Effect of Temperature, Media and pH on Growth and Sporulation of *Fusarium udum* Causing Wilt of Pigeonpea *Cajanus cajan* (L.) Millsp.

Abstract

Pigeon pea (*Cajanus cajan*) is an important legume crop worldwide, highly valued for its nutritional benefits, particularly its protein and vitamin content. However, the productivity of pigeon pea is often hindered by various abiotic and biotic stress factors. Fusarium wilt, caused by the soil-borne pathogen *Fusarium udum*, stands out as a critical biotic factor impacting pigeonpea production the disease significantly hampers yield by infecting the plant's vascular system, leading to severe wilting and eventual plant death. As the pathogen thrives in the soil, it poses a persistent challenge to crop health, resulting in considerable losses for farmers. To understand the optimal conditions for the growth of *Fusarium udum*, a laboratory experiment was conducted on the effects of different culture media, pH, and temperature on the pathogen's mycelial growth and sporulation. The study revealed that the Potato Dextrose Agar (PDA) and Czapek Dox Agar were particularly effective in supporting the growth and sporulation of the fungus. These media provide essential nutrients that facilitate the robust growth of fungal colonies. Furthermore, the optimal temperature range for mycelial growth and sporulation of *Fusarium udum* was found to be between 20-30°C. Additionally, the fungus exhibited maximum growth and sporulation at a pH range of 6 to 7, indicating a preference for slightly acidic to neutral conditions. Understanding these growth parameters is crucial for developing effective disease management strategies by manipulating factors like nutrition source, pH and temperature.

Key words: Pigeonpea, *Fusarium udum*, Mycelial Growth, Sporulation, Culture Media, Temperature.

Introduction

Pigeon pea (*Cajanus cajan*) is major grain legume in India after Chick pea. Pigeon pea is a significant pulse crop cultivated in India, contributing to 90% of the global production. India holds a dominant position, occupying more than 50% of the total pulse cultivation area and contributing 60% to the overall pulse production. Pigeon pea serves as a primary protein source in the human diet, playing a crucial role in food security and subsistence agriculture. The seeds of pigeon pea contain 20-22% protein and vitamin B **(Singh *et al.*, 2015)**. The primary factors contributing to reduced yields in pigeon pea crops include both biotic and abiotic factors. Pigeon pea is susceptible to a variety of plant pathogens, including fungi, bacteria, viruses, mycoplasma, and nematodes **(Nene *et al.*, 1996)**. Pigeon pea suffered by several diseases like Alternaria leaf spot, Fusarium wilt, Phytophthora blight, Sterility mosaic and wilt. Fusarium wilt of pigeon pea caused by *Fusarium udum* Butler, is an economically important disease in India **(Khare *et al.*, 1994)**. The pathogen can survive on infected plant debris in the soil for about three years and causes serious yield losses, sometimes up to 100% in susceptible cultivars **(Kumar and Upadhyay, 2013).** *Fusarium udum*, a soil-borne pathogen, persists in the soil primarily in the form of chlamydospores. These chlamydospores serve as the main mechanism for the pathogen's survival and are instrumental in the dissemination of Fusarium wilt disease. The disease spreads predominantly through the movement of these resilient spores, which allow the pathogen to endure unfavorable environmental conditions and perpetuate the infection cycle in host plants **(Egel and Martyn, 2013).** Environmental factors such as temperature, relative humidity, and pH significantly elevate the growth and development of this pathogen **(Yadav *et al.*, 2014).** Additionally, variations in temperature, pH, types of nitrogen and carbon sources, inoculum size, and incubation period can affect the mycelial growth and sporulation of the pathogen **(Tyagi and Paudel, 2014; Dubey, 2016)**. These factors play crucial role in determining the pathogen's proliferation and pathogenicity, underscoring the need for precise control and optimization of these parameters in experimental and practical settings. The present study explores the influence of different culture media, pH, and temperature to understand the *in vitro* activity of pathogen.

Material and method

Isolation of pathogen

The pathogen *Fusarium udum* was isolated from diseased pigeon pea plant collected from the Pulse Pathology block at the Norman E. Borlaug Crop Research Centre, G.B.P.U.A&T, Pantnagar (Uttrakhand). Pigeonpea roots with typical symptoms were used for isolation. Discolored vascular tissue from roots of diseased plants were cut into small pieces and surface sterilized with 1% sodium hypochlorite solution for 1 minute, rinsed thrice with sterile distilled water and placed on Petri plates containing potato dextrose agar medium. These plates were incubated at 28 ± 1°C in the dark for 10 days. The isolate was identified as *Fusarium udum* **(Accession No. ON479209.1)** based on morphological (presence of microconidia, macroconidia and chlamydosores) characteristics as described by **Leslie and Summerell (2006)**. A pure culture derived from single conidia of the isolate was then maintained. Comprehensive studies on the physiological and growth pattern of the *Fusarium udum* isolates were subsequently carried out under controlled laboratory conditions.

