ENZYMATIC ACTIVITY OF FUNGAL PATHOGENS IN CORN

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Abstract

Enzymatic activity e.g., pectinase, cellulase, protease and lipase of different fungal pathogens causing stalk rot disease in corn was determined. *In-vitro* studies; corn stalk rot pathogens viz., *Cephalosporium acremonium, Fusarium moniliforme, F. graminearum, F. semitectum, Macrophomina phaseolina,* st. 1, *M. phaseolina* st. 2, *Rhizoctonia solani* st.1, *R. solani* st. 2 and *Verticillium albo-atrum* showed maximum pectinase activity after 48 hrs., which then decreased with the exception of *C. acremonium. F. moniliforme, F. graminearum* and *F. semitectum* showed the same pattern of pectinase activity but in variable quantity (88.9 μml^{-1}, 66.6 μml^{-1} & 77.7 μml^{-1} respectively) after 48 hrs. In *V. albo-atrum* alongwith both strains of *Macrophomina phaseolina,* pectinase activity was also maximum at 48 hrs., and then significantly reduced at 96 hrs. Production of pectinase enzyme by *C. acremonium* started at 24 hrs., (22.2 μml^{-1}) and showed maximum activity at 72 hrs., (66.6 μml^{-1}) and then decreased significantly at 96 hrs., (11.1 μml^{-1}). Cellulase production was not observed at 24 hrs., after inoculation of the pathogens, however, all the pathogens except *R. solani* st.2 showed cellulase activity at 48 hrs. Protease enzyme was found only in *C. acremonium, F. moniliforme* and *F. graminearum.* Lipase activity was maximum at 24 hrs., post-inoculation in all pathogens with the exception of *F. graminearum, C. acremonium* and *R. solani* st.1. Differences in enzymatic activity at different intervals may suggest their specificity in causing corn stalk rot disease.

Introduction

Stalk rots are among the most destructive diseases in maize (*Zea mays* L.) throughout the world (Christensen & Wilcoxson, 1966; De Leon & Pandey, 1989). The disease caused by many soil-borne and residue-borne fungi (Byrnes & Carroll, 1986; Dodd, 1980; Gilbertson *et al*., 1985; Kommedahl *et al*., 1987) is highly influenced by low moisture stress (Dodd, 1980; Schneider & Pendery, 1983). In Pakistan, *Fusarium* spp., followed by *Macrophomina phaseolina* (Tassi) Goid have been found to produce stalk rot disease of maize (Ahmad & Aslam, 1984; Ahmad *et al*., 1995, 1996). The disease causing organisms enter the host tissue through mechanical pressure exerted by the growing germ tube or dissolving the host cell wall through secretion of toxins or enzymes (Albersheim & Jones, 1969). In *Pythium* stalk rot of maize, highly virulent isolates produced more toxins and enzymes than moderately and weakly virulent isolates (Sadik & Payak, 1983). The enzymes produced by pathogens affect chemistry of cell wall which is accompanied by cell wall degradation Albersheim & Jones (1969).

The plant cell wall is a complex structure of polymers which surrounds the cell containing cytoplasm (Karr & Albersheim, 1970). For the degradation of cell wall, exocellular enzymes like cellulolytic, hemicellulolytic, pectolytic and proteolytic enzymes are produced which are capable of attacking each of the major polymeric component

(Wheeler, 1975) and plant pathogens have been found to secrete a range of enzymes which are capable of degrading plant cell wall components (Riou, 1991). Apart from cell wall degrading enzymes secreted by a wide variety of saprophytic and phytopathogenic micro organisms (Bailey & Pessa, 1990; Rombouts & Pilnik, 1980), the pectinases also play an important role in the entry of the plant pathogen intra and inter cellularly into the host tissues thus blocking the conducting vessels resulting in the development of wilt (Soni & Bhatia, 1981). Most pathogens have the capacity to produce more cellulolytic than pectolytic enzymes (Sadik & Payak, 1983). The correlation between stalk rot and cellulase production was significant in maize (Chambers, 1987). Lipases and proteases are important enzymes in pathogenesis which attack the plasmalemma after the degradation of cell wall by proteases alongwith pectolytic and cellulolytic enzymes (Hameed *et al*., 1994). Not much work seems to have been reported on the enzyme production by pathogens causing corn stalk rot disease. Experiments were therefore carried out to study the production of enzymes by fungal pathogens associated with corn stalk rot disease and the role of these enzymes in pathogenesis.

