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PRODUCTION OF CELL WALL DEGRADING ENZYMES BY *FUSARIUM OXYSPORUM* F. SP.*VASINFECTUM*

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ABSTRACT

To test the production of cellulolytic and pectinolytic enzymes for successful pathogenesis. The virulent isolate produced higher mycelial growth and dry weight compared to avirulentisolate .The isolate ACFov is highly virulent to cause the disease in the cotton plants compared to the avirulent isolate MNFov .The virulent isolate was amplified by PCR analysis with 530 bp. The increase in the pectinolytic enzyme was increase upto 9 days after inoculation and thereafter theirdeclineat faster rate was identified by spectrophotometric ananlysis. The production of pectinolytic and cellulolytic enzymes were higher in virulent isolate.

Key words : *Cotton-cell wall degrading enzymes-cellulolytic-pectinolytic enzymes.*

Fusarium wilt caused by *Fusariumoxysporum*. f. sp. vasinfectum (Atk) Synd. and Hans (Fov) in cotton was formally identified early in the history of plant pathology and subsequently formed basis for recognition of other wilts (1). Symptoms can appear at any stage of plant development. A patchy distribution of affected plants in the field often results from seedlings that wilt and dry rapidly. Symptomatic older plants may be stunted and wilted. Affected plants shows Chlorosis of leaves and dieback that progresses from the top of the plant accompanied by the formation of characteristic brown discoloration of the vascular tissue. Some plants die, while others re-shoot from the base, but these plants do not produce bolls (2). Fusarium wilt affects all four domesticated cotton species (2). The disease is distributed globally and cause severe loss under conducive conditions.Fusarium wilt caused significant losses in the US in the 1950's after which yield losses were minimized until 1990, when the incidence started to progressively increase. Fusarium wilt was nominated as the important disease of cotton in the peoples' Republic of China in the late 70s, but that country is now able to control losses with improved, more resistant cotton varieties (3). On the other hand, Fusarium wilt continues to be Tanzania's most important cotton disease, where it is spread rapidly by the feeding of gin waste to cattle and plant infecting seed (4).

The pathogen *Fov*produces both cellulolytic enzymes (C_1 and C_x) and pectinolytic enzymes (macerating enzymes, pectin methyl esterase and endopolygalacturonase) under *in vitro* conditions. For successful pathogenesis, the pathogen has to overcome the host barriers like cell wall, pectin layer and protein matrix (5). The elaboration of an array of cell wall splitting enzymes helps the pathogen for easy penetration of the host cell wall and subsequent colonization (6).

Cellulose is a major structural constituent of the cell wall of host plants. Many phytopathogenic fungi produce cellulases in culture adaptively which hydrolyse cellulose and its derivatives (7). With this background the present study was undertaken to find the production of cellulolytic and pectinolytic enzymes for successful pathogenesis

MATERIALS AND METHODS

Standardization of culture media for the rapid growth of *F. oxysporum f spvasinfectum* : *F. oxysporum* f. sp.Vasinfectumwas grown on PDA which supported rapid growth of the pathogen. The sterilized warm medium was poured @15 ml in sterile Petri dishes and medium was allowed to solidify. A seven-day-old, five-mm culture disc of the fungus was inoculated at the centre of the plate which are collected from different districts of Tamil Nadu. The plates were incubated at room temperature (28±2ºC) and three replications were maintained for each treatment. The radial growth of the fungus was measured 7 DAI.

Growth of *F. oxysporum f. sp. vasinfectum* on different liquid media : Liquid broths *viz.,* potato dextrose broth were prepared and 100 ml of medium was distributed in 250 ml Erlenmeyer flasks. The flasks were autoclaved at1.4 kg cm^{-2} for 20 min, cooled and inoculated with seven-day-old, five-mm culture disc of the pathogen from 15 districts of Tamil Nadu. After seven days, the mycelial mat was filtered through pre weighed Whatman No.1 filter paper, dried in hot air oven at 100º C until constant weight was obtained.

Pathogenicity in Glasshouse

Multiplication of inoculum : The fungus was multiplied on sand-maize medium. The medium containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1} and filled in empty salaine bottles. The bottles were sterilized at 1.4 kg cm^{-2} pressure for two h for two alternate days. Each bottle was inoculated with two nine-mm culture disc of actively growing *F. oxysporum* f. spvasinfectumand incubated at

room temperature (28 2°C) for 30 daysto be used as inoculum.

