

**STUDIES ON WILT INCIDENCE AND CULTURAL VARIABILITY OF  
*Fusarium oxysporum* f.sp. *udum* OF PIGEONPEA COLLECTED FROM  
DIFFERENT DISTRICTS OF BUNDELKHAND REGION OF U.P**

**THESIS**

**SUBMITTED TO THE**



**BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, BANDA  
UTTAR PRADESH (INDIA)**

**BY**

**VIVEK SINGH**

**(Id. No. 1018)**

**IN PARTIAL FULFILMENT OF THE REQUIRMENTS  
FOR THE DEGREE OF**

**MASTER OF SCIENCE (AGRICULTURE)**

**IN**

**PLANT PATHOLOGY**

**2020**

**BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, BANDA-210001**

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**Certificate-I**

This is to certify that the thesis entitled “**Studies on wilt incidence and cultural variability of *Fusarium oxysporum* f.sp. *udum* of pigeonpea collected from different districts of Bundelkhand region of U.P**” submitted in partial fulfillment of the requirement for award of the degree of **Master of Science (Agriculture) in Plant Pathology**, College of Agriculture, Banda University of Agriculture & Technology (Banda), is a genuine record of bonafide research work carried out by **Mr.Vivek Singh (LD.No-1018)** under my guidance and supervision. The results of the investigation in this thesis have not so far been submitted for any other degree or diploma.

It is further certified that the help or information received during the course of investigation and preparation of the thesis have been duly acknowledged.

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## ACKNOWLEDGEMENT

I am very much grateful to GOD and MATA SARASWATI for rescuing me from mental crises whenever I faced.

I am deeply indebted to my supervisor **Dr. Virendra Kumar Singh**, Head, Department of Plant Pathology, College of Agriculture, Banda University of Agriculture and Technology, Banda for his valuable and inspiring guidance, close supervision constant encouragement and constructive criticism during the course of investigation and preparation of this thesis.

I am great full to **Dr. Dharmendra Kumar**, Associate Professor, Department of Plant Pathology **Dr. Durga Parsad** Assistant Professor, Department of Plant Pathology, **Dr. S.B. Singh** Assistant Professor, Department of Agronomy, **Dr. S.K. Singh**, Head Department of Entomology (Registrar Nominee), members of the advisory committee for their timely advice and help.

I extend my cordial gratitude and sincere regards to **Dr. U.S. Gautam**, Hon'ble Vice Chancellor, and **Dr. G.S. Panwar**, Registrar and Dean, College of Agriculture, Banda University of Agriculture & Technology, Banda to providing facilities for the research work.

I owe my sincere thanks to **Dr. Vivek Singh**, Assistant Professor and **Dr. H.S. Negi**, Assistant Professor Department of Plant Pathology for their valuable suggestions and help during my studies.

I am thankful to my friends Kshitij Gupta, Arvind Kumar, Pardeep Kumar, Ajeet Singh, Anuj Mishra, Priya Singh, Sandhya Fariya and all my juniors for their copperation. My sincere thanks to Mr. Shiv Prakash Bajpai, Stenographer and Mr. Satya Prakash for their help in lab work and typing.

I express my sincere regards to my parents, brother, sister for their affection, encouragement and blessing which boosted my moral when needed.

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## ABBREVIATION

PDA	Potato Dextrose Agar
@	at the rate
%	Per cent
+_	Plus minus
et al.	And other
°C	Degree Celsius
cm	Centimetre
g	Gram
i.e.	that is
ml	millilitre
kg	kilogram
sp.	Species
viz.	namely
DAI	Days after inoculation
Fig.	Figure
ha.	Hectare
CD	Critical difference
SEM	Standard error of mean
q	Quintal
mm	Millimeter
RBD	Randomized Block Design
CRD	Completely Randomized design
etc.	Etcetra
dia	Diameter
mg	Milligram
e.g.	exempli gratia (For Example)
cfu	Colony forming units
/	Per
conc.	Concentration

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### ABSTRACT

Pigeon pea *Cajanus cajan* (L.) is an important pulse crop grown throughout India. *Fusarium udum* is the most important disease of this crop. Survey was conducted of pigeonpea growing areas of 4 districts in Bundelkhand region of Uttar Pradesh during 2019-20. The average disease incidence ranged between 3.25% to 49.00% from district to districts. The average incidence percentage of wilted plants in Chitrakoot district was 39.06% followed by Banda district 25.67%, Hamirpur district 15.99% and Mahoba district 14.64% respectively. *Fusarium udum* was found constantly associated with the root samples. This indicates that this fungus, a well known wilt pathogen, was primarily responsible for the wilt disease of pigeonpea.

Pathogenicity of *Fusarium udum* was tested on pigeonpea variety 'Bahar' all isolates of *F. udum* showed the typical wilting symptoms and proved Koch's postulates. The height and number of leaves was maximum in mahoba isolates followed by hamirpur, banda and chitrakoot isolates. The maximum wilting was recorded in chitrakoot isolate followed by banda, hamirpur, and mahoba isolates. Chitrakoot isolate of *F. udum* was found more pathogenic and caused higher wilt incidence than other isolate.

All the isolates differed in their radial growth colony characters on both solid media. It was found that PDA was the best medium in compare to PSA. The chitrakoot isolate was fast growing followed by banda, hamirpur and mahoba isolates. Sporulation was moderate to excellent in different isolates. The highest radial growth was observed in chitrakoot isolate on PDA while minimum radial growth was observed on PDA in mahoba isolate. However, the maximum radial growth was a recorded on PSA in chitrakoot isolate and minimum radial growth in mahoba isolate. Micro conidia are single or bicelled, hyaline, mostly curved, ovoid to fusoid and scattered and measure 3.80-9.12 x 0.98-2.10µm. Macro conidia which are hyaline, thin walled, falcate with a distinct foot cell, and an apical cell that decreases in width towards the tip. The most distinguishing characteristic of the macro conidia are their strongly curved or hooked apices and measure 11-21.12 x 1.95 to 3.78µm.

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शोध शीर्षक	: उत्तर प्रदेश के बुन्देलखण्ड संभाग के विभिन्न जिलों में अरहर के उकठा रोग व्यापकता एवं संवर्धन विभिन्नता पर अध्ययन		

### सारांश

अरहर संसार के बहुत देशों में उगायी जाने वाली प्रमुख दलहन की फसल है। भारत वर्ष के कई प्रदेशों में इसकी खेती उच्च स्तर पर की जाती है। अरहर का एक प्रमुख रोग उकठा भी है उकठा रोग क बिमारी की जानकारी हेतु उत्तर प्रदेश के बुन्देलखण्ड संभाग के चार जिलों में सत्र 2019-20 के दरम्यान सर्वे किया गया। जिसमें चारों जिलों के अनुपात के अनुसार बिमारी की बहुलता 3.25 से 49.0 % थी जबकि सबसे ज्यादा बिमारी 39.06 % चित्रकूट जिला में तथा दुसरे क्रम में बाँदा जिला में 25.6 %, हमीरपुर जिला में 15.99 % और महोबा जिला में 14.6 % दर्ज की गयी। उकठा रोग से ग्रसित अरहर के जड़ व तना दोनों में देखा गया है।

रोगजनकता का अध्ययन अरहर के बहार प्रजाति के ऊपर चारों जिलों के वियोजको द्वारा पाट में किया गया। अरहर के ऊपर जो लक्षण देखे गये वे खेत में पाये जाने वाले लक्षणों की तरह थे। इससे सिद्ध हुआ कि यह उकठा रोग ही है। चित्रकूट वियोजक का प्रभाव अरहर के ऊपर सबसे प्रचंड था इसके बाद बाँदा, हमीरपुर व महोबा दर्ज किया गया। अरहर की ऊँचाई व पत्तियों की संख्या सबसे ज्यादा महोबा, हमीरपुर, बाँदा व चित्रकूट में पायी गयी।