Materials and Methods

Fungi viz., *Cephalosporium acremonium* Samra, Sabet & Hingorani, *Fusarium moniliforme* Sheld., *F. graminearum* Schw., *F. semitectum* Berk. & Rav., *Macrophomina phaseolina* (Tassi) G. Goid st. 1, *M. phaseolina* st. 2, *Rhizoctonia solani* st.1 Kuhn, *R. solani* st. 2 and *Verticillium albo-atrum* Nees., were isolated from infected corn stalks on potato-dextrose-agar (PDA) medium. These pathogens were then tested for *in-vitro* production of pectinase, cellulase, protease and lipase enzymes.

Pectinase: For qualitative tests, cultures were grown on agar medium in Petri dishes containing 500 ml of mineral salt solution, 1g of yeast extract, 15g of agar, 5g of apple pectin and 500 ml of distilled H_2O (Hankin, 1975). After 3-5 days growth at 28 ^{o}C the plates were flooded with 1% aqueous solution of hexadecyl-trimethyl-ammonium-bromide which precipitated the pectin in the medium and showed clear zones around a colony indicating degradation of pectin.

For quantitative assay of pectolytic activity the method given by Soni & Bhatia (1981) was used where 100 ml of the medium was poured in 250 ml flasks. After autoclaving, the medium was inoculated with a spore suspension of the test pathogens and incubated at 25°C for 4 days in a rotary shaker at 110 rpm. After 24, 48, 72, and 96 hours growth the filtrate was centrifuged at 5000 rpm for 20 mins., at 4°C. The supernatant was used as enzyme extract for measuring enzyme activity. The method given by Deese & Stahmann (1962) was used for the measurement of pectin methyl esterase (PME) activity. Titration was done against 0.01 N NaOH using phenolphthalein as an indicator (Vogel, 1969). PME activity was calculated as microequivalents of methoxyl groups liberated hr^{-1} by 1ml of the enzyme preparation under the specified conditions.

Cellulase: For qualitative analysis the method described by Leopold & Samsinakova (1970) was used to detect cellulolytic activity. Petri plates containing ($NaNO_3$ 0.2 g; KH_2PO_4 0.1 g; KCl 0.05 g; $MgSO_4$ 0.02 g; $CaCl_2$ 0.01 g; Yeast extract 0.05 g; Distilled H_2O 100 ml) were inoculated and incubated for 2-4 days at 28°C. The surface was then flooded with 1%

aqueous Hexadecyl-trimethyl-ammonium-bromide (Hankin *et al*., 1971). Undegraded cellulose was precipitated with this reagent making a clear zone where cellulose had been degraded.

For quantitative assay samples were grown in 250ml flasks containing 100 ml of Czapek's Dox broth on a rotary shaker at 110 rpm at 28^oC for 4 days. Samples (10ml) were taken from the flasks after 24, 48, 72 and 96 hours. Fungal cells were removed by filtration and filtrate was used for the enzyme assay. The method of Vaidya *et al*., (1984) was employed. The reaction mixture, made up of 1ml suitably diluted enzyme filtrate, 1ml of citrate acetate buffer 0.5 M (pH 5) and 2.5 ml of 1% carboxymethylcellulose (CMC) as a substrate was incubated for 1 hr. at 37^oC. This reaction mixture served as sample for determining the presence of glucose by DNS (Dinitrosalicylic acid) method (Ghose, 1969). One unit of cellulose was defined as 1.0mg of glucose released from 1% CMC hr^{-1} at 37^oC and pH 5.