Earthen pots of 25 cm-dia were filled with five kg of potting medium (red soil : sand : FYM @ 1:1:1). The pot mixture was sterilized in an autoclave at 1.4 kg cm^{-2} pressure for two h on two successive days and inoculated with 5 g inoculum of *F.oxysporum f spvasinfectum* multiplied on sand maize medium. Healthy cotton were sown in pots with proper control. The pots were maintained in glasshouse by uniform and judicious watering and plants were constantly observed for the development of symptoms. The pathogen was reisolated from plants showing symptoms of wilt.

Survey and disease assessment :A survey was conducted during 2008-2009 on the incidence ofbasal rot disease in differentcotton growing areas of Tamil Nadu, In each village, four fields were selected and four plots in each field having an average area of ten square meters were marked at random. Basal rot affected plants were counted in each plot and expressed as per cent disease incidence.

$$\text{Per cent disease incidence} = \frac{\text{Number of plant affected}}{\text{Total number of plants observed}} \times 100$$

Isolation of pathogen : The pathogen was isolated from the diseased tissues of cotton by tissue segment method. The infected portions of diseased plants were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in three changes of sterile distilled water and then placed on previously poured and solidified Petri dish containing Potato Dextrose Agar (PDA) medium. These plates were incubated at room temperature (28 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogens were identified based on their cultural and morphological characters**.**

Molecular identification of pathogens

Isolation of total genomic DNA : Total DNA was extracted from 15 isolates of *F. oxysporum* f sp. *vasinfectum* isolates according to the method of (8). For DNA extraction, the fungi were grown separately in 30 ml of potato dextrose broth for seven days at room temperature (28 ± 2ºC). The mycelia were harvested by filtration through double layers of country filter paper and dried at 25 ºC. One g of the dried mycelial mat was ground to fine powder using liquid nitrogen. Powdered mycelia were vortexed in pre-cooled CTAB buffer (N-cetyl-N,N, Ntrimethyl ammonium bromide (1%), 0.1 M TrisHcl (pH: 8.0), 1.4 M NaCl, 0.5 M EDTA (pH: 8.0), polyvinyl pyrolidone (1%), mercaptoethanol (1%) and 1 % sodium sulphite) and incubated at 65°C for 30 min followed by the addition of 750 µl of chloroform and isoamylalcohol (24:1 v/v). The contents were gently mixed by inverting the tube for 4-5 times and then centrifuged at 10,000 rpm for 10 min. The upper aqueous phase containing DNA was transferred to a new 1.5 ml micro centrifuge tube and added with equal volume of chloroform and isoamylalcohol (24:1), mixed well by inverting the tube for 4-5 times and centrifuged at 10,000 rpm for 10 min. The upper aqueous phase (300 µl) was taken without disturbing the inter phase in a new 1.5 ml microfuge tube and added with 0.5 volume of 5 M NaCl and 2 volume of ice cold ethanol, the contents were mixed well and incubated at -20°C for 1h or overnight. The contents were centrifuged at 13,000 rpm at 4°C for 10 min, the ethanol was decanted and the DNA pellet was air dried. The DNA pellet was resuspended in 50 µl of Tris-EDTA buffer (10 mMTris-HCl and 1mM EDTA, pH 8.0). The genomic DNA was checked by agarose gel electrophoresisand stored at -20°C for further use.

Sequencing of the Internal Transcribed Spacer (ITS) region of the Rdna : The purified DNA was amplified by Polymerase Chain Reaction (PCR) using universal ITS1 and ITS4 primers.

Primer type	Primer sequence	Target
Forward	(5'-TCC GTA GGT GAA CCT GCG G- 3')	18S rDNA
Reverse	(5'-TCC TCC GCT TAT TGA TAT GC- 3')	18S rDNA

Reactions were performed in 20 l mixture containing approximately 50 ng of total DNA, 5 mM each dNTPs, 20 pmol of each ITS1 forward primer and ITS2 reverse primer and 0.5 µl of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The reaction was carried out in Eppendorfepgradient S Master cycler (Eppendorf, Hamburg, Germany) programmed with

Steps	Temperature (°C)	Time
Denaturation (initial)	94	5 min
Denaturation	94	30sec
Annealing	55	30sec
Extension	72	2 min
Extension (final)	72	5 min

Amplicons were analyzed by electrophoresis in 1.5% agarose gels in TAE buffer and visualized by staining with ethidium bromide and recorded with an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA). The sizes of the PCR products were determined by comparison with standard 100 bp or 1 kb molecular marker (Bangalore Genei Pvt. Ltd., Bangalore, India).Sequencing was done at 1st Base Pvt. Ltd, Singapore. Database search was performed with the BLAST 2.0 program from the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA, World Wide Web serve.