इन वियोजको का रेडियल वृद्धि एवं कालोनी लक्षण दो तरह के माध्यम पर देखा गया । जिसमें विभिन्नता पायी गयी। पी0डी0ए0 माध्यम मुख्य रूप से पी0एस0ए0 माध्यम से अच्छा साबित हुआ । कवक की सबसे तीव्र वृद्धि चित्रकूट के बाद बाँदा, हमीरपुर व महोबा में दर्ज की गयी। सबसे ज्यादा रेडियल वृद्धि चित्रकूट वियोजक का पी0डी0ए0 माध्यम के ऊपर दर्ज किया गया। फ्यूजेरियम उडम संवर्धन को लेकर सूक्ष्मदर्शी के द्वारा सूक्ष्म कोनिडिया, वृहद कानिडिया एवं कोनिडियमधर की आकृति को देखा गया है। सूक्ष्म कोनिडिया की साइज 3.80 – 9.12 x 0.98 – 2.10 माइक्रान मीटर तथा वृहद कोनिडिया की साइज 11-21.12 x 1.95 – 3.78 माइक्रान मीटर दर्ज की गयी।

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## INTRODUCTION

Pigeonpea commonly known as red gram, arhar, tur, and it is one of the major grain legume (pulse) crops of the tropics and subtropics. Globally, it is the sixth most important legumes food crop and India is the largest producer. Pigeonpea accounts for 4.42 million hectares of area, production 2.86 million tones and yield 647 kg ha<sup>-1</sup> in India (FAOSTAT, 2011). It is largely eaten in the form of split pulse as dal. Pigeonpea is a major source of protein; enriches soil; provides fodder and fuel, wood and it is beneficial for arresting soil erosion (Ae et.al., 1990 and Saxena et al., 2002). This is the way generations of Indian people on the subcontinent have used pigeonpea for over 2000 years. Besides its main use as dal, its tender green seeds are used as vegetable, seed husk and pod wall as cattle feed, green leaves as fodder, stem as fuel wood. Extensive ground cover by pigeonpea prevents soil erosion by wind and water, encourages infiltration, minimizes sedimentation and smothers weeds. Being a legume it fixes nitrogen in to the soil in which it is grown. The plants have been described as soil plough because of their deep root system in the soil which plays a significant role in the improvement of soil structure. Extensive root system provides ample organic matter in the soil.

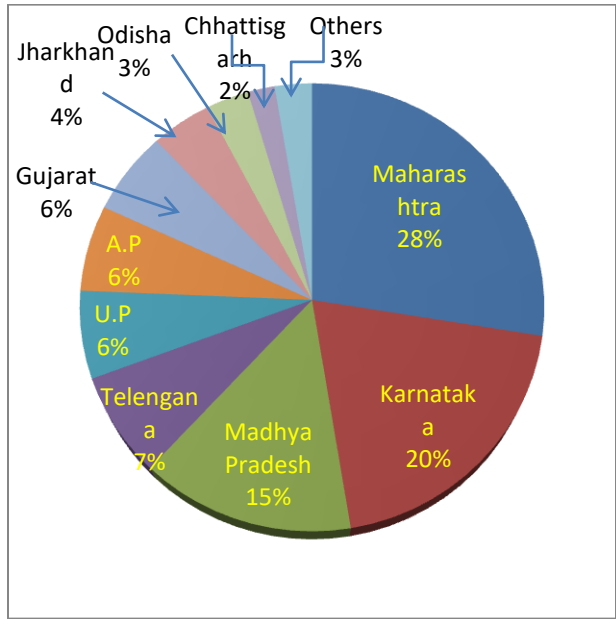
### **Area and production:**

In India, pigeonpea is the most important grain legume ranking second after chick pea (*Cicer arietinum*) in area and production. India is the world's largest producer and growers over 77% of total world production. The Major pigeonpea growing states of India are Maharashtra, Madhya Pradesh, Uttar Pradesh, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu and Bihar (Table-1 and Plate.1 and 2).

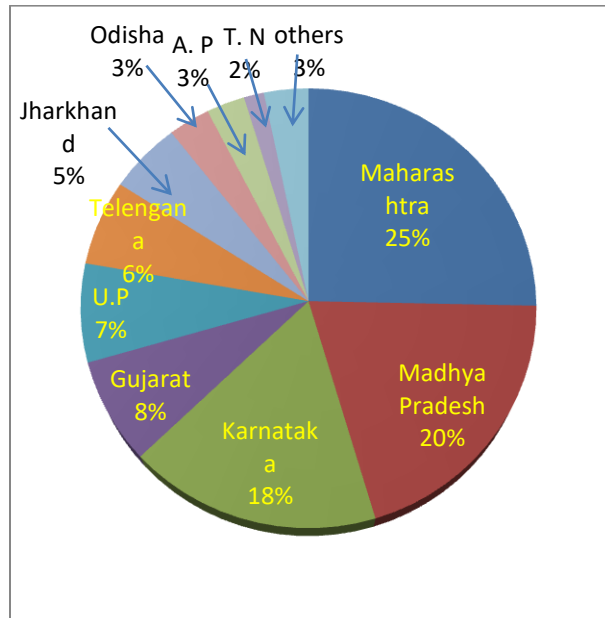
**Table 1. States contribution in area and production of pigeonpea (2017-18)****Area lakh ha, Production lakh tons**

<b>States</b>	<b>Area</b>	<b>Contribution</b>	<b>States</b>	<b>Production</b>	<b>Contribution</b>
		<b>%</b>			<b>%</b>
Maharashtra	12.29	27.56	Maharashtra	10.59	25.33
Karnataka	8.85	19.85	Madhya Pradesh	8.39	20.07
Madhya Pradesh	6.47	14.51	Karnataka	7.29	17.44
Telangana	3.31	7.42	Gujarat	3.21	7.68
Uttar Pradesh	2.82	6.32	Uttar Pradesh	3.03	7.25
Andhra Pradesh	2.78	6.23	Telangana	2.64	6.32
Gujarat	2.71	6.08	Jharkhand	2.22	5.32
Jharkhand	1.94	4.34	Odisha	1.28	3.05
Odisha	1.38	3.09	Andhra Pradesh	1.18	2.82
Chhattisgarh	0.86	1.93	Tamilnadu	0.61	1.47
Others	1.19	2.67	Others	1.36	3.25
<b>All India</b>	<b>44.59</b>		<b>All India</b>	<b>41.80</b>	

**Source:** DES, Ministry of Agri. &FW (DAC&FW), Govt. of India; 2017-18 - IIIrd Adv.Est.



**Plate .1. Contribution of states in area**



**Plate .2. Contribution of states in production**

**Diseases:**

Pigeonpea can be attacked by more than 100 pathogens (Nene et al., 1989 c). These include fungi, bacteria, viruses, nematodes and phytoplasma. However, only a few of them cause economic losses (Kannaiyan et al., 1984) and the distribution of the major diseases is geographically restricted. Such as an important crop is affected by a number of diseases and it is one of the main reasons for low productivity of pigeonpea. The diseases of considerable importance at present are sterility mosaic, fusarium wilt, phytophthora blight, macrophomina root rot, stem canker, and alternaria blight on the Indian subcontinent, wilt and cercospora leaf spot in eastern Africa, witches broom in the Caribbean and Central America.

