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Studies on Effect of Physical Factors on Protease Production in Seed-Borne Fungi of Green Gram (*Phaseolus Aureus* Roxb)

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ABSTRACT

Green gram (Phaseolus aureus Roxb.) is one of the most important legume crops grown in Marathwada region. The seeds of green gram are found to be heavily infested with variety of fungi. The fungi associated with the seeds bring about several undesirable changes making them unfit for consumption. These associated fungi are known to deteriorate the seeds and seed contents and also produce different hydrolytic enzymes. Among them, proteases have been found to degrade protein content of the seeds. In the present study, fungi were isolated from the seeds of green gram and effect of some physical factors like incubation period, temperature and pH was studied on protease production in these fungi.

KEYWORDS Physical factors, protease production, seed - borne fungi, green gram.

INTRODUCTION:

Legumes constitute a very important daily diet. The seeds of legumes are found to harbour a variety of fungi. These associated fungi are known to deteriorate the seeds and seed contents (Sinha and Prasad, 1977; Charya and Reddy, 1981). Neergaard (1977) stated that, the leguminous crops carry seed–borne diseases so commonly. The pulse seeds are reported to carry many moulds in field and during storage (Rangaswami, 1966). The fungi associated with the seeds bring about several undesirable changes making them unfit for consumption (Bhikane, 1988). Green gram (***Phaseolus aureus*** Roxb.) is one of the most important legume crops grown in Marathwada region. Many scientists reported seed mycoflora of green gram and noted that the seeds of green gram are heavily infested with various fungi (Ramnath ***et al***.,1970 ; Agarwal ***et al***., 1973; Mali ***et al***., 2008). The hydrolytic enzymes produced by the fungi like cellulases, pectinases, amylases, lipases and proteases are known to degrade food contents of seeds. This has been reported in paddy seed spoilage due to fungal enzymes (Vidhyasekaran ***et al***.,1970). Sreekantiah ***et al***. (1971) found that, ***Alternaria alternata, Fusarium solani*** f.sp. ***minus, Pleospora infectoria*** and ***Alternaria solani*** were capable of producing all the four kinds of hydrolytic enzymes, viz., pectinase, cellulase, amylase and proteinase. Balsubramanian (1972) reported that, protease along with cellulose and pectinase was found to be effective in infection by ***Rhizopus stolonifer*** within the tissue.

Keeping this in view, effect of some physical factors like incubation period, temperature and pH was studied on the protease production in the fungi isolated from the seeds of green gram.

MATERIAL AND METHODS:

Production of protease:

Production of protease was made by growing the fungi on liquid medium containing Glucose – 10 gm, Gelatin – 10 gm, K_2HPO_4 – 1.0 gm, $MgSO_4.7H_2O$-0.5 gm and Distilled Water – 1000 ml, pH-5.5. Twenty five ml of the medium was taken in 100 ml conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spore suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 °C. On 7th day, the flasks were harvested by filtering the contents through Whatmann No. 1 filter paper. The filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparations.

Enzyme assay (Cup Plate Method):

The protease activity was studied by Cup Plate Method (Hislop ***et al***., 1982). A basal medium was prepared by adding 2% agar and 1% gelatin and pH of was adjusted at 5.0. Then, it was sterilized at 15 lbs pressure for 20 minutes. About 15 ml of the medium was poured in pre-sterilized Petri plates under aseptic conditions. On solidification, 6 mm diameter cups/cavities were made in the centre of each of the agar plate with a sterilized cork borer. The cups/cavities were filled carefully with about 0.5 ml of culture filtrate (crude enzyme preparation). The plates were incubated at 25 °C for 24 hours. Then the plates were flooded with 15% mercuric chloride in 1N HCl. After 10 minutes of standing, a clear transparent zone indicated the hydrolysis of gelatin by the extracellular proteolytic enzymes, whereas the rest of the regions of the Petri plates became opaque due to coagulation of gelatin (protein) by mercuric chloride. Diameter of clear zone was used as a measure of protease activity, while non-appearance of clear zone was considered to be due to absence of protease in the culture filtrates.