Production of cell wall degrading enzymes : To study the *in vitro* production of pectinolytic and cellulolytic enzymes, the pathogens were grown on Czapek'sDox liquid medium (pH 7-7.5) wherein the carbon source was

substituted with one per cent pectin (for pectic enzymes) or one percent carboxy methyl cellulose (for cellulolytic enzymes). The media were inoculated with 9 mm diameter culture disc of the pathogens. The culture filtrates were obtained after incubation at room temperature (27 ± 1ºC) for 5, 10, 15 and 20 days and centrifuged at 3000 g for 20 min. For the assay of pectinolytic enzymes, the culture filtrates were dialysed for 18 h against distilled water at 40ºC. The dialysate served as enzyme source. As dialysis was found to reduce the activity of cellulolytic enzymes (9), the culture filtrates as such were used for the assay of cellulases.

Assay of Cellulolytic Enzymes

Cellulase (C_1) activity : Cellulase (C_1) activity was assayed by the method of Norkrans (1950). The assay mixture contained 1 ml of cellulose solution (the concentration of which was adjusted to give approximately 0.85 absorbance at 610 nm), 4 ml of 0.1 M phosphate buffer (pH 7.0) and 5 ml of enzyme source. The absorbance of the assay mixture was determined at 610 nm in a Spectronic - 20 colorimeter immediately upon the addition of the enzyme source and again after an incubation period of 24 h at 27ºC. The enzyme activity was expressed in units (1 unit = change in absorbance of 0.01)

Cellulase (C_x) activity : Cellulase (C_x) activity was assayed by the viscosimetric method of (10) using carboxy methyl cellulose as the substrate. Two ml of enzyme extract was added to 4 ml of 1.2 per cent carboxy methyl cellulose (CMC) solution buffered at pH 5.0 with sodium citrate buffer. The loss of viscosity of the CMC solution was determined by means of an Ostwald-Fenskeviscosimeter size 150 at 5 min intervals up to 15 min. Enzyme source boiled for 10 min at 100ºC served as check. The results were expressed as the per cent loss in viscosity in 15 min.

$$V = \frac{T_0 \quad T_1}{T_0 \quad T_w} \quad 100$$

Where

V	=	Per cent loss of viscosity
T_0	=	Flow time in seconds at zero time
T_1	=	Flow time of the reaction mixture at time T_1
T_w	=	Flow time of distilled water

Assay of Pectinolytic Enzymes

Macerating enzymes : Macerating enzyme activity was assessed by the method described by Mahadevan (1965). Potato discs of 9 mm diameter and 30 µ thickness were obtained by using a hand microtome. Ten potato discs were placed in a sterile Petri dish and 10 ml of the culture filtrate was added. The uninoculated medium served as control. The coherence of the potato discs was tested at different time intervals using a sterile glass rod and the enzyme activity was expressed as the time taken in hours for maceration of potato discs.

Pectin methyl esterase (PME)

Pectin methyl esterase activity was estimated following the procedure described. Pipetted out 20 ml of pectin solution and its pH was adjusted to 7.0 using 1 N sodium hydroxide. To this, 10 ml of enzyme solution was added and its pH was adjusted immediately to 7.0 in the pH meter by adding 1 N NaOH. The enzyme substrate mixture was incubated for 24 h and pH was readjusted to 7.0 with 0.02 N NaOH, which was equal to the enzyme activity and the enzymatic activity was expressed in terms of units (one unit is 0.1 ml of 0.02 N NaOH used).

Endo polygalacturonase (endo PG)

Endo PG activity was estimated by the standard viscosimetric method (Hancock *et al.*, 1964) using 3 ml of enzyme source, 1 ml of 1.2 per cent sodium polypectate and 1 ml of 0.5 M ammonium acetate buffer. The loss in viscosity of the pectate solution was determined by means of the Ostwald-Fenskevicosimeter size 150 at 5 min intervals up to 15 min. Enzyme source boiled for 10 min at 100ºC served as check. The results were expressed as the per cent loss in viscosity in 15 min.

