Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

June 2020, Volume 33 Issue 1, Page 141-156

Isolation, Identification and an overview of variability exhibited by different isolates of *Fusarium udum.* Butler

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ARTICLE INFO

Article history:
Received 23 February 2020
Revision Received 21 May, 2020
Accepted 9 July 2020

Key words: *Wilt, Cultural Characteristics, Cellwall Degrading Enzymes, Fusaric acid*

ABSTRACT

Wilt of pigeonpea is devastating disease and the pathogen causing wilt show high variation among morphological, biochemical and molecular characteristics. In the present study wilt pathogen was isolated from infected pigeonpea plant, based on the variation observed in mycelial colour, pigmentation produced on media and mycelial growth pattern test, pathogen was differentiated in to 10 isolates. Marked variation was observed among the isolates in-terms of cultural, biochemical and molecular characteristics. Partial correlation was observed between fusaric acid production and consistency of wilt symptoms, and unable to establish correlation between production of extracellular cellwall degrading enzymes and wilting. DNA sequencing reveals that isolate 3-E1 was more than 97% similar to *Fusarium udum f.sp* crotalariae and *Fusarium udum*, satisfies the cultural characteristics of *Fusarium udum* as mentioned in important literature, and is evident for the production of fusaric acid and important cell wall degrading enzymes, which establishes its pathogenecity.

1. Introduction

Pigeonpea wilt was caused by *Fusarium udum.*Butler and this pathogen was first reported from India by E. J. Butler in 1906. So it was named as *Fusarium udum.* Butler (Karimi *et al.,* 2012). Pigeonpea wilt was more common in India, East Africa and Malawi, brings about 50% loss in yield. In India, this disease was common in Uttar Pradesh, Madhya Pradesh, Maharashtra and Andhra Pradesh causing yield loss up to 97000 tonnes per year (Saxena *et al.,* 2010). 22-95% wilt incidence was reported in case of 'Bahar' variety in various parts of Uttar Pradesh (Singh *et al.,* 2013).

Isolates of *Fusarium udum* shows high variation among the cultural characteristics along with virulence levels (Panwar *et al.,* 2017) and produces wide range of cellwall degrading enzymes which acts determinants of fungal pathogenecity, helps in hyphal growth and release of nutrients from host cells thereby helps in pathogen growth (De Lorenzo *et al.,* 1997) these cellwall degrading enzyme present in both pathogenic and non-pathogenic fungi, but specialised structures which helps in penetration in to the host differentiates pathogens from non pathogenic ones (Mendgen and Deising, 1993; Struck *et al.,* 1998). Spore adhesion and germination, formation of appressorium, penetration peg, other primary infection- structures are some of the external steps for successful infection by pathogen (Carroll and Tudzynski, 2012). Accumalation of fusaric acid secreted by the pathogen shows positive correlation with wilting symptoms produced on infected plant (Singh and Upadhyay, 2014; Selim *et al.,* 2015).

Present study mainly focussed on isolation, identification by morphological and molecular characteristics, variation among the isolates between cultural and biochemical characteristics in relation to pathogenecity.

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2. Materials and Methods

2.1 Isolation, Identification and Isolate differentiation of *Fusarium udum*

Three wilt infected pigeonpea plants were collected from AICRP on pigeonpea (Plant pathology trails, Breeding trails & Pest Incidence trails respectively)-BHU; Varanasi-India. Root and apical shoot portion were discarded. Main stem region showing discolouration was cut in to small bits of 0.5cm, surface sterilised with 0.1% $Hgcl_2$ then split in two halves vertically with the help of sterilised scalpel and inoculated on PDA plates such that discoloured vascular tissue comes in contact with media. After 3-4 days, mycelium was subcultured from the inoculated plate and was observed under compound microscope at 7 days after incubation. Based on the morphological characters mentioned in the literature the test pathogen was identified.

Sclerotia like bodies produced on the media were separated and embedded in tissue freezing medium. Thin sections of 10-15μ were made using the cryomicrotome which were observed under compound microscope for presence of perethecia like structures (Fig:-2;d,e,f). These sclerotia like bodies were cultured on PDA medium for further observations.

Based on mycelial colour pigmentation produced on media and production of sclerotia like bodies, test pathogen was differentiated in to 10 isolates (Flow chart:-1 & Fig:-3).

2.2 Cultural characteristics

0.5mm mycelial bits of each isolate were inoculated on PDA plates and incubated at 25+2°C.Mycelial growth was recorded everyday and observed for variation in cultural characteristics like mycelial colour, pigmentation produced on media, mycelial growth pattern, mycelial growth rate and sporulation capacity, which were recorded at 10 days after incubation.

2.3 Pathogenecity test

Wilt susceptible variety 'Bahar' was used for the experimentation. Here pathogen inoculation was done by spore suspension method (Shashi Mishra & Vishwa Dhar, 2005) during the first time, to the 21 days old seedlings and during the second time by soil inoculation method (Shashi Mishra & Vishwa Dhar, 2005) and observations were recorded for every 3 days interval, after inoculation up to 60 days (Table:-2).

2.4 Qualitative Biochemical assays

2.4.1 Cellulase assay

0.5cm mycelial bits were inoculated on the PDA plates supplemented with 1% carboxy methyl cellulose, after 5 days of incubation the plates were stained with 0.1% congo red solution followed by destaining with 5% NaCl solution. Presence of clear halo around the mycelia indicates cellulase activity. Diameter of the clear zone was recorded was further analysis (Zakpaa *et al.,* 2009).

2.4.2 Polygalacturonase assay

0.5cm mycelial bits were inoculated on pectin agar medium, after 5 days of incubation plates were the plates were flooded with 50 mM potassium iodide-iodine solution. A clear halo zone around the colonies indicates the ability of an isolate to produce polygalacturonase. Diameter of the clear zone was recorded for further analysis (Hankin *et al.,* 1971).

2.4.3 Pectin methyl esterase assay

0.5cm mycelial bits were inoculated on the basal medium containing commercial pectin as the sole carbon source. After 5 days of incubation, plates were flooded with 1% copper acetate, formation of bluish background was recorded as positive result for this assay (Onyeocha and Ogbonna, 1983).

