in Richard Agar and Potato Dextrose Agar. Gangadhara, *et al*., (2010) [3] reported that *F. oxysporum* f. sp. *vanilla* isolate showed the best growth on Richard's agar and Potato dextrose agar. Singh *et al*., (2016) [17] studied the effects of different solid and liquid media on the radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Potato dextrose agar and Richard's agar were the optimal media for radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Maitlo *et al*., (2017) [12] studied the influence of medium on *F. oxysporum* f. sp. *ciceri* found that Richards agar and potato dextrose agar were optimal for growing isolates. Poorvasandhya *et al*., (2020) [14] showed that *Fusarium udum* isolates followed best growth (87.70 mm), fresh weight and dry weight (5.98 & 1.40 g) on PDA and PDA broth medium followed by Czapek's Dox medium. The present study also showed that potato dextrose agar and Richard's agar are the optimal media for mycelial growth, resulting in excellent sporulation of *Fusarium udum*.

Table 1: Influence of solid and liquid media on mycelial growth and sporulation of *Fusarium udum*

Media	Solid Media		Liquid Media	
	Mycelial growth (mm) after 168 hrs	Sporulation	Dry mycelial weight (mg) after 21 day	Sporulation
Asthana and Hawker's agar	49.6	++	202.2	++
Coon's medium	45.0	+	75.0	+
Czapek's Dox agar	56.8	+++	302.1	++
Potato dextrose agar	82.0	++++	348.3	++
Browns medium	47.0	++	166.1	++
Ashby's agar	46.3	+	125.6	+
Richard's agar	79.3	++++	375.0	+++
CD (0.05)	2.331		1.951	

Influence of pH

Mycelial growth and sporulation of the test pathogen was obtained at all pH values tested, but highest at pH 6.0 (84.33 mm) 168 hours after inoculation. pH 6.5 (78.33 mm) and pH 7.0 (75.16 mm) were also good (Table 2). Growth of the test organisms was reduced by increasing or decreasing the pH from 6.0. Predominantly acidic and alkaline pH values are not suitable for pathogen growth and sporulation. Our results are consistent with those of Khan *et al*., (2011) [8], who also found the optimal pH for the growth of *Fusarium oxysporum* f. sp. *ciceri* ranged from 6.5 to 7.0. Khalare and Ahmed, (2012) [9] reported the optimum pH for the growth of *Fusarium oxysporum* f. sp. *ciiceri* was 6.0 and 6.5. Tyagi and Paudel (2014) [18] reported that a pH of 6.0 is the optimum pH for fungal growth and sporulation. Further increases in pH showed a retarding effect on growth and sporulation. Singh *et al*., (2016) [17] reported maximum growth of *Fusarium oxysporum* f. sp. *lentils* at pH 6.5. Poorvasandhya *et al*., (2020) [14] studied the growth and sporulation of *Fusarium oxysporum f. sp. udum* at different pH values showed this maximum at pH 6.0 (78.0 mm) and 7.0 (75.70 mm). This result strongly supports the present study that pH 6.0 and 6.5 were optimal for mycelial growth and good sporulation of the test fungi.

Table 2: Influence of various pH on mycelial growth and sporulation of *Fusarium udum*

pH	Mycelial growth (mm) after 168 hrs	Sporulation
8.5	20.00	--
8.0	45.00	++
7.5	51.00	++
7.0	75.16	+++
6.5	78.33	++++
6.0	84.33	++++
5.5	70.33	+++
5.0	63.00	++
CD (0.05)	1.773	

Influence of temperature

Table 3 shows the results of examining the growth of *F. udum* at temperatures between 10 and 40°C. After 7 days, it was found that there was considerable variation in the growth of these strains at different temperatures. Maximum mycelial growth was recorded at 30 °C (88.23 mm), followed by 25 °C (66.83 mm), 20 °C (49.67 mm) and 35 °C (21.00 mm), although no mycelial growth was recorded at 10 and 40°C. Temperatures between 25 and 35 °C were most favorable for target pathogen growth. The highest pathogen growth was recorded at 30 °C with high sporulation. Reddy (2002) [15] reported that the growth of 40 *F. udum* isolates differed in temperature requirements varying between 20 °C and 35 °C. Scott, *et al*., (2010) [16] studied the effect of temperature on Fusarium wilt in lettuce (*Lactuca sativa*) caused by *F. oxysporum* f. sp. *lactucae*, an increase in radial growth was observed from 10 °C to an apparent maximum around 25 °C. Desai *et al*., (2016) [1] reported maximum growth of *Fusarium udum* at 28 °C. This result strongly supports current research that the optimal temperature levels for the growth of the test fungi were 25 and 30 °C. Maitlo *et al*., (2017) [12] reported the optimum temperature level for the growth of *Fusarium oxysporum* f. sp. *ciceri* was at 30°C, increased to 25 and 35°C after 7 days of inoculation, and declined sharply below 10°C and above 45 °C. Poorvasandhya *et al*., (2020) [14] observed maximum mycelial growth at 30 °C (83.80 mm) and 25 °C (64.30 mm), but no mycelial growth was recorded at 40 °C. This result strongly supports the present study that the optimal temperature levels for mycelium growth and good sporulation of the test fungi were 25 °C and 30 °C.