**Fusarium wilt (*Fusarium udum*):**

Wilt disease caused by *Fusarium oxysporum* f. sp. *udum* is the most important disease of pigeonpea worldwide. This is the most important soil born disease of pigeonpea and was first described in 1906 from Bihar state, India (Butler, 1906). Wilt is the most destructive disease of pigeonpea in India. To date *Fusarium* wilt has been reported from several countries like India, Indonesia, Ghana, Bangladesh, Kenya, Nepal, Thailand, Tanzania, Uganda and Trinidad (Nene et al.; 1989), but it is relatively more important in India and eastern Africa. It is very destructive in parts of UP, Bihar, Maharashtra, M.P, W.B, A.P, Tamil Nadu and Karnataka. The lower productivity of pigeonpea in india is due to different biotic and abiotic stress. The disease may be noticed in August in June sown crops but the highest mortality occurs at flowering and podding time from November onwards. Although the disease first appears in patches in a field, it can extend to the entire field if pigeonpea is repeatedly cultivated in same field. Even though plants are infected at an early stage, the wilt symptoms are not expressed until flowering and podding. The yield loss depends on the stage at which the plants wilt, it can approach 100%.When wilt

occurs at the pre pod stage, about 67% when wilt occurs at maturity and 30% when it occurs at the preharvest stages (Kannaiyan and Nene,1981). Singh et al., (2002) observed a yield loss of 10-50% and in some years up to 90% in pigeonpea due to *Fusarium* wilt in farmers' fields. Wilt incidence generally increases when the crop is ratooned or retained as perennial. The disease is seed borne and soils born .The annual losses due to wilt has been estimated at \$71 million in India and \$5 million in Eastern Africa (Pandey et al., 2012). The objective of this study was to survey the different wilt incidence in susceptible pigeonpea of four districts of bundelkhand region of Utter Pradesh with this in view the present study was under taken on the following aspects:

- Exploration and collection of wilt disease infecting pigeonpea from different districts
- Isolation, characterization and Identification of pathogen from collected samples
- Study on cultural variability of selected isolates of *Fusarium udum*
- Purification and maintenance of fungal isolates
- Pathogenicity test

## REVIEW OF LITERATURE

### Survey for wilt incidence at different districts of Bundelkhand region:

Lilly and Barnett (1951) demonstrated that every microorganism requires food for its growth and survival. There is no single medium upon which can grow. Prasad and Chaudhary (1974) studied the production of fusaric acid and sporulation by different isolates of *Fusarium oxysporum* f.sp. *udum*. They found that fusaric acid liberated during rapid growth phase of the mycelium from pigeonpea. Padwick (1940) stressed that one of the differences between *Fusarium udum* and *Fusarium vasinfectum* is that the former produces macro conidia which are hooked at the apex. Subramanian (1951) studied the nine isolates of *Fusarium udum* of which six had abundant aerial mycelium, one had aerial mycelium forming a thin mat and two had no aerial mycelium. Out of these nine isolates one produced neither sporodochia nor pinnotes while the other eight produced both sporodochia and pinnotes. Cent percent grain losses has been reported due to Fusarium wilt when occurred at pre- pod stage, 67 per cent at pod maturity stage and 29.5 per cent at pre harvest stage (Kannaiyan, 1981). The existence of variants /races of *F. udum* have been reported and have been cited as a major drawback in the development of pigeonpea varieties resistant to Fusarium wilt (Okiror and Kimani, 1997). The survey of *Fusarium udum* incidence of pigeonpea worked by Pawar et al, (2013) Sushreeta et al, (2017) and Maurya et al., (2020).

### Cultural variability and radial growth of *Fusarium udum* :

Subramanian (1955) grouped one hundred isolates of *Fusarium udum* in seven groups on the basis of mycelium growth, presence or absence of stroma, sclerotia, sporodochia and pinnotes. Cultures varied from complete absence to presence and fluffy development of aerial mycelium, through intermediates where in the aerial mycelium was developed in patches and formed islands. The majority of the isolates had little or no aerial mycelium. Jeswani, et al., (1977) showed the morphological variability in *Fusarium lateritium* f. *cajani*. They found different isolates have the capacity of secreting differing quantities of metabolic products. Subramanian (1955) recorded the morphology of *F. udum* as follows. The micro conidia one-celled, hyaline elliptical, sometimes curved, 4-14 x 1-4 um in size. Booth (1971) gave the size of macroconidia as 15-30 x 2.5-3.5 um and that of micro conidia as 6-11 x 2-3 um. He described the chlamydosores to be intercalary in the mycelium and 8-10 um in diameter. Mahesh et al., (2010) studied the morphological and

cultural variability of six isolates of *Fusarium udum* collected from Bangalore, Kolar, Hoskote, Ramanagar, Anekal and Jagalur. All the isolates showed the significant variations with respect to morphological characters viz, the size of macro conidia and micro conidia varied from 10.51-18.70 x 1.27-3.10  $\mu\text{m}$  and 3.62-8.12 x 0.96-1.80  $\mu\text{m}$  respectively. Number of septa of macro conidia and micro conidia varied from 2.12-2.93 and 0-0.61 respectively. The experimental report of Gaur and Sharma (1989) point out the high degree of cultural variability's among isolates of *F. udum* regardless of their geographical origins. Kiprof et al., (2002) cultural characteristics, pathogenicity and vegetative compatibility of *Fusarium udum* isolates from pigeonpea in Kenya. Shalini and Dohroo (2003) found that morphological and cultural variability existed among isolates of *Fusarium oxysporum* f. sp. *pisi* collected from pea. The isolates showed slow to rapid growth, variable pigmentation and morphology of the hyphae, micro conidia, macro conidia and chlamydospores. The cultural and morphological variability of *Fusarium oxysporum* f.sp. *ricini* isolates from castor growing areas ( Sangava et al., 2018). Gupta et al., 1988, Mahesh et al., 2010, Ghante et al., 2018, Shushreeta et al., 2018 showed the morphological and cultural variability in *Fusarium udum*.

#### **Pathogenicity test:**

Being a soil born pathogen, *Fusarium udum* and the fungus enters the host vascular system at root tip through wound leading to progressive chlorosis of leaves, branches, wilting and collapse of the root system (Jain and Reddy, 1995). Sarojini (1951) the pathogenicity test with *Fusarium udum* isolates show that the strain 1, strain 11 and strain 111 were more virulent than other strains. The strain 5 and 6 cause pre-emergence wilt. Singh and Husain (1970) showed the filterates of the fungus *Fusarium lateritium* f.sp. *cajani* induced irreversible wilting, maceration of stems and browning of basal tissues of young pigeonpea cuttings. Baldev and Amin (1974) studied on the existence of races in *Fusarium udum* and concluded that a number of pathogenic races of *Fusarium udum* exist. Upadhyaya and Rai (1982) reported the perfect state of *F. udum* on wilted and dead pigeonpea plant near Varanasi in Uttar Pradesh and identified it as a new species of *Gibberella* and named it as *Gibberella indica*.

## MATERIALS AND METHODS

The materials used and techniques adopted in accomplishing the objectives of the present investigations are described under: survey, collection, identification, disease incidence, cultural variability and morphological characters of *Fusarium udum* collected from different districts of Bundelkhand region and pathogenicity were carried out under this study.

### **Experimental site:**

Pot and lab experiment was conducted in the department of Plant Pathology, Collage of Agriculture, Banda University of Agriculture and Technology, Banda, U.P and survey of pigeonpea fields at Banda, Mahoba, Hamirpur, and Chitrakoot districts of U.P.

### **Survey for wilt incidence at different districts of Bundelkhand region:**

Survey of pigeonpea fields was conducted at Banda, Mahoba, Hamirpur, and Chitrakoot districts of Bundelkhand region of Uttar Pradesh during kharif season in 2019-20 to know the wilt disease incidence of pigeonpea. Random roving method of survey was carried out to record the severity of *Fusarium* wilt in pigeonpea. The area having maximum wilt incidence was selected from those areas. Observations on disease incidence of the surveyed plots were recorded. The percent disease incidence was assessed by the formula:

$$\text{Disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of observed plants}} \times 100$$

### **Collection, isolation, purification, maintenance of *Fusarium udum* isolates:**

After collection of diseased samples, the collar region of each samples were washed thoroughly in running tap water to remove the soil particles. Each sample was cut into small bits and at least 5 bits were surface sterilized by dipping in 0.1% mercuric chloride for 1 minute and rinsed three times in sterilized distilled water. The surface sterilized bits were then transferred to separate petriplates containing PDA medium under aseptic condition. The procedure was repeated for each sample. The petriplates was incubated at 25+<sub>-</sub>2<sup>0</sup>C till visible fungal colonies appeared. From the colonies a small bit of actively growing mycelium were cut and transferred to separate sterilized petriplates. Each isolate was purified by adopting single spore isolation technique

(Kiprof et al., 2002). The cultures of all isolates were maintained on PDA slants at  $25 \pm 2^{\circ}\text{C}$  for further studies.