Protease: For qualitative analysis of proteolytic activity, Casein soluble medium containing dextrose 40g; peptone 10g; agar 15g; yeast extract 3g; casein 8g; distilled H_2O 1000ml; pH 7.0 as described by Hameed (1984) was used. Inoculated plates containing the medium were incubated at 28^oC for 5-7 days. Formation of clear zones around the colonies showed protease activity. These zone formations were enhanced by flooding the plates with 10% glacial acetic acid.

For quantitative analysis of proteolytic activity, samples were grown in 250ml flasks containing 100ml of Sabouraud dextrose and yeast extract medium containing dextrose 40g; peptone 10g; agar 15g; yeast extract 3g; distilled H_20 1000 ml adjusted at pH 7.0 on a rotary shaker at 110 rpm at 28^oC for 4 days. Ten ml samples were taken after 24, 48, 72 and 96 hours by filtration and the filtrate was used to determine the proteolytic activity using the method of Kunitz (1965). One ml of 1% casein soluble was taken with one ml of enzyme extract and then incubated for one hour at 45^oC. After incubation 3ml of 5% Trichloroacetic acid (TCA) was added and kept in ice for 15 minutes. These samples were centrifuged at 5000 rpm for 30 mins., at 4^oC. Blank was prepared in the same way and 3ml of TCA was added before incubation. O.D. was measured on Shimadzu Model recording spectrophotometer under UV 240 at 280 nm. One enzymatic unit represents the quantity of enzyme which liberates 1 μg of tyrosine under enzyme assay conditions.

Lipase: To detect lipolytic enzyme activity qualitatively, the medium containing difco peptone 10g; NaCl 5g; $CaCl_2$. $2H_2O$ 0.1g; agar 20g; distilled H_2O 1000ml at pH 6.0 as described by Sierra (1957) was used. Olive oil was used as the lipid substrate. Plates were incubated at 28^oC for 4 days. Clear zones which formed around the colonies indicated degradation of substrate due to lipolytic activity.

Zvyagintseva's method (1972) was used for quantitative assay. Samples were grown in 250ml flasks containing 100ml of autoclaved medium containing $(NH_4)_2SO_4$ 3.0g; $MgSO_4$ 0.7g; NaCl 0.5g; $Ca(NO_3)_2$ 0.4g; KH_2PO_4 1.0g; K_2HPO_4 0.1g; glucose 5.0g; yeast extract 1.0g at pH 5 and kept on a rotary shaker at 30^oC and 110 rpm for 4 days.

Olive oil was sterilized separately in dry oven and 1ml of olive oil was added per 100 ml of sterile medium. Ten ml samples were taken after every 24 hrs., for 4 days. The cells were removed by centrifugation at 5000 rpm for 20 mins., and the supernatant was used for determining lipase activity. For this 1.3 ml of olive oil, 1 ml of phosphate buffer 0.066 M at

pH 7.0 (Gomori, 1955), 3ml of supernatant and 1.5 ml of distilled H_2O were shaken for 3 mins., then placed in an incubator shaker at 30^oC and 150 rpm for 5 hrs. After incubation 15 ml of ethanol was added to the reaction mixture, then 12.5 ml of diethyl ether (commercial ether) was added to destroy the emulsion. The mixture obtained was titrated against 0.1N NaOH solution using Thymolphthalein as an indicator (Vogel, 1969). The control samples were treated in the same manner but without incubation. The results of the titration of the control samples were subtracted from the results of the test samples. Lipase activity was expressed in ml of 0.1N NaOH used in the titration of the fatty acid formed where 1ml of alkali corresponds to 500 arbitrary units of lipase activity (Bier, 1955).