2.4.4 Amylase assay

0.5cm mycelial bits were inoculated on the starch agar medium, after 5 days of incubation plates were the plates were flooded with potassium iodide-iodine solution (1gm iodine and 5gm of potassium iodide in 330ml distilled water). Formation of clear halo zone indicates amylase activity and diameter of halo zone was recorded for further analysis (Hankin & Anagnostakis, 1975).

2.4.5 Xylanase assay

0.5cm mycelial bits were inoculated on the mineral salt medium containing commercial xylose as the sole carbon source. After 5 days of incubation, the plates were flooded with 0.01% of 10-15ml congo red solution and allowed to stand for 10 to 15 min and destained with 1% NaCl and the zone of hydrolysis was recorded for further analysis (Teather and Wood, 1982).

2.4.6 Laccase assay

0.5cm mycelial bits were inoculated on the PDA medium supplemented with 0.02% guaiacol. After 3-4 days of incubation the laccase positive fungal strains showed reddish brown coloured halo on media (Adiveppa *et al.,* 2015).

2.4.7 Fusaric acid estimation

Fusaric acid was extracted by following the procedure given by Stefan Eged (2005) and estimated by using thin layer chromatography with 75:15:10 v/v of butanol-formic acid-water as the solvent and 5µl of 0.2% of commercial fusaric acid as standard (Shinde and Deshmukh, 2014).

2.5 DNA Extraction and PCR amplification

DNA of isolates of test pathogen was extracted according to the procedure given by Patel *et al.,* (2016) and the extracted DNA was subjected to PCR amplification (Sambrook *et al.,* 2001) with ITS 1& 4 primers.

2.6 Molecular Identification of Test pathogen

Using the FASTA sequences of the samples provided after DNA sequencing, FASTA sequences of related species were obtained from NCBI-BLAST website (https://blast.ncbi.nlm.nih.gov/). On comparision with the closely related species based on query cover, alligment score and percent identity, samples were identified.

3 Results and Discussion

3.1 Isolation, Identification and Isolate differentiation of *Fusarium udum*

White coloured mycelium was observed on the inoculated stem bits of pigeonpea. Micro and macroconidia of the *Fusarium spp.* were observed when the mycelium of test pathogen was subjected to compound microscopic observation. Microconidia are ovoid to fusoid, single celled to bicelled formed on false heads, whereas macroconidia were sickle shaped with distinct hook like apical cell and foot like basal cell. Based on this conidial characteristics (Fig:-1), isolated species was identified as *Fusarium udum.*

The results were in agreement with the conidial characters mentioned in 'The genus *Fusarium*' by Booth (1971), 'the *Fusarium* Laboratory manual', by John F Leslie and Brett A Summerell (2006), which was illustrated by Suzanne Bullock and in 'Contribution to the genus Fusarium in Egypt, with dichotomous keys for identification of species' by Ismail *et al.,* (2015). On subculturing the test pathogen on potato dextrose agar media, variation in mycelial colour, pigmentation produced on media and mycelial growth pattern was observed. After one month of storage under refrigeration at 4-6°C, growth of sclerotia like bodies (SLB) were observed on PDA slants (Fig:-2;b), on inoculating these sclerotia like bodies on to the petridish with PDA media, it produced whitish mycelium. On microscopic observation it shows macroconidia similar to that of *Fusarium udum.* Cryomicrotomy of these sclerotia like bodies (10-15µ thick sections) shows aggregation of mycelium to form fungal tissue like structures (Fig:-2; d,e,f). Based on these characteristics, test pathogen was divided into ten isolates which were mentioned in the form of flowchart and pictures were given in figure-3.

3.2 Cultural Characteristics

Under the cultural characteristics, variation was observed in mycelial colour, mycelial growth pattern, average growth rate of mycelium, pigmentation produced on media and sporulation capacity of isolates and the observations were presented in table:-1.

Ten isolates of test pathogen were grouped into three categories based on mycelial colour, they were orange coloured mycelium (O-G, O-E), white coloured mycelium (SLB, 1-E1, 3-E1, WH-1, WH-2) and yellow coloured mycelium (Yellow and 3-E2). Four categories based on pigmentation produced on PDA medium, they were purple pigmentation on media (SLB and WH-2), yellow pigmentation on media (3-E1, 3-E2 and 1-H1), orange pigmentation on media (WH-1) and normal or no pigmentation on media (O-G, O-E, Yellow and 1-E1) and three groups based on sporulation capacity and types of spores observed (figure-4) when focussed under 10x, namely excellent sporulation capacity with all three types of spores i.e. macro conidia, micro conidia and chlamydospore visible (O-G, O-E), excellent sporulation capacity but only one or two types of spores are visible (Yellow, 3-E1, 3-E2, WH-1, WH-2) and moderate sporulating capacity with one or more types of spores (SLB and 1-E1). Isolates of test pathogen similar in one characteristic will vary for remaining other cultural characteristics.

These results were in agreement with the conclusions of Purohit *et al.,* (2017); Sanjeev & Upadhyay (2013); Rashmi and Chattannavar, (2016); Sahu Santram *et al.,* (2017); they also classified isolates of *Fusarium udum* based on mycelial growth, mycelial colour and substrate pigmentation. They also observed that isolates similar in one cultural characteristics will vary for other cultural characteristics and proposed that cultural characteristics were not associated with the geographical region.

In present study, some of isolates were isolated from single pigeonpea plant (O-G and SLB from one plant, O-E, Yellow, 1-E1, 3-E1, 3-E2 from one plant and 1-H1, WH-1 and WH-2 from one plant) and these isolates show marked variation among the cultural characteristics, which was supported by the study of Kiprop *et al.,* (2002) that MK 08 and MK 09 are isolates of *Fusarium udum* collected from single pigeonpea plant and isolates

MAL01a and MAL01b acquired from the similar culture were placed in different groups were based on cultural characteristics. Variation in isolates collected from single plant or similar culture were due to the heterothallic nature of fungi (Rashmi and Chattannavar, 2016) or because of existence of physiological races of pathogen (Mahesh *et al.,* 2010).