Assay of PectateLyase (PL) activity

PL activity was determined by monitoring the formation of C_4 and C_5 unsaturated products spectrophotometrically at 235 nm (11). Five hundred µl of 0.1 M TrisHC1 (pH 9) buffer containing 0.5 mM $CaCl_2$ was rapidly mixed in a 1.5 ml cuvette with 370 µl of distilled water, 100 µl of a polygalacturonic acid solution (1% w/v) in water, and 30 µl of supernatant obtained by grinding infected aloe vera leaf tissue in a pestle and mortar using 0.1M TrisHcl (pH9) buffer. The reaction mixture was incubated at 30°C. One unit was defined as the amount of enzyme which produced 1 µm of unsaturated product. Activity was expressed in micromoles of unsaturated product liberated per minute per ml of supernatant and specific activity was expressed in m of unsaturated product liberated per minute per ml of OD at 580 nm.

Assay of Pectin Trans Eliminase (PTE) activity

PTE activity was estimated by the viscometric method described by (12). Four ml of the substrate and one ml of the enzyme were pipetted into the viscometer. The loss in viscosity of the pectin solution was determined by using of Vinsell Viscometer of size 300. The activity was expressed as per cent reduction in viscosity.

$$V = \frac{T_0 \quad T}{T_0 \quad T_{H_2O}} \quad 100$$

Where

V -Per cent loss in viscosity

Table-1 : Virulence analysis of *Fusariumoxysporum* f. sp. Vasinfectum.

Sl. No.	Isolate code	Place of collection	Districts	Disease Incidence (%)	Expression of symptom (days)	Mycelial growth (cm) on 7th day	Mycelial Dry weight (mg) on 7th day)
1.	ACFov	Alandurai	Coimbatore	66.00(54.33)	30	9.00[a]	914.00[a]
2.	TCFov	Thottipalayam	Coimbatore	44.00 (41.55)	65	8.30[a]	851.00[c]
3.	KCFov	Keranatham	Coimbatore	20.00 (26.57)	70	8.90[a]	574.00[j]
4.	NSFov	Navakurichi	Salem	42.00 (40.40)	35	7.00[a]	781.00[d]
5.	PSFov	Periyeri	Salem	23.00 (28.66)	70	9.00[b]	914.00[a]
6.	RNFov	Rasipuram	Namakkal	54.90 (47.81)	28	7.50[g]	542.00[k]
7.	MTFov	Manachanallur	Trichy	65.98 (54.27)	24	7.50[g]	742.60[a]
8.	KEFov	Kodumudi	Erode	56.78 (48.85)	76	9.22[a]	899.89[b]
9.	PMFov	Palamedu	Madurai	43.34 (41.15)	67	8.32[d]	530.90[m]
10.	UMFov	Usilampatti	Madurai	56.76 (48.85)	23	7.00[a]	765.90[f]
11.	TTFov	Thiruvidaimaruthur	Thanjavur	36.78 (37.29)	22	7.50[g]	699.80[g]
12.	ANFov	Akkur	Nagapattinam	59.23 (50.30)	27	7.60[f]	650.87[h]
13.	VSFov	virudhunagar	Srivilliputhur	64.23 (53.25)	65	8.30[e]	560.80[i]
14.	ANFov	Ananthamangalam	Nagapattinam	54.33 (47.47)	63	8.50[c]	540.89[l]
15.	MNFov	Mayiladuthurai	Nagapattinam	15.16 (26.12)	63	8.00[a]	523.90[n]

Values are means of three replications, Figures in the parentheses represent arcsine transformed values. The common letters show non-significant differences among the treatments based on DMRT.

Table-2 : Production of cellulase enzyme by different FOV isolates in cotton.

Sl. No.	Isolate code	Cellulase enzyme (After 7th day)	
		C1*	CX**
1.	ACFov	9.42[a]	9.24[o]
2.	TCFov	3.68[h]	20.13[n]
3.	KCFov	4.34[g]	24.86[m]
4.	NSFov	7.12[b]	73.42[f]
5.	PSFov	8.66[bc]	86.16[a]
6.	RNFov	1.66[i]	80.44[c]
7.	MTFov	1.24[l]	75.64[d]
8.	KEFov	4.34[g]	63.38[j]
9.	PMFov	5.63[e]	70.16[h]
10.	UMFov	1.22[m]	80.78[b]
11.	TTFov	1.56[k]	67.99[i]
12.	ANFov	4.67[f]	60.98[l]
13.	VSFov	6.78[c]	74.56[e]
14.	ANFov	4.90[e]	62.55[k]
15.	MNFov	5.66[d]	70.54[g]

*Enzyme activity in units, **percent loss of viscosity values are means of three replications. The common letters show non-significant differences among the treatments based on DMRT.