Pathogenicity test: For pathogenicity test, 6 corn cultivars viz., Pool-10 (source local selections x Satipo 31-CIMMYT), Islamabad (source Islamabad local farmer's land race), Gauher (source population 7930 selection), EV-1085 (source CIMMYT population 31), Sarhad yellow (source Akbar x Vikrim) and Akbar (source synthetic Pa, M14, WF9, W9, A495, A556) having three maturity groups., early (E), medium (M) and late (L) were hill planted in 15 m^2 plots. Hills were spaced 0.25 m apart within 0.75 m spaced rows. The trial was a split-split plot arrangement in randomized complete block design with three replications.

Corn stalks were inoculated with 9 fungal pathogens viz., *C. acremonium, F. moniliforme, F. graminearum, F. semitectum, M. phaseolina st. 1, M. phaseolina st. 2, R. solani* st.1, *R. solani* st. 2 and *V. albo-atrum* using the toothpick method of inoculation (Young, 1943). Round toothpicks, six cm long, were washed five times in boiling water, air dried and soaked in potato dextrose broth (potato 200 g; dextrose 20 g; water 1000 ml) contained in 300 ml glass jars were sterilized at 15 p.s.i., at 120^oC for 20 min. These were acidified with five drops per 100 ml of 25% lactic acid (Tuite, 1969) to inhibit bacterial growth and then inoculated with test fungi. Toothpicks were incubated at 25^oC for 3 weeks.

Corn plants were inoculated two weeks after tasseling (9 weeks after planting). Infected toothpicks carrying inoculum were inserted into holes drilled in the first elongated basal internode. The severity of disease was determined by measuring the lesions around the inserted toothpicks 8 weeks after inoculation at harvesting by splitting the stalks lengthwise and recording the extent of the spread of rot on a 0-5 scale (Hooker, 1957). Grain yield recorded was based on weight of shelled grains at 15% moisture using the following formula.

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{Field weight (100 - moisture \%) 0.8 x10,000}}{\text{Area harvested x 85}}$$

Where 0.8 was the shelling percentage (80% grains and 20% cob) and 85 was adjusted to 15% moisture.

Disease data on 0-5 scale were then transformed to percentages for statistical analysis as follows:

$$\text{Disease index} = \frac{\text{Disease rating in plants examined}}{\text{Total no. of plants examined}}$$

$$\text{Disease severity \%} = \frac{\text{Disease Index}}{5} \times 100$$

Data were analyzed by analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and correlation was calculated to examine the relationship between disease severity and grain yield losses as well as with each enzyme production.

Results

In-vitro tests all pathogens showed maximum pectinase activity after 48 hrs., post-inoculation which then decreased except in *C. acremonium* which showed maximum activity after 72 hrs., post-inoculation. All *Fusarium* spp., showed the same pattern of pectinase activity but in variable quantity whereas, *F. moniliforme, F. graminearum* and *F. semitectum* produced 88.9 μml^{-1}, 66.6 μml^{-1} and 77.7 μml^{-1} pectinase, respectively, after 48 hrs., growth. In both strains of *Rhizoctonia solani* the same enzyme activity was observed after 24 hrs., which reached its peak after 48 hrs., (77.7 μml^{-1}, and 88.8 μml^{-1} respectively) followed by a decline at 72 hrs., and 96 hrs., interval. In *V. albo-atrum* and also in both strains of *M. phaseolina,* pectinase production was found maximum after 48 hrs., and then significantly declined after 96 hrs., interval. Production of the same enzyme by *C. acremonium* was observed after 24 hrs., (22.2 μml^{-1}) with a maximum activity after 72 hrs., (66.6 μml^{-1}) followed by a decline 11.1 μml^{-1} after 96 hrs., interval (Table 1).