3.3 Pathogenecity test

Germination percentage, day of initiation of wilting symptoms and wilt incidence at 60 days after pathogen inoculation were recorded and presented in table:-2. Days of initiation of wilting symptoms recorded almost same in two types of pathogen inoculation, which was in agreement with the results of Vishwa Dhar and Shashi Mishra (2005). Characteristic wilt symptoms like yellowing of leaves, loss of turgidity of leaves, epinasty, vascular dicolouration were observed (figure: -5) similar to that of Mesapogu *et al.,* (2012) during pathogenecity test especially in case of pathogen inoculation by spore suspension method. Isolates O-E, O-G, SLB, 3-E1, 3-E2 and WH-1 exhibited typical wilting symptoms. Based on wilt incidence at 60 days these isolates were classified into moderately virulent (SLB, O-G, O-E, 1-E1, 3-E1 and WH-1) and less virulent or avirulent (3-E2, 1-H1, WH-2). Wilt incidence was high incase of isolate SLB (50%) and least incase of WH-2 and 1-E1 (21.42%). This marked difference in wilt incidence produced by the isolates of test pathogen was supported by the results of Kiprop et al. (2002); Sinha *et al.,*(2008), Vishwa Dhar *et al.,* (2012), Mesapogu *et al.,*(2012), Naik *et al.,*(2017), Panwar *et al.,*(2017) , Purohit *et al.,*(2017) and Ghante *et al.,*(2018). Being collected from same region i.e., within the BHU main campus the isolates of test pathogen show variation in pathogenecity test, these results were in agreement with the study of Kiprop *et al.* (2002), that *Fusarium udum* isolates namely MK08, MK09; NY02, NY03; and TK05, TK06 collected from same location or of same geographical origin shows difference in virulence nature towards KAT 60/8 variety of pigeonpea.

3.4 Qualitative Biochemical assays

All the ten isolates of test pathogen shows activity of cellulase, polygalacturonase, pectin methyl esterase and xylanase. Amylase activity was not evident in O-G isolate, laccase activity was observed in SLB, 1-E1, 1-H1, WH-1, isolates of test pathogen. Pectin methyl esterase, fusaric acid assays were purely qualitative and activities of other enzymes were estimated in form of relative clear zone size, these results were presented in the table:-3.WH-2 isolate shows high activity of polygalacturonase (0.060), Amylase (0.480) and xylanase (0.227). Cellulase activity was highest in 1-E1 isolate.

SLB shows highest laccase activity (4.20). there is no signicant variation among the enzyme activities exhibited by the isolates of test pathogen and the enzyme activity doesn't show any correlation with pathogenecity of the isolates. But the isolates SLB, WH-1 shows activity of fusaric acid (figure:-6), laccase and consistent production of seedling death and wilting symptoms. As the normal cellwall degrading enzymes present in both pathogenic and non pathogenic isolates, it is difficult to correlate the activities of cellwall degrading enzymes with virulence nature of pathogen. Mann, (1962); Puhalla and Howell, (1975); Cooper, (1988); Scott-Craig *et al.,* (1990); Apel *et al.,* (1993); Schaeffer *et al.,* (1994); Sposato *et al.,* (1995) and Vinodkumar *et al.,* (2007). Production of specialised infection structures i.e., laccases which helps formation of appressorium, conidial pigmentation, reduces the host tissues lignification, protecting the pathogen from secondary metabolites of host plant (Mendgen and Deising 1993; Struck *et al.* 1998; Mayer *et al.,* 2002; Baldrian, 2006 ; Caneroand Roncero, 2008) and secretion of fusaric acid, which plays major role in destruction of photosynthetic process, leaf yellowing, leaf necrosis, finally leads to wilting of plant (Singh & Upadhyay, 2014; Selim and El- Gamma,, 2015; Singh *et al.*, 2017) are the two important factors contributing for the virulence of pathogen in present study. Considering all these, SLB isolate which is evident for production of all cellwall degrading enzymes, laccase, fusaric acid as well as shows consistency in symptoms in both the methods of pathogen inoculation, was proved to be pathogenic isolate.

3.5 DNA Extraction and PCR amplification

Along with SLB, two other isolates viz., O-G and 3-E1 showing consistency in production of wilting symptoms in both the methods of pathogen inoculation and in fusaric acid production, were carry forwarded for DNA extraction and PCR amplification of DNA with ITS-1 and ITS-4 primers. Amplified DNA bands of isolates travel approximately 500-600bp of distance on the gel when compared with the ladder used.

3.6 Molecular identification of test pathogen

FASTA sequences of the samples were subjected to BLAST by entering the organism name as *Fusarium fujikori* complex (taxid:171627), results reveals that among the three species, 3-E1 shows 73% query cover, alligment score more than 200 and more than 97% identity with *Fusarium udum* f.sp *crotalaria* and *Fusarium udum* .Butler. 3-E1 shows white mycelial colour with creamy yellow pigmentation on PDA. Variation was observed in terms of cultural characteristics as mentioned by Kiprop *et al.*, (2002) and Purohit *et al.*, (2017) who classified isolates of *Fusarium udum* based on mycelial growth, mycelial colour and substrate pigmentation.

This isolate produces fusaric acid as mentioned by John F Leslie and Brett A Summerell (2006), Selim and El-Gamma, 2015; Singh and Upadhyay, 2017; Singh, *et al.*, 2017. With all these evidences we can partially confirm that 3-E1 belongs to *Fusarium udum*. For the complete confirmation, further molecular identification of isolates should be performed by targeting other conserved genes such as beta tubulin, and Tef-1 alpha as done by other researchers (Herron *et al.,* 2015; Nosratabadi *et al.,* 2018).

4 Acknowledgements

We are very thankful to Dr. Asha Sinha, Dr. H.B. Singh and Dr. B.K. Sarma for providing us access to the equipment required for this research work and we acknowledge Dr.Sudheer, Dr.Shadroopa, and Ph D. scholars of our department; Ms. Raina , Mrs. Prachi, Ms. Jyothi, Ms. Vandana, Mr. Rahul, Ms. Jhumi shree, Mr. Rashid , and Ms. Jharjari for helping me by teaching how to operate the equipment required for conducting this research.

