

## PRODUCTION OF CELLULOLYTIC ENZYME (CX) BY DIFFERENT SEED BORNE PATHOGENS

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Eight different seed borne pathogens viz : *Alternaria alternata* (AA), *Verticillium* sp. (V.sp), *Macrophomina phaseolina* (MP), *Rhizoctonia bataticola* (RB), *Gliocladium virens* (GV), *Aspergillus niger* (AN), *Phoma* sp. (P.sp) and *Botryodiplodia theobromae* (BT) were obtained from five different legume hosts. All the pathogens were tested for cellulase production *in vitro*. *Verticillium* sp. produced highest whereas *Phoma* sp. produced lowest amount of cellulase. All the pathogens showed highest production during 9-12 days of incubation.

**Keywords:** Cellulase; Legume; Seed borne pathogens.

Cellulases are involved in softening and disintegration of host cell walls and they facilitate the penetration and invasion of host by the pathogen, and cause collapse of tissues. Several workers have reported that cellulolytic enzymes are important in disease development<sup>1-3</sup>. Cell wall degrading enzymes have been implicated in pathogenesis<sup>4,5</sup>.

The present studies were undertaken to investigate the cellulolytic enzyme producing capacity of the eight seed borne pathogens, *in vitro*, to know the role of these enzymes in pathogenesis.

Eight seed borne pathogens viz; *Rhizoctonia bataticola*, *Gliocladium virens*, *Aspergillus niger* were isolated from diseased seeds of *Phaseolus vulgaris* (PV). *Phoma* sp. and *Botryodiplodia theobromae* were obtained from diseased seeds of *Trigonella foenumgraecum* (TFG) whereas, *Alternaria alternata*, *Verticillium* sp. and *Macrophomina phaseolina* were isolated from the diseased seeds (D) of *Glycine max* (GM), *Lathyrus odoratus* (LO) and *Phaseolus radiatus* (PR) respectively.

The pathogens were cultured in Richard's broth medium and harvested after 3,5,7,9 and 12 days of incubation for the estimation of cellulolytic enzyme (Cx) The culture filtrates were collected which served as crude enzyme source. Liberation of sugar, as glucose, was measured by DNS method<sup>6</sup>.

From Table 1 and Fig. 1 it is evident that all the eight isolates were able to produce higher amounts of cellulase during their later growth phase (9-11 days).

*Verticillium* sp. produced highest amount (6407) of cellulase followed by *A. niger* (58.25), *A. alternata* (54.45), *R. bataticola* (50.00), *B. theobromae* (38.73), *M. phaseolina* (31.32), *G. virens* (29.60) and *Phoma* sp. (29.17).

All the 8 pathogens showed difference in cellulase production activity. On the basis of cellulase production all the isolates differed in their generic level. Cellulolytic enzymes secreted by the pathogen play an important role by softening of the cell wall material thus facilitating the penetration by the pathogen. In all the pathogens the production of cellulolytic

**Table 1. *In vitro* production of cellulase (Cx) by the isolates of different seed borne pathogens at different incubation periods.**

Isolate	Glucose liberated ( $\mu\text{g/ml}$ )							Mean	Units of Cx produced Days of incubation				Mean	
	3	5	7	9	11	3	5		7	9	11			
<i>A. alternata</i>	98.0	187.3	626.3	840.2	971.0	987.3	987.3	987.3	9.80	18.73	62.63	84.02	97.10	54.45
<i>Verticillium</i> sp.	110.0	360.0	748.2	998.1	987.3	987.3	987.3	987.3	11.00	36.00	74.82	99.81	98.73	64.07
<i>M. phaseolina</i>	70.0	176.0	423.0	615.0	282.2	282.2	282.2	282.2	7.00	17.60	42.30	61.50	28.22	31.32
<i>R bataticola</i>	123.3	348.2	528.7	780.0	720.0	720.0	720.0	720.0	12.33	34.82	52.87	78.00	72.00	50.00
<i>G virens</i>	40.0	180.0	482.2	640.0	138.2	138.2	138.2	138.2	4.00	18.00	48.22	64.00	13.82	29.62
<i>A. niger</i>	130.3	480.0	592.3	820.3	890.3	890.3	890.3	890.3	13.03	48.00	59.23	82.03	89.00	58.25
<i>Phoma</i> sp.	23.3	76.6	287.3	589.0	482.3	482.3	482.3	482.3	2.33	7.66	28.73	58.90	48.23	29.17
<i>B. theobromae</i>	82.3	256.4	378.4	599.3	620.4	620.4	620.4	620.4	8.23	25.64	37.84	59.93	62.04	38.73

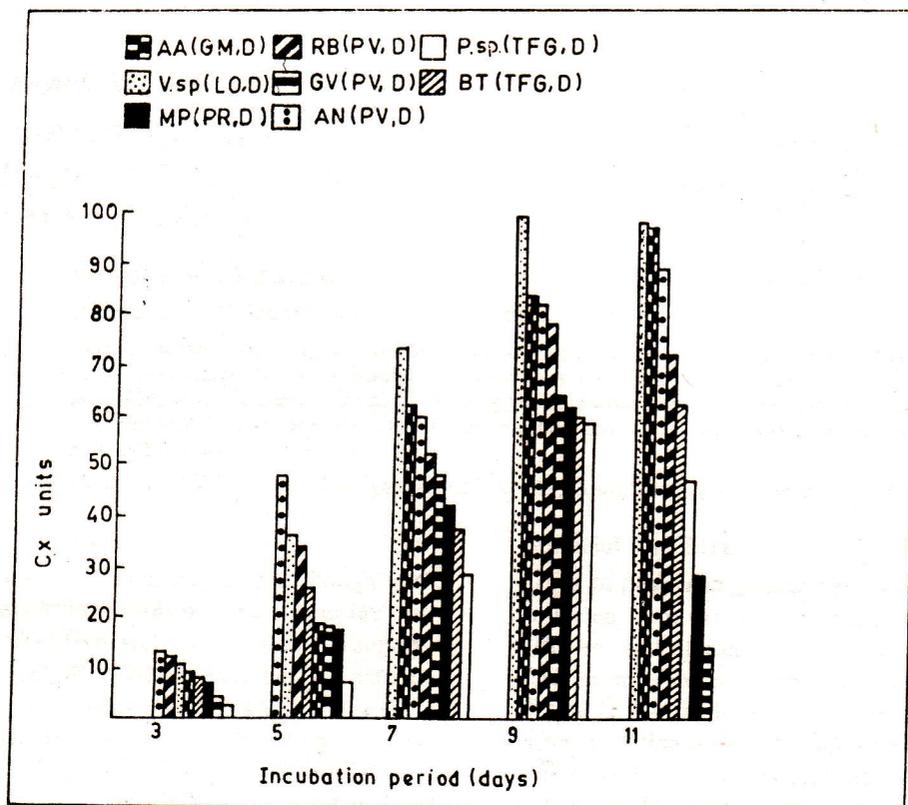


Fig. 1. Production of cellulase (Cx) by the isolates of different seed borne Pathogens.

enzyme indicated their possible role in pathogenesis, by causing degradation of cellulolytic substances present in the cell walls of the host. The result obtained during this investigation indicated the ability of different seed borne pathogens to produce cellulase and hence can be considered to play an important role in pathogenesis.

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