Cellulase production was not observed in the pathogens under test after 24 hrs., interval whereas all the pathogens except *R. solani* st.2 showed enzymatic activity after 48 hrs. *C. acremonium* showed maximum cellulase activity after 48 hrs., (0.37 μml^{-1}) which slightly reduced after 72 and 96 hrs., interval (0.35 μml^{-1} and 0.31 μml^{-1}, respectively). *F. moniliforme, F. graminearum* and *F. semitectum* showed maximum cellulase activity after 72 hrs., with (0.42, 0.18, and 0.56 μml^{-1}, respectively. Both strains of *M. phaseolina* as well as *R. solani* showed maximum cellulase activity after 96 hrs., with 1.3, 0.56 and 0.73 μml^{-1}, respectively except *M. phaseolina* st. 2 where the same enzyme activity reached its peak after 72 hrs., (0.42 μml^{-1}). Similarly in *V. albo-atrum* maximum activity of this enzyme was observed after 72 hrs., (0.96 μml^{-1}) (Table 2).

In-vitro production of protease enzyme was found only in *C. acremonium, F. moniliforme* and *F. graminearum* with maximum protease activity of 2.4, 8.6 and 11.2 μml^{-1}, respectively observed after 72 hrs., interval which decreased to respectively 1.4, 6.2 and 5.6 μml^{-1} after 96 hrs., interval (Table 3).

Lipase activity was maximum after 24 hrs., post-incubation in all pathogens, with the exception of *C. acremonium, R. solani* st.1. and *F. graminearum.* Of these *C. acremonium* and *R. solani* st.1., produced more lipase activity after 48 hrs., post-inoculation (200 μml^{-1} and 600 μml^{-1} respectively), whereas, *F. graminearum* showed maximum activity after 72 hrs., post-inoculation (600 μml^{-1}). *M. phaseolina* st.1 produced more lipase enzyme (400 μml^{-1}) after 24 hrs., as compared to 150 μml^{-1} by *M. phaseolina* st.2 which gradually decreased after 96 hrs., interval. These results were also found related with the virulence of pathogens in producing corn stalk rot disease (Table 4).

Table 1. *In-vitro* production of pectinase enzyme by different pathogens causing corn stalk rot at different intervals of growth.

Pathogens	Unit of activity (µml^{-1}) at hours			
	24	48	72	96
Cephalosporium acremonium	22.2	55.5	66.6	11.1
Fusarium moniliforme	22.2	88.9	44.4	22.2
Fusarium graminearum	16.6	66.6	44.4	16.6
Fusarium semitectum	22.2	77.7	33.3	27.7
Macrophomina phaseolina st. 1	11.1	66.6	44.4	11.1
Macrophomina phaseolina st. 2	22.2	88.9	61.1	11.1
Rhizoctonia solani st.1	11.1	77.7	22.2	11.1
Rhizoctonia solani st. 2	5.5	88.8	33.3	11.1
Verticillium albo-atrum	22.2	88.8	44.4	22.2

Table 2. *In-vitro* production of cellulase enzyme by different pathogens causing corn stalk rot at different intervals of growth.

Pathogens	Unit of activity (µml^{-1}) at hours			
	24	48	72	96
Cephalosporium acremonium	0.0	0.37	0.35	0.31
Fusarium moniliforme	0.0	0.21	0.42	0.39
Fusarium graminearum	0.0	0.13	0.18	0.19
Fusarium semitectum	0.0	0.49	0.56	0.38
Macrophomina phaseolina st. 1	0.0	0.23	0.40	1.3
Macrophomina phaseolina st. 2	0.0	0.35	0.42	0.31
Rhizoctonia solani st.1	0.0	0.39	0.47	0.56
Rhizoctonia solani st. 2	0.0	0.0	0.06	0.73
Verticillium albo-atrum	0.0	0.68	0.96	0.72

Table 3. *In-vitro* production of protease enzyme by different pathogens causing corn stalk rot at different intervals of growth.