### **Cultural variability and radial growth of *Fusarium udum* :**

The study was conducted at Department of Plant Pathology, Banda University of Agriculture and Technology, Banda during 2019-20. To study the variability among the isolates of *Fusarium oxysporum* f. sp. *udum* on PDA and PSA solid media. The isolates were collected from different farmer's fields of Bundelkhand districts of U.P. *Fusarium udum* was grown on Potato dextrose agar (PDA) and Potato sucrose agar (PSA). Radial growth and colony characters were recorded on PDA and PSA solid media. To culture *Fusarium udum* isolates on solid media, petriplates of uniform size (90 mm) were used. These were sterilized in hot air oven at  $160^{\circ}\text{C}$  for 1 hour. 20 ml of sterilized media was transferred in each sterilized petriplates. A disc of 5mm was cut with the help cork borer from the edge of actively growing 7 days old culture and placed in the center of the petriplate containing different media. All isolates were grown on PDA and PSA plates in triplicate. Inoculated petriplates were inoculated at  $25 \pm 2^{\circ}\text{C}$  for 21 days. Three were maintained in each media and for each isolates. The growth of the fungus was recorded as diameter in mm. The colony characters were noted on the after 21 days. The pattern of growth was also noted, fluffy partially submerged and submerged (Jeswani et al, 1977 and Gaur and Sharma, 1989).

### **Morphological character:**

Pigeonpea wilt pathogen was identified on the basis of their cultural and morphological characteristic. Slides were prepared with cotton blue stain and examined under the compound microscope for morphological characters of the fungus.

### **Potato Dextrose Agar (PDA) medium composition:**

Peeled Potato –	200 gm
Agar-Agar -	20 gm
Dextrose -	20 gm
Distilled water -	1000 ml
pH -	6-6.5

**PDA preparation procedure:**

The required quantities of peeled potatoes were cut in to small pieces, boiled in 500 ml of distilled water till the pieces become soft. The potato extract was filtered through muslin cloth and the filtered was collected in the beaker. Rest of the 500 ml water was made warm 20 gm agar and 20 gm dextrose was added properly by shaking through glass rod. Both solutions were mixed in beaker and volume was maintained up to 1000 ml by adding required amount of distilled water. Two hundred ml of this solution was dispensed in each conical flask of 250 ml capacity. Flasks were plugged with non absorbent cotton plugs and wrapped with silver foil. Flasks containing medium were sterilized at 121°C for 15 minutes in autoclave.

**Potato Sucrose Agar (PSA) medium composition:**

Potato extract	–	500 ml
Agar-Agar	-	20 gm
Sucrose	-	20 gm
Distilled water	-	500 ml
p <sup>H</sup>	-	6- 6.5

**PSA preparation procedure:**

Cook 200 g diced potatoes in 500 ml of water for 10 minutes and filter through muslin cloth. The agar is melted in other 500 ml of the water and added to sucrose and the final volume made up to 1.0 liter. Flasks containing medium were sterilized at 121°C for 15 minutes in autoclave.

**Pathogenicity test:**

The pathogenicity of *Fusarium udum* was tested with 'Bahar' variety of pigeonpea. Each isolate was grown on PDA plates. Two actively grown mycelia discs (5mm dia.) from the periphery of 7 days old culture of each isolates were separately inoculated in 500 ml conical flasks containing 100 g pigeonpea meal medium. The flasks were incubated at 25±2°C for 20 days. A fungus-soil mixture was prepared by mixing 200 g of inoculums with 2 kg of autoclaved sand: soil mixture (3:5). Fifteen cm diameter earthen pots were sterilized by formalin (0.1%). These

were then filled with fungus-soil mixture. Seeds sterilized with mercuric chloride (1%) were sown in each pot. Seeds sown in uninoculated pots served as control (Haware and Nene, 1994). Soil moisture was maintained by adding water on throughout the period. Re -isolation of *Fusarium udum* was made from such infected plants and culture were compared with original culture.

## RESULTS

### Survey of wilt disease of pigeonpea in different districts of Bundelkhand region of Uttar Pradesh

Survey was conducted of pigeonpea growing areas of 4 districts in Bundelkhand region of Uttar Pradesh during 2019-20. A total of 56 villages were taken from Bundelkhand region in 4 districts of Uttar Pradesh (Plate-3 and 4). The disease incidence was found in 4 districts of Bundelkhand region of Uttar Pradesh (Table-2 and Fig-1). The average disease incidence ranged between 3.25% (Bari village of Mahoba) to 49.00% (Khoh village of Chitrakoot) from district to districts. The average percentage of wilted plants in Banda district was (25.67%) minimum (13.23 %) being in Hardouli, village of Banda while maximum (39.43%) in Bargahani of village of Banda.

The average percentage of wilted plants in Mahoba district was (14.64%) minimum (3.25%) being in Bari village of Mahoba while maximum (41.00%) in Suraicha village of Mahoba. Hamirpur district showed 15.99% average disease incidence, the minimum (3.50%) being in Sijvahi village of Hamirpur, maximum (38.00%) in Chilli village of Hamirpur. The average percentage of wilted plants in Chitrakoot district was 39.06% the minimum (26.00%) being in Kalla village of Chitrakoot, while maximum (49.00%) in Khoh village of Chitrakoot. The detailed survey inferred that maximum disease incidence was recorded in Chitrakoot district and followed by Banda, Hamirpur and Mahoba.

#### Colony character:

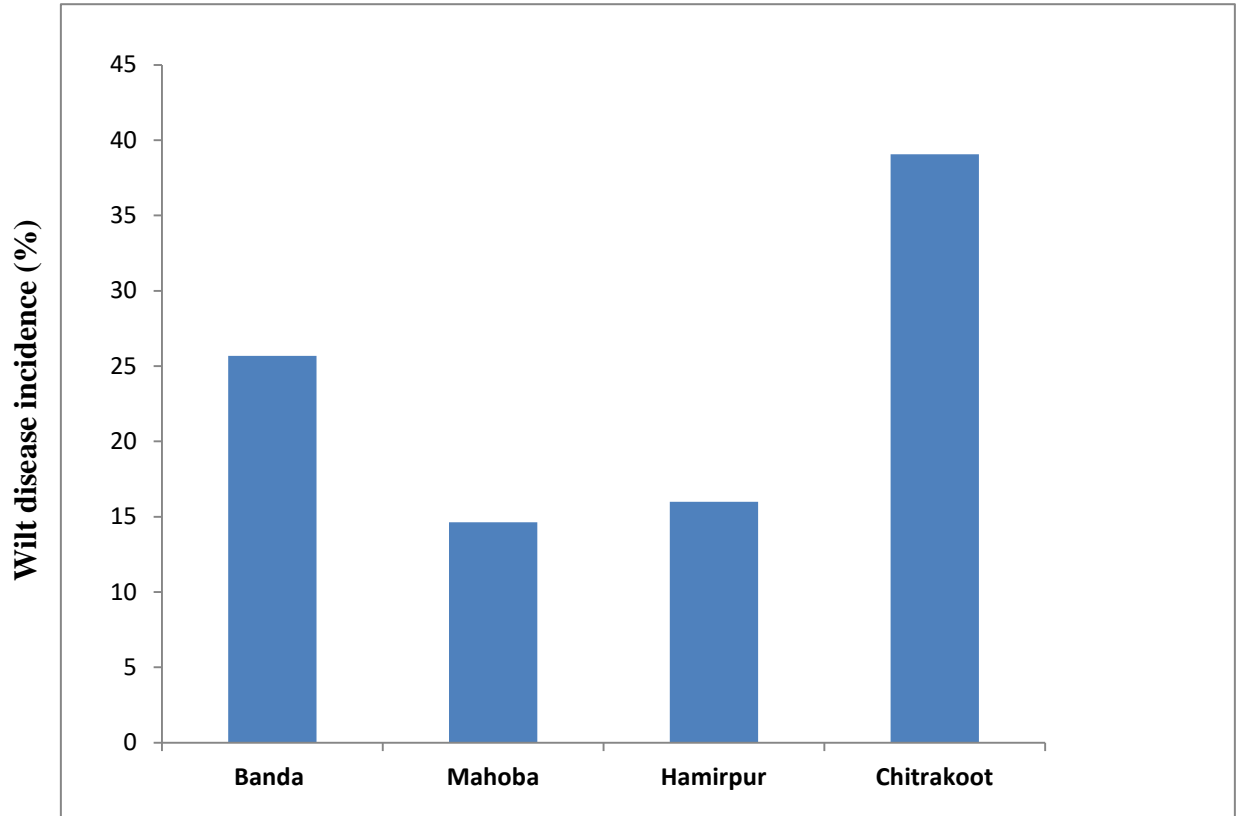
The colony characteristics of different isolates of *Fusarium udum* exhibited on the PDA and PSA media were recorded and presented in Table-3 and Plate 5 and 6.