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Table:-1 Variation in cultural characteristics of isolates of test pathogen

Location	Isolate	Avg. Growth rate	Colony colour	Pigmentation on media	Mycelial growth	Spores found	Sporulation capacity
Pigeonpea plots-Dept of GPB	O-G	1.21cm/day	Orange	Normal	Wavy margin,radial zones,more or less compactly woven , uniformly grown aerial fluffy mycelium.	Micro,macro conidia and chlamydospores	++++
Pigeonpea plots-Dept of GPB	SLB	1.88cm/day	Pale white	Salmon to purple	Circular margin with radial zones, loosely woven completely appressed mycelium,less dense at centre and moderately dense at margins	Micro conidia	++
Pigeonpea plots-Dept of EAZ	O-E	1.58cm/day	Orange	Normal	Wavy margin,radial zones,more or less compactly woven, uniformly grown ,aerial mycelium.	Micro,macro conidia and chlamydospores	++++
Pigeonpea plots-Dept of EAZ	Yellow	1.81cm/day	Creamy yellow	Normal	Circular margin, more or less compactly interwoven, dense,web like aerial mycelium	Macro conidia and chlamydo spores	++++
Pigeonpea plots-Dept of EAZ	1-E1	1.04cm/day	Pale white	Normal	Circular margin, loosely interwoven, foamy and appressed mycelium	Both micro and macro conidia	++
Pigeonpea plots-Dept of EAZ	3-E1	1.07cm/day	Creamy white	Creamy yellow	Circular margin, aerial, compact, cottony mycelium radiating from centre.	Both micro and macro conidia	++++
Pigeonpea plots-Dept of EAZ	3-E2	1.09cm/day	Pale white to yellow	Creamy yellow	Circular margin with circular zones, more or less compactly woven, web like mycelium radiating from centre	Micro conidia	++++
Pigeonpea plots-Horticulture farm	1-H1	1.05cm/day	Pale white	Pale yellow	Wavy margin, completely appressed on onside and partly on another, loosely interwoven , foamy ,web like mycelium	Both micro and macro conidia	++++
Pigeonpea plots-Horticulture farm	WH-1	0.87cm/day	Pale white	Orange to pink	Wavy margin, appressed near the centre and moderately appressed at the borders, loosely interwoven, foamy web like mycelium.	both micro and macro conidia	++++
Pigeonpea plots-Horticulture farm	WH-2	0.98cm/day	Pale white	Purple to violet	Wavy margin, slightly dense at centre, sparsely dense at borders, loosely interwoven and radiating from the centre.	Both micro and macro conidia	++++

Sporulation Score Grade Description

1 ++++ Excellent >100 spores/microscopic field (10x)
2 +++ Good 50-100 spores/microscopic field (10x)
3 ++ Moderate 10-50 spores/ microscopic field (10x)
4 + Poor sporulation 1-10 spores/ microscopic field (10x)
5 - No sporulation < 1 spores / microscopic field (10x)

Average growth rate= $\frac{\text{sum of mycelial growth recorded every day till completion of the growth}}{\text{Total no of days taken to complete the growth(to cover entire petridish)}}$

*** Spore count observations was taken at 10 days after inoculating the 0.5cm mycelial bits on petridish.**

Table:-2 Pathogenecity test of isolates of test pathogen

Method of pathogen Inoculation	Spore suspension Method				Soil Inoculation method			
Isolates	Germination % @ 21 DAS	Initiation of wilting symptoms	% wilted plants after 60 DAI	Virulence	Germination % @ 21 DAS	Initiation of wilting symptoms	% of wilted plants after 60 DAI	Virulence
O-G	71.42	28 DAI	35.71	M	78.57	24 DAI	36.36	M
SLB	78.57	35 DAI	50	M	28.57	38 DAI	50	M
O-E	92.85	22 DAI	42.8	M	92.85	21 DAI	46.15	M
Yellow	71.42	-	-	L	28.57	-	-	L
1-E1	85.71	38 DAI	21.42	M	42.85	32 DAI	33.33	M
3-E1	85.71	26 DAI	35.71	M	71.42	28 DAI	45.45	M
3E-2	78.57	-	-	L	64.28	35 DAI	33.33	M
1-H1	71.42	30 DAI	21.42	L	71.42	-	-	L
WH-1	71.42	23 DAI	28.57	M	64.28	31 DAI	22.22	M
WH-2	85.71	36 DAI	21.42	M	42.85	-	-	L
Control	85.71	-	-	-	85.71	-	-	-