RESULTS AND DISCUSSION:

Table 1: Effect of incubation period on protease production in seed-borne fungi.

Incubation Period (days)	Fungi						
	Aal	Fox	Rso	Cla	Phy	Clu	Ani
	Activity Zone (mm)						
4	17	15	12	10	17	14	16
8	20	15	16	17	19	16	18
12	18	13	14	18	20	18	21
16	13	11	15	16	13	14	16
20	13	11	14	14	12	13	13

Aal – ***Alternaria alternata***
Phy – ***Phytophthora sp.***

Fox – ***Fusarium oxysporum***
Clu – ***Curvularia lunata***

Rso – ***Rhizoctonia solani***
Ani – ***Asperillus niger***

Cla – ***Cladosporium sp.***

Table 2: Effect of temperature on protease production in seed-borne fungi.

Temperature (^{0}C)	Fungi						
	Aal	Fox	Rso	Cla	Phy	Clu	Ani
Activity Zone (mm)							
5	-	-	-	-	-	-	-
10	11	-	-	-	-	-	12
15	11	12	13	-	-	14	15
20	14	14	16	15	18	15	18
25	16	15	17	18	21	17	22
30	17	18	17	17	20	16	20
35	14	14	16	13	16	14	15

Table 3: Effect of pH on protease production in seed-borne fungi.

pH	Fungi						
	Aal	Fox	Rso	Cla	Phy	Clu	Ani
Activity Zone (mm)							
3.0	-	-	-	08	10	-	-
3.5	14	-	18	11	14	-	13
4.0	20	16	20	14	18	14	18
4.5	20	19	20	16	18	14	23
5.0	18	17	19	18	20	16	23
5.5	16	17	18	19	20	17	22
6.0	16	16	18	17	18	16	21
6.5	14	15	16	15	16	14	21
7.0	12	13	16	12	14	13	16
7.5	12	12	14	10	12	12	15
8.0	12	12	14	10	12	10	14

It is clear from table-1 that, all the seed-borne fungi showed maximum protease production on incubation of 8-12 days. From the literature, it becomes clear that, micro-organisms show variations in their optimum period of incubation for protease production. Incubation period is recorded two days in case of ***Aspergillus flavus*** (Malathi and Chakraborty, 1991). ***Alternaria alternata*** required incubation period of 4-5 days (Patil and Shastri, 1982). Micro-organisms which required optimum period more than a week for production are ***Fusarium oxysporum*** and ***Rhizoctonia solani*** (Charya and Reddy, 1982), ***Aspergillus niger*** (Ashour ***et al***., 1996) and ***Penicillium*** sp. (Singh and Saxena, 1988). Sharma and Saxena (1981) recorded 4-5 days of incubation period for protease production in ***Chaetomium globosum*** and ***Fusarium moniliforme***.

It is clear from table-2 that, the temperature range 20-30^{0}C was optimum for protease production in all the fungi studied. Fungi having optimum temperature range between 20-30^{0}C are ***Alternaria alternata*** (Patil and Shastri, 1982) and ***Alternaria tenuissima*** (Jonsson, 1968) and ***Rhizopus oryzae*** (Banerjee and Bhattacharya, 1992). The fungus having optimum temperature range between 35^{0}C -40^{0}C is ***Aspergillus*** sp. (Nehra ***et al***., 1998).

It is clear from table-3 that, the pH range 4-6 was found to be stimulatory for protease production in all fungi. Acidic pH is required for protease production in ***Penicillum*** sp. (Singh and Saxena, 1988), ***Fusarium oxysporum*** (Rajamani, 1990), while alkaline pH is required in ***Alternaria tenuissima*** (Jonsson, 1968) and ***Aspergillus*** sp. (Nehra ***et al***., 1998).

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