It is clear from the table that isolates differed in their colony characters on different media. Sporulation was moderate to excellent in different isolates. All the isolates were in good growth on both media. There was a considerable variation among all the isolates of *Fusarium udum*.

**Table 2. Wilt incidence of *Fusarium udum* collected from different districts of Bundelkhand region of U.P**

<b>District</b>	<b>Village/ Location</b>	<b>History of field</b>	<b>PDI (%)</b>
Banda	Jamunipura	Mung/Wheat/Urd/Gram/Pigeon pea	28.76
	Gureh	Mung/Mustard/Pigeonpea/Pigeon pea	25.74
	Mahokhar	Sorghum/Gram/Mung/Gram Pigeon pea	26.33
	Kanvara	Sesame/Gram/Sorghum/Mustard/Pigeon pea	18.06
	Bargahani	Pigeonpea/pigeonpea/pigeonpea	39.43
	Jari	Sorghum/Gram/Urd/Wheat/Pigeon pea	18.29
	Mawaibujurg	Mung/Lentil/maize/wheat/pigeonpea	21.82
	Badeha	Urd/Wheat /Pigeon pea /Pigeon pea	34.53
	Khairada	Pigeon pea /Urd/Gram /Pigeon pea	38.00
	Chandvara	Mung/Gram /Pigeon pea/Pigeon pea	37.36
	Munderi	Sorghum/Wheat /Mung/Gram /Pigeon pea	22.29
	Hardouli	Pigeon pea/Sorghum/Wheat/Pigeon pea	13.23
	Bhabhua	Urd/Mustard/Mung/Gram/Pigeon pea	18.52
	Pailani	Urd/Wheat /Urd/Wheat/Pigeon pea	17.06
Mahoba	Suraicha	Pigeon pea/Pigeon pea/Pigeon pea	41.00
	Revai	Urd/Mustard/Pigeon pea/Pigeon pea	14.57
	Pahara	Urd/Mustard /Pigeon pea/Pigeon pea	17.83
	Sukoura	Mung/wheat/Sorghum/Lentil/Pigeon pea	5.53
	Kharka	Pigeon pea/Sesame/Wheat/Pigeon pea	8.44
	Barbai	Pigeon pe /Pigeon pea /Pigeon pea	36..00
	Sirsi	Mung/Wheat/Sorghum/Mustard/Pigeon pea	7.75
	Mawaikhurd	Urd/Mustard/Sorghum/Gram/Pigeon pea	15.00
	Chichara	Pigeon pea /Pigeon pea/Pigeon pea	17.61
	Suraha	Sorghum/Gram/Urd/Wheat/Pigeon pea	13.79
	Bari	Urd/Wheat/Sorghum/Gram/Pigeon pea	3.25
	Baberi	Urd/Mustard/Sorghum/Linseed/Pigeon pea	8.06

	Barany	Sesame/Mustard /Sesame/wheat/Pigeon pea	12.00
	Kharela	Sesame/Gram/Sesame/Wheat/Pigeon pea	3.56
Hamirpur	Biharka	Sesame/Mustard/Sorghum/Gram/Pigeon pea	8.03
	Patanpur	Sesame/Lentil/Pigeon pea /Pigeon pea	26.57
	Sisolar	Sorghum/Pea Urd/Wheat/Pigeon pea	9.17
	Gusiyari	Pigeon pea/Pigeon pea /Pigeon pea	37.60
	Marauli	Sorghum/Lenti/Sesame/Linseed/Pigeon pea	13.69
	Fattepur	Mung/mustard/sorghum/wheat/Pigeon pea	7.34
	Bhaulami	Sesame/Linseed/Mung/Mustard/Pigeon pea	9.82
	Makarav	Pigeon pea/Pigeon pea/Pigeon pea	37.81
	Tikari Bujurg	Sorghum/Gram/Urd/Linseed/Pigeon pea	4.33
	Sijvahi	sesame/Pea/Mung/Wheat/Pigeon pea	3.50
	Kapsa	Mung/Mustard/Sorghum/Gram/Pigeon pea	7.80
	Chandi	Sorghum/Pea/Mung/Mustard/Pigeon pea	8.61
	Mashgav	Urd/Pea/Sorghum/Gram/Pigeon pea	11.66
	Chilli	Pigeon pea/Pigeon pea/Pigeon pea	38.00
	Chitrakoot	Bhabhai	Pigeon pea/Pigeon pea/Pigeon pea
Rayrani ka chakla		Urd/Gram /Urd/Gram/Pigeon pea	42.25
Banadi		Rice/Gram/Urd/Wheat/Pigeon pea	48.42
Sidhapur		Pigeon pea/Sunflower/Pigeon pea	39.00
Maphikarvi		Rice/Mustard/Pigeon pea/Pigeon pea	36.63
Bagaiha		Urd/Wheat/Rice/Gram /Pigeon pea	38.00
Shivrampur		Urd/Gram/Urd/Wheat/Pigeon pea	41.00
Khoh		Pigeon pea /pigeon pea /Pigeon pea	49.00
Sangrampur		Mung/Mustard/Urd/Gram/Pigeon pea	47.90
Raipurva		Sorghum/Wheat/Pigeon pea/Pigeon pea	35.00
Manohrgang		Pearl millet/wheat/Pigeon pea /Pigeon pea	40.00
Kalla		Mung/Mustard/Pigeon pea /Pigeon pea	26.00
Sikarisani		Urd/Mustard/Mung/Wheat/Pigeon pea	38.00
Pahadi		Pigeon pea /Sorghum/Gram/Pigeon pea	28.00