Pathogens	Unit of activity (µml^{-1}) at hours			
	24	48	72	96
Cephalosporium acremonium	0.0	1.7	2.4	1.4
Fusarium moniliforme	0.0	4.2	8.6	6.2
Fusarium graminearum	0.0	7.3	11.2	5.8
Fusarium semitectum	0.0	0.0	0.0	0.0
Macrophomina phaseolina st. 1	0.0	0.0	0.0	0.0
Macrophomina phaseolina st. 2	0.0	0.0	0.0	0.0
Rhizoctonia solani st.1	0.0	0.0	0.0	0.0
Rhizoctonia solani st. 2	0.0	0.0	0.0	0.0
Verticillium albo-atrum	0.0	0.0	0.0	0.0

Table 4. *In-vitro* **production of lipase enzyme by different pathogens causing corn stalk rot at different intervals of growth.**

Pathogens	Unit of activity (μml^{-1}) at hours			
	24	**48**	**72**	**96**
Cephalosporium acremonium	100	200	100	50
Fusarium moniliforme	250	100	100	50
Fusarium graminearum	50	100	600	200
Fusarium semitectum	150	100	100	50
Macrophomina phaseolina st. 1	400	200	100	50
Macrophomina phaseolina st. 2	150	50	0.0	0.0
Rhizoctonia solani st.1	100	600	300	150
Rhizoctonia solani st. 2	100	100	50	0.0
Verticillium albo-atrum	300	100	100	50

Pathogenicity tests: *C. acremonium* produced maximum infection (90.8%) in the E cv. Islamabad followed by the cvs., Pool-10 (60.5%), Akbar (59.2%), Gauher (58.3%), Ev-1085 (40.2%) and Sarhad yellow (53.0%). Of the *Fusarium* spp., *F. moniliforme* was found most damaging with an average disease severity of 75.1% in L cv., Akbar followed by cvs., Sarhad yellow (L) 66.1%, Islamabad (E) 64.4% and Ev-1085 (M) 60.3%. Infection by *M. phaseolina* st.1 , was comparatively higher than st.2 in different cvs., except in Islamabad where *C. acremonium* was most damaging. *M. phaseolina* st.1 showed disease severity of 87.8, 85.5, 82.2 and 75.5% respectively in cvs., S. yellow, Akbar, Pool-10, and Ev-1085. *M. phaseolina* st.2 produced disease severity of 76.7, 73.7 and 72.8% in cvs. Pool-10, Akbar and Ev-1085 whereas, cvs. S. yellow and Islamabad showed 68.9% infection. It is interesting to note that cv. Gauher was found comparatively resistant against both strains of *M. phaseolina* (Table 5). *R. solani* st.1 caused more disease severity in cvs., Sarhad yellow (L) 67.5% and E cvs. Islamabad (63.9%) and Pool-10 (61.7%), while, *R. solani* st.2 caused more disease (73.8%) only in one cv. Pool-10, whereas, *V. albo-atrum* was found least damaging in other cvs. tested (Table 5).

In cv. Pool-10, *F. moniliforme* and *M. phaseolina* st.1 respectively showed 73.9% and 81.9% yield loss followed by cv. Islamabad which showed yield losses of 69.3% by *C. acremonium* and 66.5% by *M. phaseolina* st.2. Cultivar Akbar also showed an yield loss of 69.8% and 59.5% by *C. acremonium* and *F. moniliforme*, respectively. *M. phaseolina* st.1 was found to cause higher yield loss of 81.9% in cv. Pool-10 (Table 6). Grain yield loss was observed in three cvs., viz., Islamabad, Akbar and Pool-10 whereas the other three cvs. viz., Gauher, Faisal and Sarhad yellow were least affected (Table 6).

In corn cv. Pool-10, highest grain yield was observed in plants inoculated with *V. albo-atrum* which also showed the lowest yield loss of 26.2% as compared to plants inoculated with *C. acremonium* showing least grain yield and also higher yield loss of 69.2%. *M. phaseolina* and *R. solani* st.2 showed 52.0 and 52.4% yield losses respectively. Yield loss produced by *R. solani* st.1 in Islamabad cv. was 43%. On the other hand, cv. S. yellow showed low yield losses produced by all the pathogens with maximum yield loss of 31.4% produced by *V. albo-atrum* and least yield loss of 7.3% by *R. solani* st.1. *C. acremonium, M. phaseolina* st.1 and *R. solani* st.2 showed yield losses of 25.6, 21 and 25.5%, respectively.