Growth of *Fusarium udum* on different culture media

The growth characteristics and sporulation of *Fusarium udum* was evaluated on ten distinct culture media namely Oat Meal Agar, Potato Dextrose Agar, Corn Meal Agar, Czapek Dox Agar, Host Root Extract, Potato Carrot Agar, Malt Extract Agar, Richard's Agar, Glucose Peptone Agar and T-2 (Asparagine), to identify the most effective one (Table 1). Each medium was prepared in 1 liter of distilled water and sterilized in autoclave at 121°C for 15 minutes, these were cooled to 45°C and then poured into 90 mm Petri dishes to solidify. After solidification, the Petri dishes were inoculated with 5 mm discs of the test pathogen and incubated at 27±1°C. Each treatment was replicated three times, with observations recorded after ten days. Sporulation of the pathogen was assessed using a hemocytometer. To prepare the spore suspension, five 5 mm discs of the pathogen were excised and suspended in 10 ml of distilled water to achieve a uniform spore suspension. One drop of this suspension was then placed on the hemocytometer for spore counting (fungal dilution 10^{-6}).

Table 1: Quantity and Composition of different culture media

The composition and preparation of the following mentioned synthetic and semisynthetic media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by **Hawks worth *et al.* (1983).** The composition and preparation of the media is given below**.**

Medium		Composition and Quantity
Oat Meal Agar (OMA)	:	Rolled Oats (30g) and Agar (15g); Distilled water (1000ml)
Potato Dextrose Agar (PDA)	:	Peeled and sliced potato (200g); Dextrose (20g) and Agar (20g); Distilled water (1000ml)
Corn Meal Agar (CMA)	:	Cornmeal (20g); Peptone (20g); Glucose (20g) and Agar (15g); Distilled water (1000ml)
Czapeks Dox Agar (CDA)	:	Di potassium hydrogen phosphate (1g); Sodium nitrate (2g); Magnesium sulphate (0.5g); Potassium chloride (0.5g); Sucrose (30g); Ferrous sulphate (0.01g) and Agar (20g); Distilled water (1000ml)
Malt Agar (MA)	:	Malt extract powder (20g); Glucose (20g); Peptone (1.0g) and Agar (20g); Distilled water (1000ml)
Potato Carrot Agar (PCA)	:	Peeled and sliced potato (300g); Carrot (25g) and Agar (15g); Distilled water (1000ml)
T-2 Agar (T-2)	:	L- Asparagine (10g); Sucrose (100g); Yeast extract (0.1g); Potassium dihydrogen phosphate (0.25g); Magnesium Sulfate (0.25g); Ferrous sulphate heptahydrate (0.02g); Zinc Sulphate Heptahydrate (0.015g); Potassium chloride (0.12g); Calcium Nitrate Tetrahydrate (1.0g) and Agar (20g); Distilled water (1000ml)
Host root extract (HRE)	:	Host root (150g) and Agar (20g); Distilled water (1000ml)
Richard's Agar (RA)	:	Potassium dihydrogen phosphate (5g); Magnesium sulphate (2.5g); Ferric chloride (0.02g); Sucrose (50g); Potassium nitrate (10g) and Agar (20g); Distilled water (1000ml)
Glucose-Peptone Agar (GPA)	:	Peptone (20g); Dextrose (10g); Sodium chloride (5g) and Agar (20g); Distilled water (1000ml)

Growth of *Fusarium udum* on different temperature

The experiment aimed to investigate the impact of temperature on the mycelial growth and sporulation of *Fusarium udum in vitro*. Sterilized potato dextrose agar was poured into 90 mm Petri dishes, which were then inoculated with 5 mm discs of the test pathogen using a sterile cork borer. The inoculated plates were incubated at temperatures ranging from 5°C to 40°C, with each temperature condition replicated three times. Observations were made on both mycelial growth and sporulation. Sporulation was quantified using a hemocytometer, and mycelial growth was measured in diameter with the help of a measuring scale after ten days of incubation.