**Fig -1. Different districts of Bundelkhand region of U.P**



**Banda**



**Mahoba**



**Hamirpur**



**Chitrakoot**

**Plate.3. Wilt disease of pigeonpea in different districts of Bundelkhand region of U.P at farmer's fields**



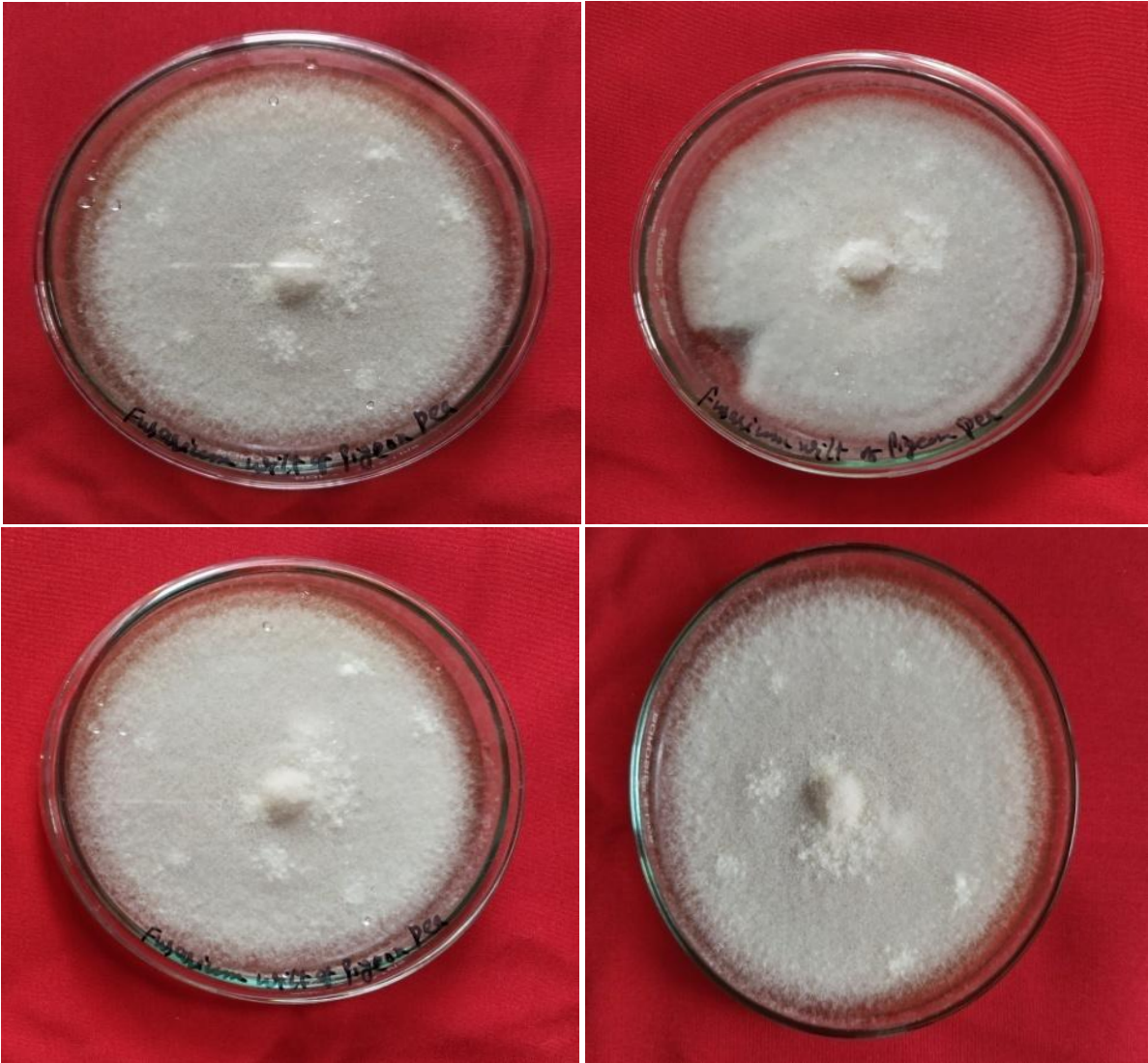
**Plate.4. Wilt disease of pigeonpea at farmer's fields**

**Table-3 Colony characters of different isolates of *Fusarium udum* on solid media**

Media	Isolates	Colour		Aerial mycelium
		Mycelium	Substrate	
PSA	<b>Banda</b>	White	Light Yellow	Fluffy forming concentric rings,
	<b>Hamirpur</b>	White	Light Yellow	Cottony, forming sector
	<b>Mahoba</b>	White	Yellowish	Fluffy, sticky in the middle
	<b>Chitrakoot</b>	White	Light Yellow	Fluffy, sticky
PDA	<b>Banda</b>	White	Yellowish White	Fluffy, Cottony
	<b>Hamirpur</b>	White	Yellowish White	Fluffy, Cottony with sticky mycelium in the middle, forming sectors
	<b>Mahoba</b>	White	Yellowish White	Sticky, Cottony at the point of inoculation
	<b>Chitrakoot</b>	White	Whitish Yellow	Fluffy, sticky



**Plate.5. Colony character of different isolates of *Fusarium udum* on PDA**



**Plate 6. Colony character of different isolates of *Fusarium udum* on PSA**

### Radial growth:

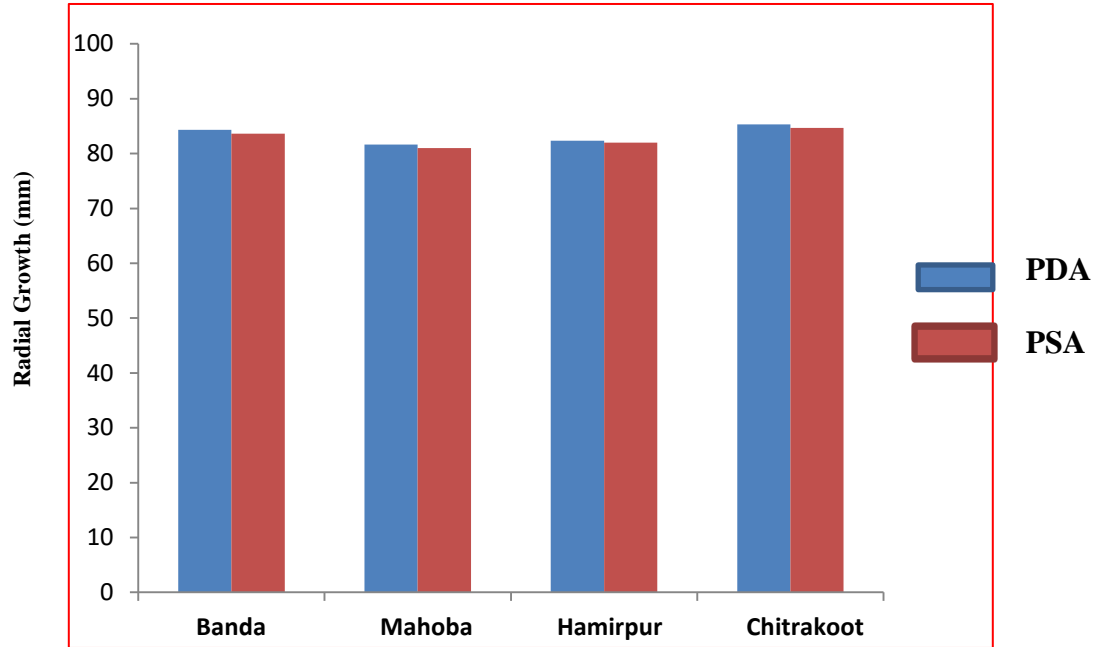
The data on radial growth is tabulated in Table-4 and Fig-2. Radial growth of fungal mycelium was studied and observations were recorded on PDA and PSA media on 21 days of inoculation. The radial growth of chitrakoot isolate was maximum on PDA and PSA media consistently on 21 days of inoculation followed by banda, hamirpur and mahoba isolates. Data of the observations were taken after 21 days of inoculation. Chitrakoot isolate showed the highest radial growth i.e 85.33 mm on PDA followed by banda 84.33 mm, hamirpur 82.33 mm and mahoba 81.66 mm. The mean of radial growth on PDA and PSA media in chitrakoot isolate showed highest 84.99 mm followed by banda 83.99 mm, hamirpur 82.16 mm and mahoba 81.33 mm after 21 days of inoculation. Also the highest radial growth was observed on PDA in case of chitrakoot isolate while least radial growth was observed on PDA in mahoba isolates. The maximum radial growth was recorded on PSA in chitrakoot isolate and minimum in mahoba isolate. The *Fusarium udum* isolates exhibited high variation in radial mycelial growth and sporulation on PDA.