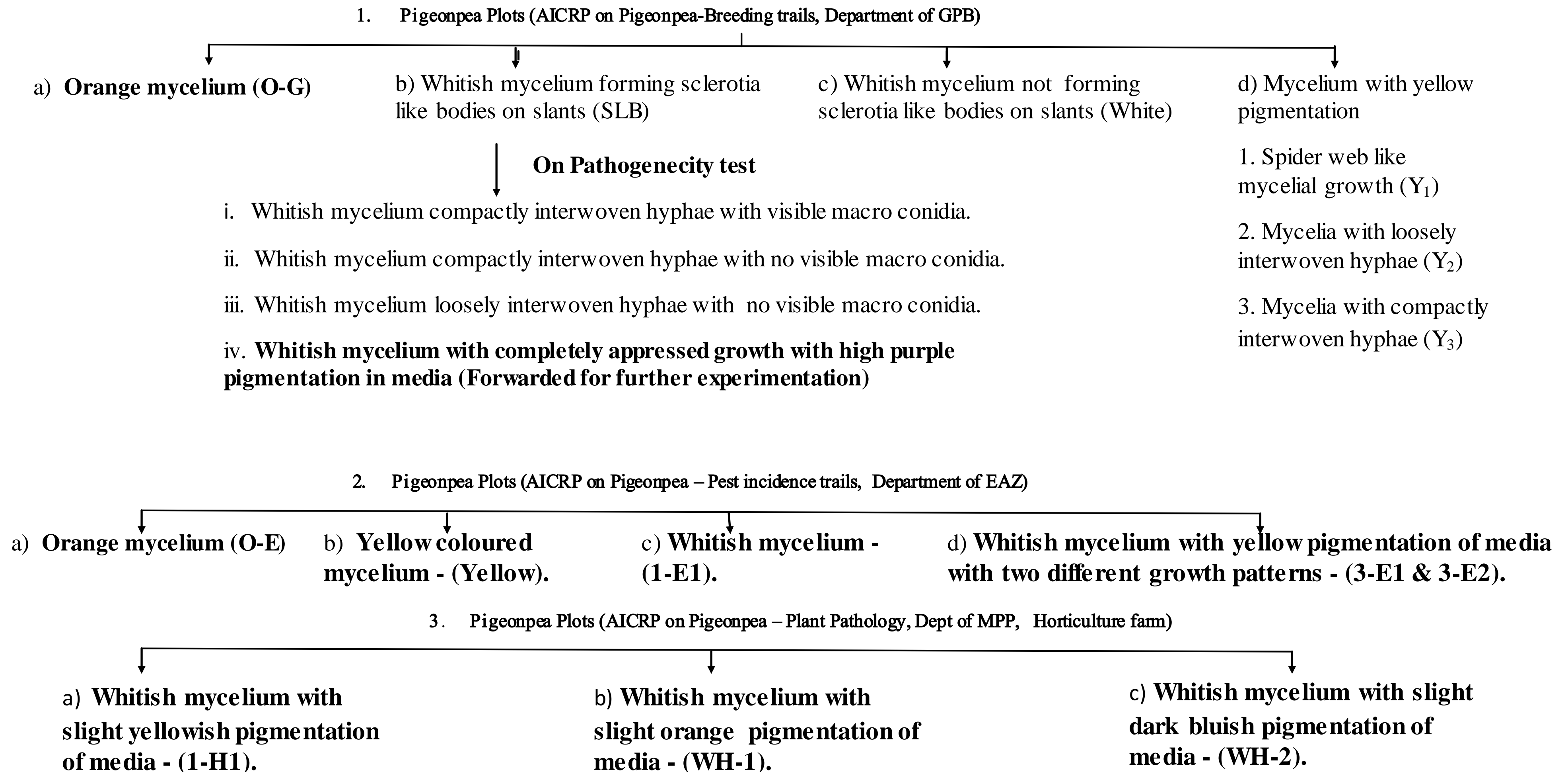
Grading of wilt incidence *(I %)* = 0–20%- avirulent (L), >20–50% - moderately virulent (M); >50% - highly virulent (H); *DAI= Days after inoculation, DAS= Days after sowing.

Table:-3 Activities of different cellwall degrading enzymes (Average relative clear zone) produced by the isolates of test pathogen (figure:-7)

Isolates	Cellulase	Polygalcturonase	Amylase	Xylanase	Laccase
O-G	0.087	0.045	N.E	0.033	N.E
SLB	0.093	0.050	0.030	0.045	4.20
O-E	0.110	0.055	0.134	0.032	N.E
Yellow	0.137	0.022	0.022	0.032	N.E
1-E1	0.297	0.027	0.043	0.133	1.40
3-E1	0.162	0.058	0.440	0.108	N.E
3-E2	0.141	0.044	0.111	0.111	N.E
1-H1	0.277	0.054	0.045	0.142	0.60
WH-1	0.283	0.030	0.093	0.200	1.00
WH-2	0.292	0.060	0.480	0.227	N.E
C.D.	**0.006**	**0.004**	**0.015**	**0.005**	**0.027**
SE(m)	**0.002**	**0.001**	**0.005**	**0.002**	**0.009**
SE(d)	**0.003**	**0.002**	**0.007**	**0.002**	**0.013**
C.V	**0.340**	**0.225**	**0.823**	**0.267**	**1.253**

* N.E = Not Evident

Flowchart :-1 Flow chart showing Specimen collection and isolate differentiation

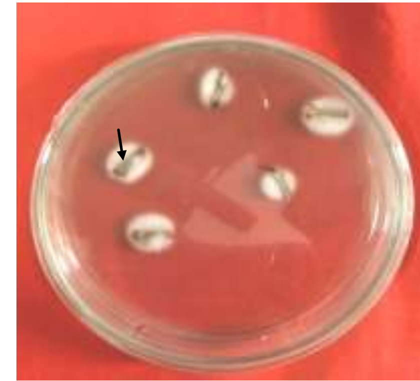


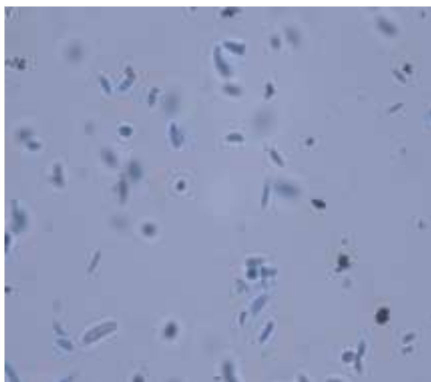
*Above highlighted isolates were carry forwarded for further experimentation

Wilt infected Pigeonpea plant

Vascular discolouration

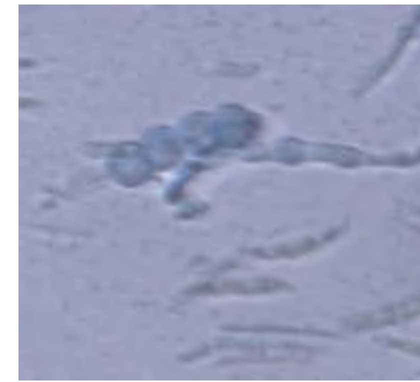
Petriplate inoculated with infected stem-bits