Growth of *Fusarium udum* on different pH levels

To study the effect of different pH level (4-8.5 pH) on mycelial growth and sporulation of pathogen *Fusarium udum* was determined by adjusting the pH of potato dextrose agar media, with a difference of 0.5 between each pH levels. Each pH was adjusted by adding HCL or NaOH using pH meter before autoclaving the media. Sterilized potato dextrose agar (PDA) media was poured into 90 mm Petri plates. These plates were inoculated with a 5 mm disc of the test pathogen using a sterilized cork borer and incubated at pH ranges from 5-8.5. Each treatment was replicated three times. Mycelial growth was measured after fourteen days of inoculation, and sporulation was assessed using a hemocytometer.

Result and discussion

Isolation of pathogen

Fusarium udum was isolated from the infected roots of pigeonpea plants collected from Pulse pathology block G.B. Pant University of Agriculture and Technology, Pantnagr (Uttrakhand). The pathogen was identified on the basis of presence of microconidia, macroconidia and chlamydospores under light microscope.

Growth of *Fusarium udum* on different culture media

To investigate the optimal growth conditions for *Fusarium udum*, a study was conducted using ten different culture media. The findings revealed that Potato Dextrose Agar (PDA) facilitated the maximum colony diameter (90.0mm) and exhibited abundant sporulation. The second most effective media was Czapek dox agar, which achieved a colony diameter of 88.33 mm, followed by the host root extract medium with a colony diameter of 83.00 mm. Conversely, the least colony diameter and poor sporulation were observed on T-2 medium, with a diameter of 56.67 mm (Table 2; Graph 1 and Plate 1). However, the most conducive medium for growth and sporulation for *Fusarium udum* was PDA. These results align with the observations of **Anjaneya Reddy (2002),** who also reported maximum growth of *Fusarium udum* on PDA, followed by Richard's agar medium. Similarly, **Gangadhara *et al.* (2010)** found that PDA and Richard's agar were the optimal media for the growth of *F. oxysporum* f. sp. *vanillae*. Recent findings by **Chaudhary *et al.* (2018)** also corroborate the results of the present study.

Growth of *Fusarium udum* on different temperature

The study investigated the growth and sporulation of the pathogen *Fusarium udum* from a temperature range of 5-40°C. The optimal temperature for mycelial growth (89mm diameter) and sporulation was determined at 25°C, with a slightly lesser colony diameter of 75.67mm observed at 20°C. The minimal mycelial growth and sporulation, measuring 0 mm, were noted at 40°C (Table 3; Graph 2 and Plate 2). The study demonstrated that the growth and sporulation of the test pathogen was highest at temperature 20-30°C. These findings align with the research of **Khan *et al.* (2011),** which identified the optimal temperature for mycelial growth and sporulation of

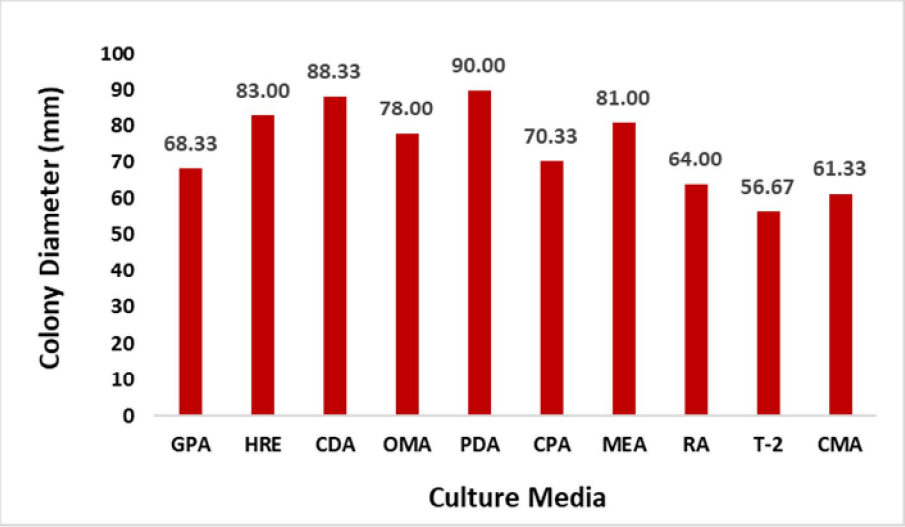
Fusarium oxysporum f.sp. *ciceri* as 30°C, followed by 25°C. Similarly, **Khilare and Ahmed (2012)** reported that the ideal temperature for the growth of *Fusarium oxysporum* f.sp. *ciceri* was 30ºC after fourteen days of inoculation, with a decline in growth observed below at 15ºC and above 35ºC. Furthermore, recent research by **Desai *et al.* (2016)** supports the present study by recording maximum growth of *Fusarium udum* at 28ºC.