**Table. 4. Radial growth of different isolates of *Fusarium udum* on solid media (mm)**

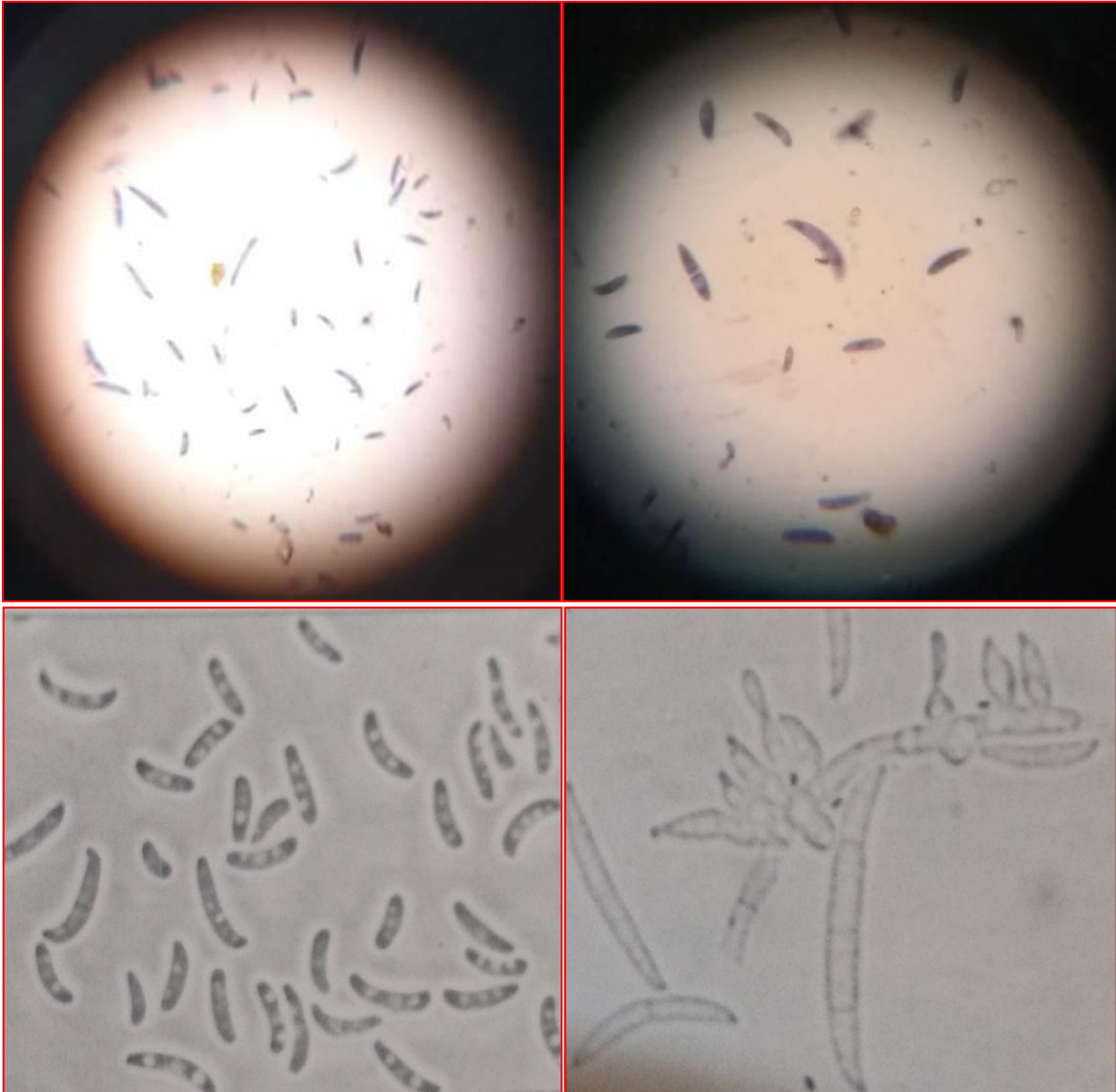
Media	Isolate				
	Banda	Mahoba	Hamirpur	Chitrakoot	Mean
PDA	84.33	81.66	82.33	85.33	83.41
PSA	83.66	81.00	82.00	84.66	82.83
Mean	83.99	81.33	82.16	84.99	

### Morphological character:

The plate 7 revealed that fungus produces mycelium, micro conidia, macro conidia and conidiophores, which are formed from simple or branched conidiophores. Micro conidia are single or bicelled, hyaline, mostly curved, ovoid to fusoid and scattered and measure 3.80-9.12 x 0.98-2.10  $\mu\text{m}$ . Macro conidia which are hyaline, thin walled, falcate with a distinct foot cell, and an apical cell that decreases in width towards the tip. The most distinguishing characteristic of the macro conidia are their strongly curved or hooked apices. They are mostly 3 septate, less frequently 4-5 septate, very rarely 5 septate and measure 11-21.12 x 1.95 to 3.78  $\mu\text{m}$ .



**Fig. 2.** Radial growth of different isolates of *Fusarium udum* on solid media



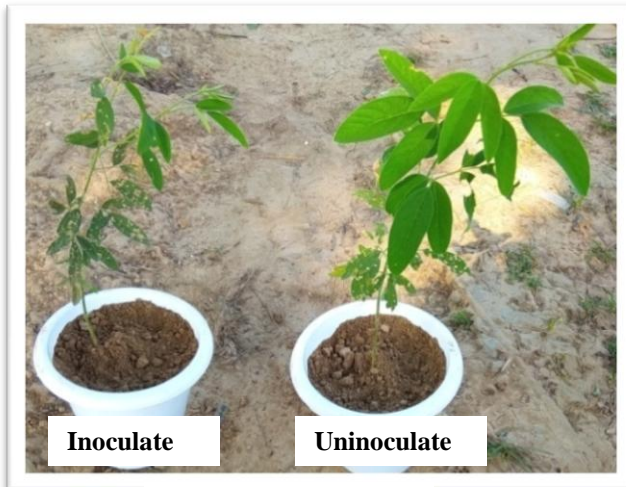
**Plate 7. Microscopic view of micro conidia, macro conidia and conidiophores of *Fusarium udum***

### Pathogenicity test:

Pathogenicity of *Fusarium oxysporum* f.sp. *udum* was tested on pigeonpea variety 'Bahar' after isolation and purification of *Fusarium udum* collected from banda, mahoba, hamirpur and chitrakoot isolates. All isolates of *F. udum* showed the typical wilting symptoms and proved the Koch's postulate and results were shown in (Table 5 and Plate.8). *Fusarium* wilt symptoms on pigeonpea plants were observed from the ten days of inoculation and the fungus re-isolated from the stems and roots of infected plants on the 16 days. The height and number of leaves was maximum in mahoba district followed by hamirpur, banda and chitrakoot isolate. However, chitrakoot isolate was found to be highly pathogenic followed by banda, mahoba and hamirpur isolates. The hamirpur isolate was low pathogenic in compare to chitrakoot, banda and mahoba isolates.