Table 3: Influence of different temperature on mycelial growth and sporulation of *Fusarium udum*

Temp. ºC	Mycelial growth (mm) after 168 hrs	Sporulation
40	0	--
35	21.83	+
30	89.23	++++
25	66.33	+++
20	49.67	++
15	27.33	+
10	11.66	--
CD (0.05)	2.441	

Conclusion

The present study demonstrated the effects of different media, pH values, and temperature on mycelial growth, sporulation, and mycelial dry weight of *Fusarium udum*. The test pathogen grew and sporulates best on PDA and Richards agar medium. The optimal pH for growth of the test pathogens was 6.0 and 6.5, which showed excellent sporulation. Growth of *F. udum* highest at 30 °C after 7 days of inoculation and decreased sharply below 10 °C and above 35 °C.

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References

1. Desai UA, Andoji YS, Shivaji SS. Influence of temperature and different culture media on growth of *Fusarium udum* (Butler), causal organism of wilt of pigeonpea. International Journal of Biological Research. 2016;4(1):42-45.
2. Dubey SC. Race profiling, genetic diversity, diagnostics and management of *Fusarium oxysporum* f. sp. *ciceris* causindg will of chickpea. Indian Phytopathology. 2016;69(3):210-217.
3. Gangadhara NB, Nagaraja R, Basavaraja MK, Krishna NR. Variability studies of *Fusarium oxysporum* f. sp. *vanillae* isolates. International Journal of Science and Nature. 2010;1(1):12-16.
4. Ingole MN. Estimation of losses, variability among isolates and management of pigeon pea wilt caused by *Fusarium udum* Butler. M.Sc. (Ag.) Thesis, Dr. PDKV, Akola; c1995. p. 146.
5. Joshi PK, Parthasarathy Rao P, Gowda CLL, Jones RB, Silim SN, Saxena KB, *et al*. The world Chickpea and Pigeonpea Economies: Facts, Trends, And Outlook. In: Shiferaw B, Silim S, Muricho G, Audi P, Mligo J, Lyimo S, You L. and Chris-tiansen JL. 2005. Assessment of the Adoption and Impact of Improved Pigeonpea Varieties in Tanzania. Journal of SAT Agricul-tural Research. 2001;3:1.
6. Kannaiyan J, Nene YL, Raju TN. Host Specificity of Pigeonpea Wilt Pathogen *Fusarium udum*. Indian Phytopathology. 1985;38:553.
7. Kannaiyan J, Nene YL, Reddy MV, Ryan JG, Raju TN. Prevalence of Pigeonpea Diseases and Associated Crop Losses in Asia, Africa and America. Tropical Pest Management. 1984;30:62–71.
8. Khan IHS, Saifulla M, Mahesh SB, Pallavi MS. Effect of different media and enviromental conditions on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing Fusarium wilt of chickpea. International Journal of Science and Nature. 2011;2:402-404.
9. Khilare VC, Rafi A. Effect of different media, ph and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. International Journal of Advanced Research in Biological Sciences. 2012;2(1):99-102.
10. Kiprop EK, Baudoin JP, Mwangombe AW, Kimani PM, Mer-geai G, Maquet A. Characterization of Kenyan isolates of *Fusarium Udum* from Pigeonpea [*Cajanus Cajan* (L.) Millsp.] Cultural characteristics, Aggressiveness and AFLP Analysis. Journal of Phytopathology. 2002;150: 517-527.
11. Leslie JF, Summerell BA. The Fusarium, Laboratory Manual, Blackwell Publishing; c2006. p. 1–388.
12. Maitlo S, A Rajput Q, Naz Syed R, Khanzada MA, Rajput NA, Lodhi AM. Influence of physiological factors on vegetative growth and sporulation of *Fusarium*

oxysporum f. sp. *ciceris*. Pakistan Journal of Botany. 2017;49(SI):311-316.
13. Nene YL, Sheila VK, Sharma SB. A world List of Chickpea and Pigeonpea Pathogens. Legume Pathology Progress Report. 1989;7:23.
14. Poorvasandhya R, Sinha B, Sobita Devi Ph. Effect of Media, Temperature and pH on Growth and Sporulation of *Fusarium oxysporum* f. sp. *udum* under *in vitro* Condition. International Journal of Current Microbiology and Applied Sciences. 2020;9(4):2406-2412
15. Reddy AB. Variability of *Fusarium udum* and evaluation of pigeonpea (*Cajanus cajan* (L). Mills) genotypes. M.Sc (Agri) Thesis, Univ. Agril. Sci. Bangalore; c2002. p. 115.
16. Scott JC, Gordon TR, Shaw DV, Koike ST. Effect of temperature on severity of Fusarium wilt of lettuce caused by *Fusarium oxysporum* f. sp. *lactucae*. Plant Disease. 2010;94(1):13-17.
17. Singh R, Laxmikant, Singh M. Management of root rot of pea (*Pisum sativum* L.) through commercially available bioagent formulation and fungicide. 6th international Conference "Plant, Pathogens and People" February 23-27, New Delhi, India; c2016.
18. Tyagi S, Paudel R. Effect of different pH on the growth and sporulation of *Fusarium oxysporum*: The causal organism of wilt disease of Tomato. International Journal of Basic and Applied Biology. 2014;2:103–106.
19. Yadav RS, Tyagi S, Javeria S, Gangwar RK. Effect of different cultural condition on the growth of *Fusarium moniliforme* causing Bakanae disease. European Journal of Molecular Biotechnology. 2014;4(2):95-100.