Growth of *Fusarium udum* on different pH level

To determine the optimal pH level for the growth and sporulation of *Fusarium udum*, an experiment was conducted across a pH range from 4.0 to 8.5. The results indicated that the fungus exhibited the most robust growth and sporulation at a pH of 6.5, where the colony diameter reached 89.67 mm. This was closely followed by growth at pH levels of 6.0 and 7.0, with colony diameters of 83.33 mm and 82.78 mm, respectively. Conversely, minimal colony growth was observed at pH levels below 4.5 and above 8.5. The study revealed that the growth and sporulation of *Fusarium udum* was negatively affected as pH level deviated from the range of 6.0-7.0 pH. Extreme acidic and alkaline pH level were found unsuitable for test pathogen. These findings are consistent with the research of **Khilare and Ahmed (2012),** who reported that the optimal pH for *Fusarium oxysporum* f.sp. *ciceri* was between 6.0 and 6.5. Additionally, **Tyagi and Paudel (2014)** corroborated these results, demonstrating that the ideal pH for *Fusarium oxysporum* growth was also around 6.0.

Table 2: Effect of different culture media on growth of *Fusarium udum* on Potato Dextrose Agar.

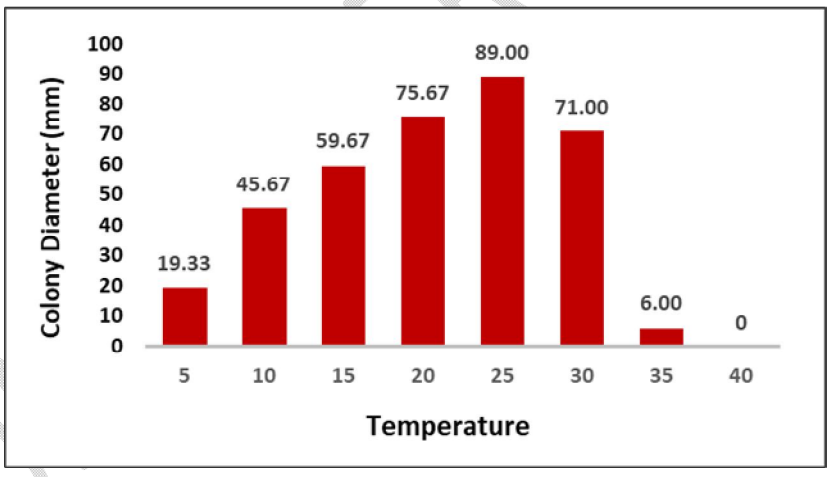
Treatments (Media)	Colony Diameter (mm)	Sporulation
Glucose peptone agar media (GPA)	68.33	+
Host root extract media (HRE)	83.00	+++
Czapek dox agar media (CDA)	88.33	++++
Oat meal agar media (OMA)	78.00	+++
Potato dextrose agar media (PDA)	90.00	++++
Carrot Potato agar media (CDA)	70.33	+
Malt extract agar media (MEA)	81.00	+
Richard's media (RA)	64.00	++
T2 media (T2)	56.67	-
Corn meal agar media (CMA)	61.33	+
C.D.	1.905	
C.V.	1.483	
S.Em.	0.641	



Graph 1: Effect of different culture media on mycelial growth of *Fusarium udum*

Table 3: Effect of different temperatures on growth of *Fusarium udum* on Potato Dextrose Agar.

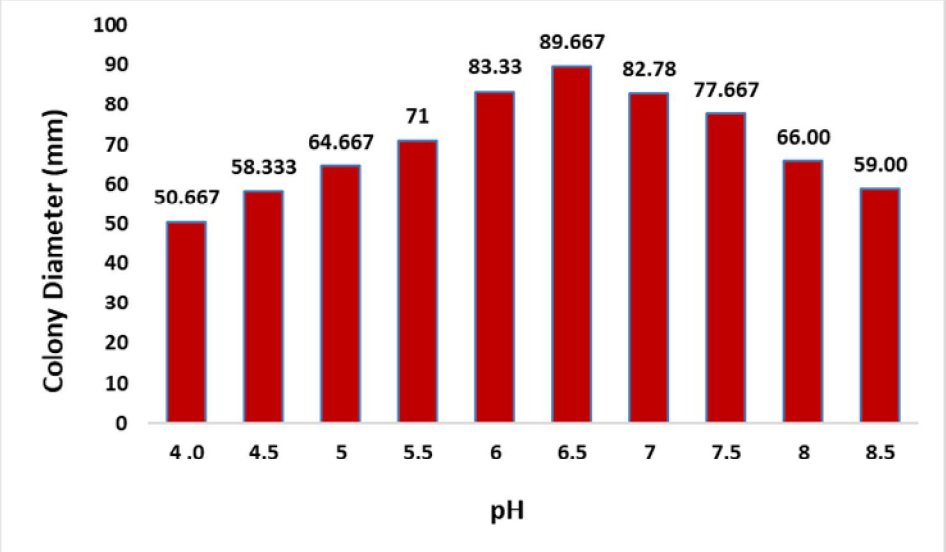
Treatments (Temperature °C)	Colony Diameter (mm)	Sporulation
5	19.33	-
10	45.67	-
15	59.67	+
20	75.67	++
25	89.00	++++
30	71.00	+++
35	6.00	-
40	0	-
C.D.	1.285	
C.V.	1.607	
S.Em.	0.425	