Microconidia

Macroconidia

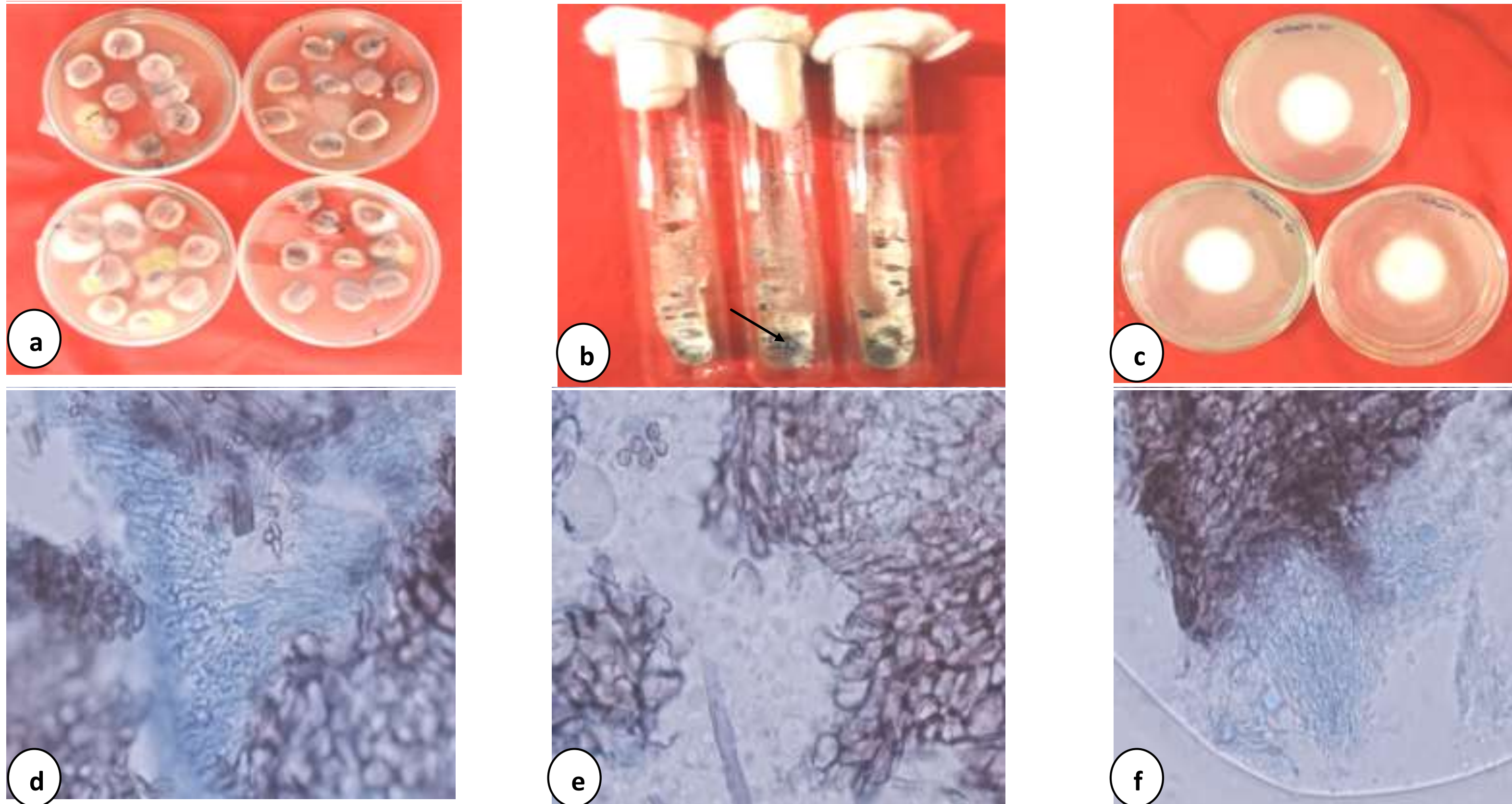


Chlamydospore

Figure: - 1 Pictures showing wilted plant, its inoculation on petriplate and three types of spores produced by the pathogen

Arrow mark in first and second pictures shows vascular discolouration , and in third picture shows growth of mycelium from inoculated stem bits.

Inoculated plates showing variation in mycelia colour **Formation of sclerotia like bodies on PDA slants** **Mycelia growth from sclerotia like bodies inoculated on PDA plates**



Pictures of cryomicrotomy of sclerotia like bodies showing aggregation of mycelium to form fungal tissue like structures (observed under Nikon Eclipse 50I microscope)

Figure :- 2 Isolate differentiation of test Pathogen; Arrow mark in picture 'b' showing dark coloured sclerotia like bodies on PDA slants and images d, e, f showing different structures observed on cryo microtome section of sclerotia like bodies, observed under Nikon Eclipse 50I microscope.