T_o-Flow time in seconds at zero time

T-Flow time of reaction mixture at time T

T_{H2O}-Flow time of distilled water.

Collection andisolation of pathogen : The cotton wilt pathogen *Fusariumoxysporum*f. sp. *vasinfectum* was isolated from the diseased root of collected from 15 different places of Tamil Nadu. The pathogen was isolated and purified. These isolates were maintained in PDA slants for further studies

Virulence of different isolates of *Fusariumoxysporum* f.sp. *Vasinfectum* : A pot culture experiment was conducted to confirm the virulence of different isolates collected from different parts of Tamil Nadu. Among the fifteen isolates tested isolate ACFov collected from Alandurai of Coimbatore district was found to be most virulent and confirm its virulent by recording 66.00 PDI variety were the avirulent isolate recorded the PDI of 26.12. Final examination vascular discolouration confirmed and the pathogen was reisolated (Table-1).

Standardization of culture media for the rapid growth of *F. oxysporum* f.sp.*Vasinfectum* : With the view to find out the growth of Fovon solid media an *in vitro* study was conducted and the result showed that the highest mycelial diameter was recorded 9.0 cm by the virulent isolate ACFovalso found to promote more mycelial biomass with a mean dry weight of 914.00 mg compared to other isolates

Molecular characterization of isolates of *Fusariumoxy sporum* f. sp. *Vasinfectum* : Representative isolates were identified by sequencing the Internal Transcribed Spacer (ITS) region of the rDNA and comparing the sequences with those in GenBank database using BLAST searches. The ITS region, which includes the ITS regions 1 and 2, the 5.8S rRNA genes was amplified with primers ITS1 and ITS4 (White *et al*., 1990). The PCR amplified products were approximately 560 bp in size. The PCR products from *Fusariumoxy sporum* f. sp. *vasinfectum* analysis of the nucleotide sequences of the ITS regions of isolates using NCBI-BLAST search showed 100% and 99% similarity.

Table-3 : Pectatelyase activity (PL) by different FOv isolates in cotton.

S. No.	Isolates	*Macerating enzymes (hrs)	**PL-OD value at 580nm*			
			Incubation time (Days)			
			3	5	7	9
1.	ACFov	14	2.715a	2.897a	3.010a	2.879a
2.	TCFov	12	2.689b	2.803b	2.924b	2.754b
3.	KCFov	10	2.688b	2.769c	2.854c	2.725c
4.	NSFov	11	2.626c	2.738d	2.831d	2.720d
5.	PSFov	10	2.621d	2.734e	2.826e	2.714e
6.	RNFov	11	2.613e	2.724f	2.825e	2.703f
7.	MTFov	18	2.613e	2.723g	2.813f	2.699g
8.	KEFov	24	2.609f	2.716h	2.808g	2.687h
9.	PMFov	No maceration	2.604g	2.715i	2.802h	2.671i
10.	UMFov	No maceration	2.597h	2.713j	2.801h	2.655j
11.	TTFov	No maceration	2.587i	2.691k	2.794i	2.634k
12.	ANFov	No maceration	2.562j	2.689l	2.741j	2.632l
13.	VSFov	No maceration	2.549k	2.655m	2.739k	2.596m
14.	ANFov	No maceration	2.541l	2.652n	2.738k	2.575n
15.	MNFov	No maceration	2.496m	2.564o	2.645l	2.504o

10*Time taken for maceration, **Enzyme activity in units Mean of three replication. The common letters show non- significant differences among the treatments based on DMRT.

Table-4 : Production of Pectin Methyl Esterase (PME) by different isolates of F.o.f. sp. vasinfectumin cotton plants.