Graph 2: Effect of different temperature on mycelial growth of *Fusarium udum*

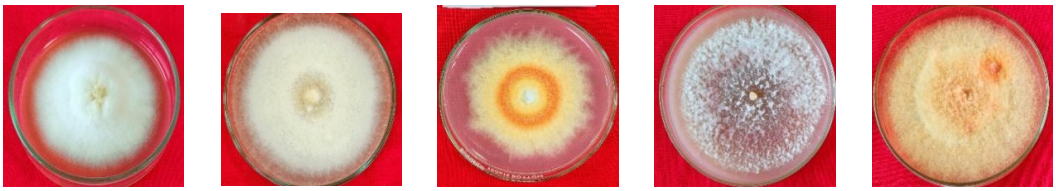
Table 4: Effect of different pH levels on growth of *Fusarium udum* on Potato Dextrose Agar

Treatments (pH)	Colony diameter (mm)	Sporulation
4 .0	50.667	-
4.5	58.333	+
5.0	64.667	++
5.5	71.00	++
6.0	82.78	++++
6.5	89.667	++++
7.0	83.333	+++
7.5	77.667	++
8.0	66.00	+
8.5	59.00	-
C.D.	2.887	
C.V.	0.972	
S.Em.	0.972	



Graph 3: Effect of different pH levels on mycelial growth of *Fusarium udum*

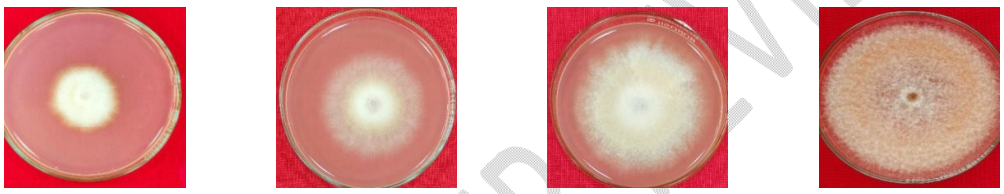
Conclusions



GPA HRE RA OMA PDA

CPA MEA CDA T-2 CMA

Plate 1: Effect of different culture media on mycelial growth of *Fusarium udum*

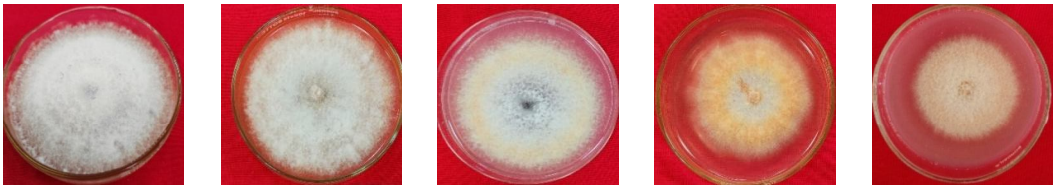


5°C 10°C 15°C 20°C

25°C 30°C 35°C°C 40°C

Plate 2: Effect of different temperature on mycelial growth of *Fusarium udum*

4.0 4.5 5.0 5.5 6.0



6.5 7.0 7.5 8.0 8.5

Plate 3: Effect of different pH levels on mycelial growth of *Fusarium udum*

Conclusion

Fusarium udum isolated from pigeonpea plant roots exhibited varying growth responses to different temperature, media, and pH levels. Potato Dextrose Agar (PDA) was found most congenial medium, achieving maximum colony diameter and PDA also provided the most conducive condition for sporulation. The pH range of 6.0-7.0 was found to be optimal for the growth and sporulation of *Fusarium udum*. Temperature trials indicated that the ideal range for maximum mycelial growth and sporulation is between 20-30°C. These findings highlight critical factors for future research and management strategies to mitigate the spread of pigeonpea wilt. The consistency of these results with prior studies strengthens their reliability and offers valuable insights for developing effective culturing and control measures for *Fusarium udum*.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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