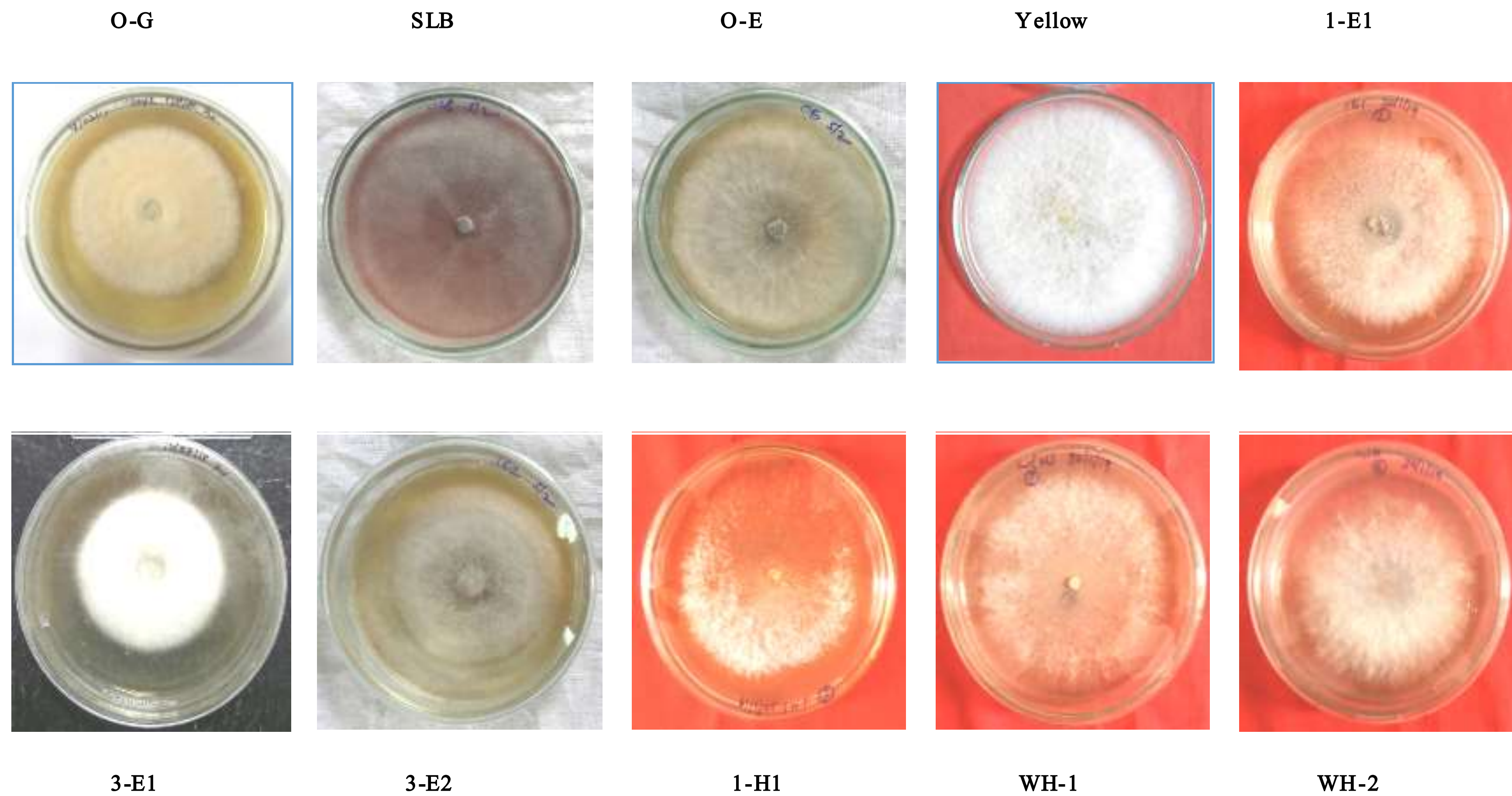


Figure :-3 Isolates of test pathogen showing variation in cultural characteristics on potato dextrose agar media

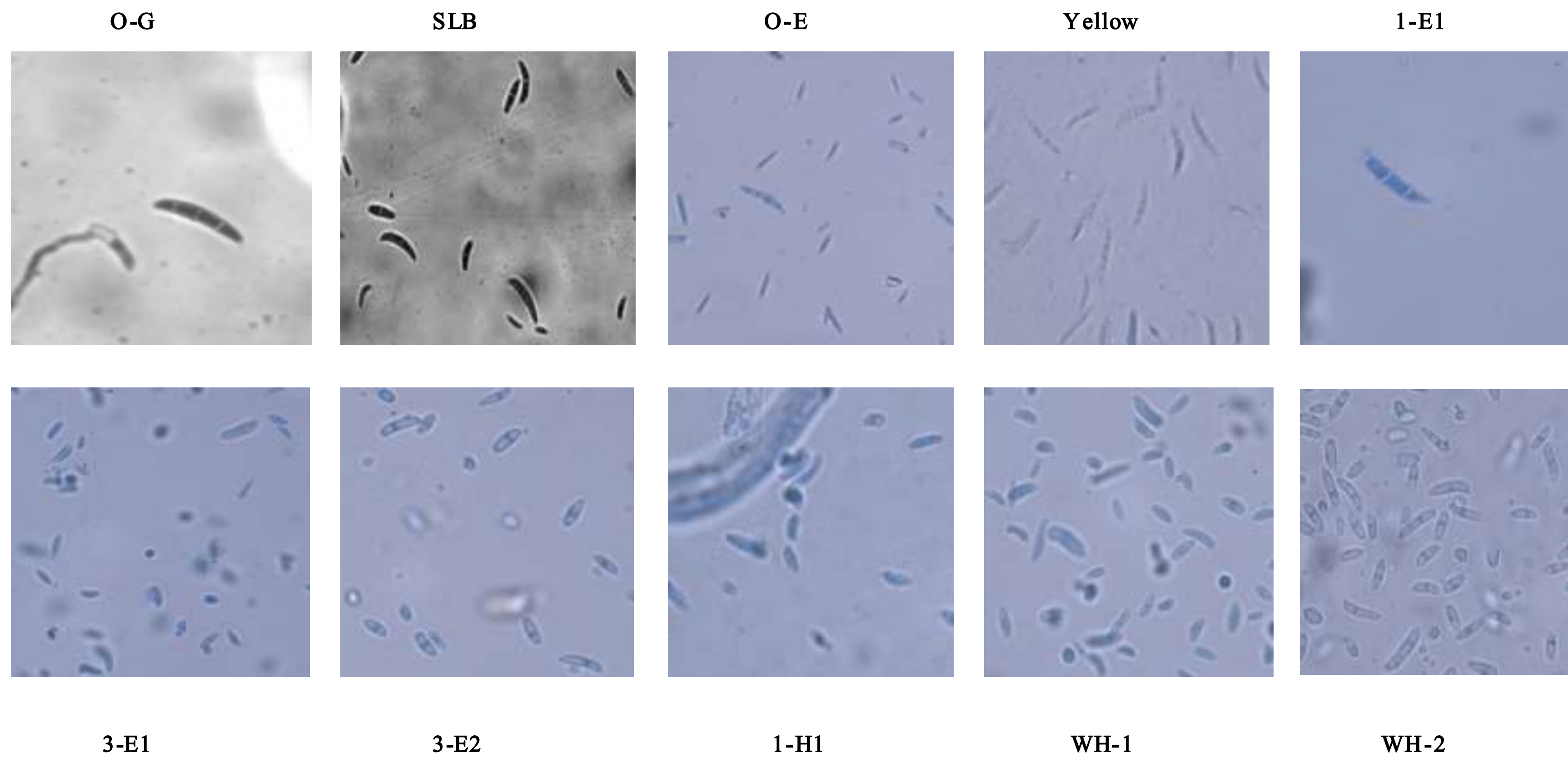


Figure:-4: Conidia of different isolates of test pathogen observed under Carl Zeiss microscope and Nikon Eclipse 50I microscope