S. No.	Isolates	*PLPME (mole hydrogen ion min^{-1} ml^{-1})			
		Days after inoculation*			
		3	5	7	9
1.	ACFov	0.20^{a}	0.29^{a}	0.38^{a}	0.44^{a}
2.	TCFov	0.18^{b}	0.27^{b}	0.37^{b}	0.37^{b}
3.	KCFov	0.18^{b}	0.26^{c}	0.37^{b}	0.37^{b}
4.	NSFov	0.18^{b}	0.26^{c}	0.37^{b}	0.36^{c}
5.	PSFov	0.16^{c}	0.25^{d}	0.36^{c}	0.36^{c}
6.	RNFov	0.16^{c}	0.24^{e}	0.36^{c}	0.35^{d}
7.	MTFov	0.15^{d}	0.24^{e}	0.35^{d}	0.35^{d}
8.	KEFov	0.15^{d}	0.24^{e}	0.35^{d}	0.34^{e}
9.	PMFov	0.15^{d}	0.24^{e}	0.34^{e}	0.34^{e}
10.	UMFov	0.15^{d}	0.23^{f}	0.33^{f}	0.33^{f}
11.	TTFov	0.14^{e}	0.23^{f}	0.33^{f}	0.33^{f}
12.	ANFov	0.13^{f}	0.23^{f}	0.33^{f}	0.33^{f}
13.	VSFov	0.13^{f}	0.22^{g}	0.32^{g}	0.32^{g}
14.	ANFov	0.04^{g}	0.22^{g}	0.31^{h}	0.32^{g}
15.	MNFov	0.04^{g}	0.20^{h}	0.22^{i}	0.31^{h}

*Enzyme activity in units Mean of three replication. The common letters show non- significant differences among the treatments based on DMRT.

The results obtained in the present study indicated that the pathogen *Fov* produced C_1 and C_x*in vitro* and the activity of these enzymes increased with increase in age of the culture. The virulent isolate of *FoV*produced more cellulolytic (C_1 and C_x) enzymes than the avirulent one. Cellulose is a major structural constituent of the cell wall of host plants. Many phytopathogenic fungi produce cellulases in culture adaptively which hydrolyse cellulose and its derivatives (Reese *et al*., 1950, Reese, 1956 and Marimuthu*et al*., 1974). The results obtained in the present study indicated that the pathogen *Fov*produced C_1 and C_x*in vitro* and the activity of these enzymes increased with increase in age of the culture. The virulent isolate of *Fusariumoxysporum f spvasinfectum* produced more cellulolytic (C_1 and C_x) enzymes than the avirulent one (Table-2).

Lasiodiplodiatheobromae and *Rhizopusstolonifer* had the highest percentage of pathogenic indices (96 per cent) followed by *Macrophominaphaseolina* and *Fusariumpalli doroseum* with 83 per cent and 80 per cent respectively and least was *Penicillium* spp (5 per cent) (14). This is in line with the results of (15) in their work on the

Table-5 : Production of Poly Galacturonase (PG) by different isolates of F. o. f. sp. vasinfectum in cotton plants.

S. No.	Isolates	*Polygalacturonase (per cent reduction in viscosity)			
		Days after inoculation*			
		3	5	7	9
1.	ACFov	9.55a	9.86a	15.45a	15.24a
2.	TCFov	9.33b	9.77b	14.78b	14.52b
3.	KCFov	9.06c	9.76b	14.56c	14.33c
4.	NSFov	9.04d	9.67c	14.30d	14.31d
5.	PSFov	9.03d	9.56d	14.22e	13.89e
6.	RNFov	8.77e	9.55d	14.11f	13.75f
7.	MTFov	8.77e	9.39e	13.89g	13.55g
8.	KEFov	8.67f	9.34f	13.78h	13.43h
9.	PMFov	8.66f	9.34f	13.67i	13.42h
10.	UMFov	8.55g	9.33g	13.67i	13.33i
11.	TTFov	8.52h	9.23h	13.56j	13.33i
12.	ANFov	8.44i	9.20i	13.00k	13.27j
13.	VSFov	8.38j	9.12j	12.88l	12.99k
14.	ANFov	8.22k	9.12j	12.67m	12.78l
15.	MNFov	8.12l	9.08k	12.22n	12.57m

**Enzyme activity in units Mean of three replication. The common letters show non-significant differences among the treatments based on DMRT.

Table-6 : Production of Pectin Trans Eliminase (PTE) by different isolates of F. o. f. sp. vasinfectumin cotton plants.