Discussion

Cell wall which is composed of pectin, polysaccharides, cellulose, hemicellulose, lipids, proteins is degraded by the enzymes produced by a virulent pathogen (Wheeler, 1975). There are reports where more enzymes are produced by highly virulent isolates of *Pythium* stalk rot of corn than the weakly virulent ones (Sadik & Payak, 1983). In the present study where fungi viz., *Fusarium* spp., *M. phaseolina, R. solani, V. albo-atrum* and *C. acremonium i*solated from corn stalk rot were used, PME activity was highest at 48 hrs., after which it gradually decreased except in *C. acremonium* which showed highest activity at 72 hrs., (66.6 μml^{-1}) and thus was more significant in yield loss and disease severity (90.8%) in early corn cv., Islamabad. *C. acremonium* was also found host specific of this cv.

Similarly highest cellulase activity was observed in cultures of *Fusarium* spp., *M. phaseolina* st. 2 and *V. albo-atrum* after 72 hr. and by both strains of *R. solani, M. phaseolina* st.1 and *F. graminearum* after 96 hrs. Production of cellulase enzyme was also correlated with yield loss and disease severity in different corn cvs. presumably the pathogens travelled relatively slowly through the host tissues and therefore, there was slow degradation by the cellulases produced by the pathogens. The study of slow moving pathogens and their production of cellulases has also been reported by Wood (1960). Stalk rot of corn is significantly correlated with cellulase production (Chambers, 1987). Comparatively higher disease severity and yield losses by *C. acremonium* may be related to greater cellulase production at 48 hrs., at an earlier stage and thus it spread more in the stalk.

F. moniliforme and *F. graminearum* as well as *C. acremonium* produced protease which increased with the time of incubation sharing maximum activity at 72 hrs., which then declined, whereas, other fungal isolates did not produce protease enzyme but produced lipase enzyme in variable quantity (Table 4). This variation may be due to host specificity of the different pathogens which was maximum at 24 hrs., for *M. phaseolina* st.1, *R. solani* st.2 and *V. albo-atrum,* while, *C. acremonium* and *R. solani* st.1 showed maximum activity at 48 hrs., interval. Lipases and proteases are very important enzymes in pathogenesis which attack the plasmalemma after the degradation of cell wall by protease alongwith pectolytic and cellulolytic enzymes. Like proteases, membrane degradation by lipase depends on the involvement of specific type of enzyme and membrane (Condrea & de Vries, 1965).

The results of the present study showed positive correlation (r = 0.38) between disease severity and enzymes viz., pectinase, cellulase and lipase (r = 0.23, 0.10 and 0.15 respectively), whereas negative correlation was found between disease and protease enzyme activity (r = -0.02). Moreover, low levels of positive correlation was also observed between yield losses and in the production of pectinase, cellulase, protease and lipase enzymes (r = 0.18, 0.11, 0.10 and 0.04 respectively).

Our results indicated that for the development of stalk rot disease three factors viz., virulence of pathogen, susceptibility of host and suitable environmental conditions are required which may enhance the production of enzymes which were produced by the pathogens under test. Pectinase was the most important enzyme in initiating the process of cell wall degradation as it showed highest activity at 48 hrs. Once the pathogen entered the host and the cell wall was degraded by the action of pectinases and cellulases, further spread of the pathogen occurred when the cell membrane was influenced by enzymes viz., proteases and lipases. The variation in enzymatic activity at different intervals of post incubation by different pathogens may suggest their specificity for pathogenesis at proper timings. There is need for further purification and characterization of these enzymes which will help in better understanding the exact role of these enzymes in pathogenesis and host specificity based

enzymatic activity in host-pathogen interaction at different stages of plant growth.

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