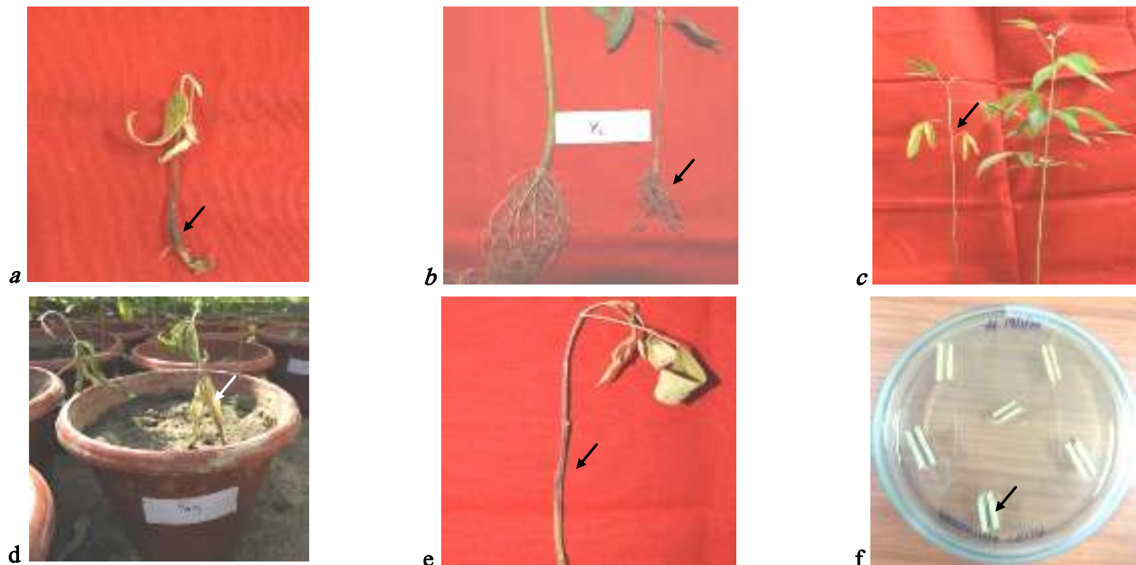


Figure:-5 images showing wilting symptoms observed during the pathogenecity test

a) Tap root infection and discolouration at collar region (indicated by arrow). b) Root symptoms on 45 days old control (left) and infected plant (right). c) Yellowing (indicated by arrow) with control plant on right side. d) Loss of turgidity in leaves and wilting. e) Discolouration of main stem in young plants (indicated by arrow). f) Internal vascular discoloration

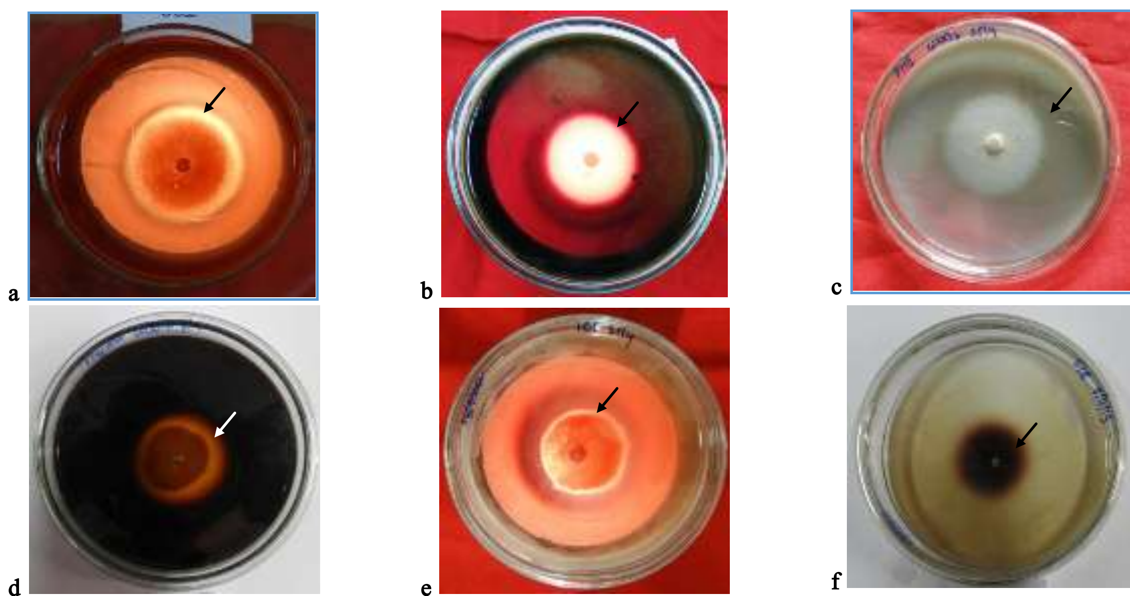


Figure:-6 images showing qualitative biochemical assay of different cellwall degrading enzymes

a) Cellulase assay b) Polygalacturonase assay c) Pectin methyl esterase assay d) Amylase assay e) Xylanase assay f) Laccase assay

Arrow mark in images a,b,d,e ; indicates zone of hydrolysis or halo zone produced due to enzyme activity. Arrow mark in image 'c' indicates bluish background produced due to activity of pectin methyl esterase and arrow mark in image 'f' shows reddish brown halo due to activity of laccase.

St d	WH- 2	Yello w	O-E	1-E1	3-E2	3-E1	SLB	O-G	Std	WH- 1	1-H1

Figure:-7 Picture showing qualitative estimation of fusaric acid produced by test pathogen isolates

Arrow marks showing the mild black bands indicates the presence of fusaric acid