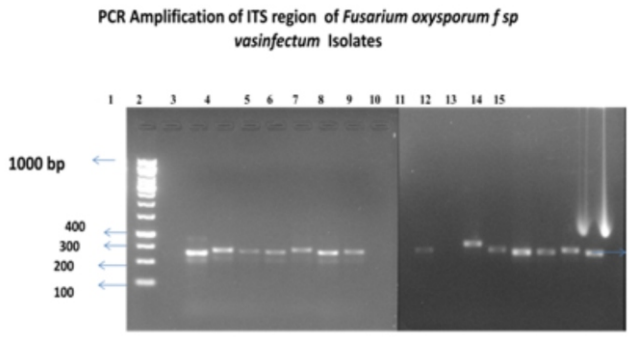
S. No.	Isolates	*Pectin trans-eliminase (per cent reduction in viscosity)			
		Days after inoculation*			
		3	5	7	9
1.	ACFov	46.45^{a}	50.86^{a}	58.45^{a}	58.64^{a}
2.	TCFov	43.55^{b}	50.67b	57.87^{b}	56.83^{b}
3.	KCFov	39.45^{c}	48.76^{c}	53.56^{c}	56.77^{c}
4.	NSFov	38.45^{c}	48.54^{d}	53.23^{d}	55.77^{c}
5.	PSFov	37.90^{d}	47.54^{d}	52.66^{e}	55.55^{d}
6.	RNFov	37.14^{e}	46.36^{e}	52.45^{f}	53.74^{e}
7.	MTFov	36.67^{f}	45.98^{f}	51.34^{g}	52.83^{f}
8.	KEFov	36.55^{g}	45.78^{g}	50.56^{h}	50.99^{g}
9.	PMFov	34.67^{h}	45.65^{h}	50.54^{i}	50.67^{h}
10.	UMFov	34.33^{i}	44.25^{i}	49.45^{j}	50.34^{i}
11.	TTFov	34.28^{j}	43.42^{j}	48.78^{k}	49.02^{j}
12.	ANFov	33.49^{k}	42.93^{k}	48.56^{l}	47.67^{k}
13.	VSFov	32.87^{l}	42.53^{l}	47.67^{m}	47.21^{l}
14.	ANFov	32.87^{l}	41.56^{m}	45.89^{n}	43.77^{m}
15.	MNFov	30.88^{m}	40.89^{n}	45.67^{o}	43.55^{n}

**Enzyme activity in unitsMean of three replication. The common letters show non- significant differences among the treatments based on DMRT.

effect of *Pythiumaphanidermatum* and *Sclerotiumrolfsii* in Tomato and Pepper. It has been reported by (13) and Padmaja and Balagopal (1985) also found that *Rhizopusoryzae* produced cellulases capable of degrading the cassava tuber cellulose.

The virulent isolates of Fov inducedmore macerating enzymes, pectin methyl esterase (PME), Pectin trans eliminase (PTE), pectatelyase (PL) and endopolygalacturonase (endo-PG) *in vitro* than the avirulent ones (Table-4 and 5). All the pectinolytic enzymes were highly active in the culture filtrate up to 10 days of age and thereafter the activity decreased.

The maceration of potato discs increased with increasing age of the culture *in vitro* up to 9 days. Maximumpectinolytic enzymes in the culture filtrate occurred up to 9 days indicating the role of these enzymes in pathogenicity (Table-3). The enzyme PG hydrolytically cleaves pectin in such a manner that the á1, 4-glycosidic bonds of the chain are split (9). PME removes the esterified methyl group from the pectin chain hydrolytically



(6). Since the fungus is *endophytic which* are intercellular in the host, the production of these enzymes appears to facilitate the dissolution of host cell wall and middle lamella and help entry and establishment of the pathogen in the host and are possibly responsible for playing a vital role in pathogenesis through cell wall degradation and disintegration of tissues.

Pectinolytic enzymes constitute the major group of cell wall degrading enzyme; they are polygalacturonase and pectin methyl esterase. They involved mainly in the catabolism as well as the solubility of the polysaccharides and also in tissue maceration which leads to rot disease. This was also observed in the works with different test pathogens used by (15). They observed tissue softening as a results of pectinolytic enzyme activities. This could result in the reduction of some contents of the cell wall, (such as galactan, araban and polyuronide), solubilization and partial depolymerization of some substances, which lead to tissue maceration. These observations are in line with those of (16), where tissue maceration was observed due to the enzymatic activities of the pectin methyl esterase and polygalacturonase enzymes.

In the present investigation, the virulent pathogens produced more cellulolytic and pectinolytic enzymes than the avirulent ones indicating the importance of the cell wall degrading enzymes in pathogenesis.

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