**Table.5. Pathogenicity test of different isolates of *Fusarium udum* in pot condition**

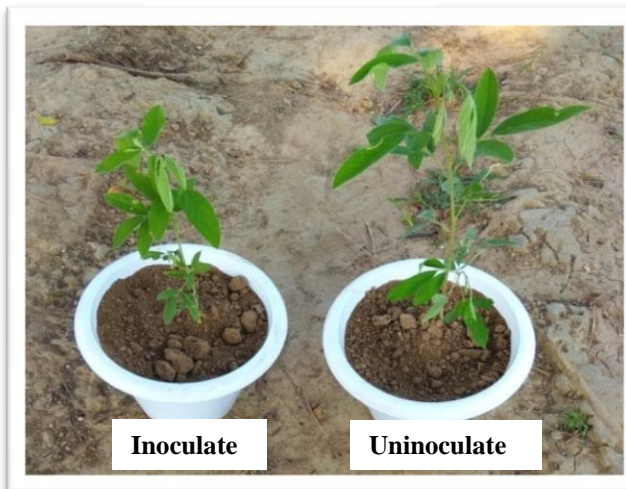
S.N	Name of Isolates	Height of uninoculated plant (cm)	Height of inoculated plant (cm)	No of leaves in uninoculated plant	No of leaves in inoculated plant	Wilting symptom
1.	Banda isolate	39.2	34.2	34.2	23.4	Positive
2.	Mahoba isolate	38.0	35.4	35.0	27.2	Positive
3.	Hamipur isolate	38.8	35.0	34.5	25.5	Positive
4	Chitrkoot isolate	39.0	34.0	34.0	20.5	Positive



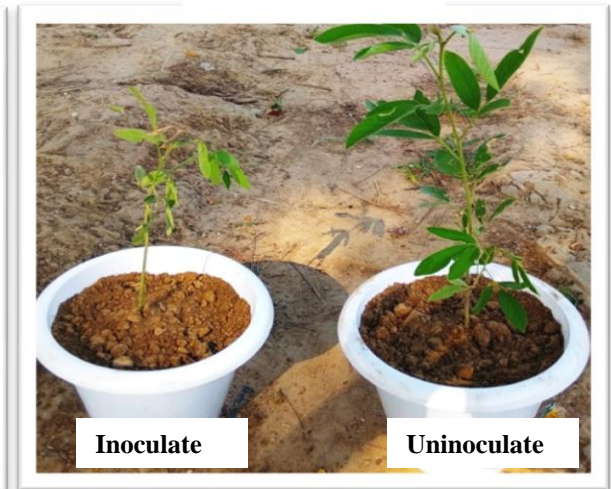
(Banda isolate)



(Mahoba isolate)



(Hamirpur isolate)



(Chitrakoot isolate)

**Plate.8. Pathogenicity test on pigeonpea**

## DISCUSSION

### Survey of wilt disease of pigeonpea in different districts:

The average disease incidence ranged between 3.25% (Bari village of Mahoba) to 49.00% (Khoh village of Chitrakoot) from district to districts. The average percentage of wilted plants in Banda district was (25.67%) minimum (13.23%) being in Hardouli village of Banda while maximum (39.43%) in Bargahani village of Banda. The average percentage of wilted plants in Mahoba district was (14.64%) minimum (3.25%) being in Bari village of Mahoba while maximum (41.00%) in Suraicha village of Mahoba. Hamirpur district showed 15.99% average disease incidence of wilting, the minimum (3.50%) being in Sijvahi village of Hamirpur, maximum (38.00%) in Chilli village of Hamirpur. The average percentage of wilted plants in Chitrakoot was 39.06% the minimum (26.00%) being in Kalla village of Chitrakoot, while maximum (49.00%) in Khoh village of Chitrakoot. The similar finding of wilt disease of pigeonpea (Pawar et al., 2013, Sushreeta et al., 2017 and Maurya et al., 2020). The detailed survey inferred that maximum disease incidence was recorded in Chitrakoot and followed by Banda, Hamirpur and Mahoba. The incidence of disease has been reported 30-60% at crop maturity and flowering stages by Kannaiyan and Nene (1981). Okiror (2002) suggested that this disease depends upon the stage of the crop infection, which approach over 50% and even upto 100% when wilt occurs at the pre pod stage. Datta et al. (2013) reported maximum yield loss up to 90% caused due to *Fusarium udum* in pigeonpea.

### Colony character:

The isolates differed in their colony characters on different media. Sporulation was moderate to excellent on PDA and PSA isolates. The results obtained during the present study showed that PDA and PSA media were best for the growth of *Fusarium udum*. The several workers have been reported that mycelial growth and colony characters on PDA and PSA media. Our results are in agreement with the findings of Subramanian (1955), Booth (1971), Prasad and Choudhury (1974), Gemawat (1977), Reddy and Choudhury (1985), Kiprof et al., (2002) and Sangava (2018).

**Radial growth and morphological character:**

Radial growth of fungal mycelium was studied and observation was recorded on PDA and PSA media on 21 days of inoculation. The radial growth of chitrakoot isolate was maximum on PDA and PSA media consistently on 21 days of inoculation followed by banda, hamirpur and mahoba isolates. Data of the observations were taken after 21 days of inoculation. Chitrakoot isolate showed the highest radial growth i.e 85.33 mm on PDA followed by banda 84.33 mm, hamirpur 82.33 mm and mahoba 81.66 mm. The mean of radial growth on PDA and PSA media in chitrakoot isolate showed highest 84.99 mm followed by banda 83.99 mm, hamirpur 82.16 mm and mahoba 81.33 mm after 21 days of inoculation. Also the highest radial growth was observed on PDA in case of chitrakoot isolate while least radial growth was observed on PDA in mahoba isolates. The maximum radial growth was recorded on PSA in chitrakoot isolate and minimum in mahoba isolate. Kiprop et al., (2002), Gwata et al., (2006), Singh et al., (2013), Sushreeta et al., (2017) and Sangava et al., (2018) agreed with most of the available reports which relates different isolates to different races.

**Pathogenicity test:**

Pathogenicity of *Fusarium udum* was tested on pigeonpea variety 'Bahar' after isolation and purification of *Fusarium udum* collected from banda, mahoba, hamirpur and chitrakoot isolates. All isolates of *F. udum* showed the typical wilting symptoms and proved the Koch's postulate. The height and number of leaves was maximum in mahoba isolate followed by hamirpur, banda and chitrakoot isolates. However, chitrakoot isolate was found to be highly pathogenic followed by banda, mahoba and hamirpur isolates. The hamirpur isolate was low pathogenic in compare to chitrakoot, banda and mahoba isolates. The similar finding were found by Reddy et al., (1990), Jain et al., (1995) Okiror and Kimani (1997), Kiprop et al.,(2002) and Sushreeta et al., (2017) .

## SUMMARY AND CONCLUSION

Pigeonpea is an important pulse crop grown throughout India. Survey was conducted of pigeonpea growing areas of 4 districts in Bundelkhand region of Uttar Pradesh during 2019-20. The average disease incidence ranged between 3.25% to 49.00% from district to districts. The average incidence percentage of wilted plants in Chitrakoot district was 39.06% followed by Banda district 25.67%, Hamirpur district 15.99% and Mahoba district 14.64% respectively.

Pathogenicity of *Fusarium udum* was tested on pigeonpea variety 'Bahar' all isolates of *F. udum* showed the typical wilting symptoms and proved Koch's postulates. The height and number of leaves was maximum in mahoba isolates followed by hamirpur, banda and chitrakoot isolates. The maximum wilting was recorded in chitrakoot isolate followed by hamirpur, banda and chitrakoot isolates.

All the isolates differed in their radial growth colony characters on both solid media. It was found that PDA was the best medium in compare to PSA. The chitrakoot isolate was fast growing followed by banda, hamirpur and mahoba isolates. Sporulation was moderate to excellent in different isolates.

The highest radial growth was observed in chitrakoot isolate on PDA while minimum radial growth was observed on PDA in mahoba isolate. However, the maximum radial growth was a recorded on PSA in chitrakoot isolate and minimum radial growth in mahoba isolate.

Micro conidia are single or bicelled, hyaline, mostly curved, ovoid to fusoid and scattered and measure 3.80-9.12 x 0.98-2.10 $\mu$ m. Macro conidia which are hyaline, thin walled, falcate with a distinct foot cell, and an apical cell that decreases in width towards the tip. The most distinguishing characteristic of the macro conidia are their strongly curved or hooked apices and measure 11-21.12 x 1.95 to 3.78 $\mu$ m.

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## Bio-Data

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