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RESEARCH ARTICLE

Studies on Effect of pH, Temperature & Light on Protease Production in Leguminous seeds.

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ABSTRACT

Leguminous seeds are one of the important dilatory food of human being. It is the major cultivated food crop in Maharashtra and India. Ten species were isolated from ten different seeds of Legume, out of these *Fusarium* species only three *Fusarium* species were tested for the study of the effect of PH, Temperature and Light on Protease Production. The results show that the pH 5.5 and 6.5 is more favourable for Protease activity in *F. moniliforme* and *F. oxysporium*. Whereas, *F. roseum* shows poor activity for Protease Production. The Performance of Temperature on Protease Production was found to be very good at 20c in tested Fusarium species and for Light, the Continuous light showed strong support in *F. moniliforme*, *F. oxysporum* and *F. roseum.* The detail results are shown in the paper.

Keywords: Leguminous seeds, Fusarium, Protease production

INTRODUCTION

Leguminous seeds are one of the important dietary food of human being. It is the major cultivated food crop in Maharashtra. Ten different Legume Seeds were collected from the Murum and Nilanga area in Marathawada. From these Legume seeds, ten Fusarium species were isolated. Out of these species, only three Fusarium species were tested for the study of the effect of PH, Temperature and Light on Protease Production.

MATERIALS AND METHODS

Collection of Seed Samples

The seed samples were collected by the method described by Neergaard (1977). According to, a random sample of different varieties of seeds was collected from Field, Store houses, Market places and Seed Companies. A Composite sample of each variety was prepared by mixing the individual samples together preserved in cloth bags at Laboratory during the studies.

Detection of Seed Mycoflora

The Seed mycoflora was isolated by using Standard moist blotter test method (BTM) and Agar Late method (APM) as recommended by Seed Testing Association, INSTA (1966), Neergaard (1977) and Agarwal (1981).

Standard Blotter Method (SBM)

A pair of white blotter paper of 8.5 cm diameter was jointly soaked in sterile distilled water and was placed in pre-sterilised Corning Petri plate of 10 cm diameter. Ten Seeds of test samples per Petri plates were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment.

The plates were incubated at 25+-2 c under the diurnal condition for 7 days.

Agar Plate Method (APM)

In this method, pre-sterilized Corning Glass Petri plates of 10cm diameter were poured with 15ml of autoclaved potato dextrose agar (PDA) medium. On cooling the medium 10 seeds per Petri plates of the test Sample placed at equal distance aseptically, incubation condition and other detailed were same as described for the blotter test method.

Production of Hydrolytic Enzymes
Protease.
Production
Production of Protease was made by growing the Fusarium on liquid medium containing glucose 10 gm, gelatin 10 gm, dipotassium hydrogen sulphate 1.0 g, mgso4.7H2o,0.500mg, and distilled water 1000 ml. The PH of the medium was adjusted at 5.5.25ml of the medium was poured in 100ml Erlenmeyer conical Flasks and autoclaved at 15 lbs pressure for 20 minutes the Flasks on cooling were inoculated separately with 01ml Standard spore/ mycelial suspension of test Fusarium species prepared from seven days old cultures grown on PDA. The Flasks were incubated for six days at 25 +1c with the diurnal periodicity of light on the seventh day the flask were harvested by filtering the contents through what man's filter no 1. The filtrates were collected in the pre-sterilised bottles and termed as crude enzyme preparation.

Assay (Cup-Plate Method)

Determination of Protease as activity was done with the help of a cup plate method adopted by Hislop et.al (1982) and Rajamani (1990). A nasal medium was prepared by adding D/W=1000,=20gm (W/V) agar and 10gm(W/V) gelatine. The pH of the medium was adjusted at 5.6 with McIlvaine's buffer. Then it was sterilized at 15lbs pressure for 20min. 15ml of the medium was poured in pre-sterilised Petri plates aseptic conditions. On solidification, 6mm diameter cups/cavities were made in the centre of each agar plate with a sterilized cork better (No.4). The cups of cavities were filled carefully with about 0.5 ml culture filtrate (crude enzyme preparations). The plates were incubated at 25c for 24 hours. Then the latest was flooded with 15% mercuric chloride in 7 M (Molar) HCL. After 10 minutes of standing, a clear transparent zone indicated the hydrolysis of gelatin by extracellular proteolytic enzymes whereas the rest of the region of the Petri plates become opaque due to the Coagulation of gelatin(Protein)by mercuric chloride. Diameter of the clear of the zone was used as a measure (mm) of Protease activity, while the non-profit appearance of clear zone considered the absence of Protease in the culture filtrates.

RESULTS AND DISCUSSION

In order to understand the study of Protease in particular by using different parameters like PH, temperature and Light, the results were very interesting. It was clear that the PH 5.5 and 6.5 is more favourable for Protease activity in *F. moniliformae* and *F. oxysporium.*whereas *F. roseum* have observed as a comparatively poor activity for Protease Production. The PH 7.5 and 8.5 was found to be more favourable for Protease Production. In the case of *F. oxysporium.*While the pH 4.5 and 8.5 responsible to show very less activity in tested *Fusarium* species.

The performance of Temperature on Protease Production was found to be very good at 20°C in tested species of Fusarium. Whereas, the Protease Production was observed very poor in *F. oxysporium* under at 40oc temperature and it was satisfactory in *F. moniliformae* and *F. roseum.*

It was clear from the result, *F. moniliformae*, *F.oxysorum* and *F. roseum* showed strong support for Protease Production in presence of Continuous Light whereas, the alternative Light showed a low response in the three species of *Fusarium.* Whereas, the response was observed in dark light for Protease Production.

CONCLUSION

It was interesting to note that all the species of Fusarium were found to be capable of Protease enzyme.

The pH 5.5 and 6.5 is more favourable for Protease activity in *F. moniliforme* and *F. oxysporum.*The PH 7.5 and 8.5 is found to be more favourable for Protease Production in *F. oxisporium* while the PH 4.5 and 8.5 responsible to show very less activity in tested Fusarium species.The performance of Temperature on Protease Production was

Table 1: Effect of pH, Temperature and Light on Protease production

Sr. No.	Species	pH					Temperature				Light		
		4.5	**5.5**	**7.5**	**8.5**	**8.5**	**20^0**	**30^0**	**40^0**	**50^0**	**Alternative**	**Continuous**	**Dark**
01	*F. moniliforme*	1.0	4.7	0.5	0.4	0.4	0.5	3.1	1.6	-	2.1	3.0	0.1
02	*F. oxysporium*	1.5	4.5	3.6	2.1	2.1	0.2	2.6	1.0	-	1.6	2.4	0.1
03	*F. roseum*	1.0	2.3	0.9	1.0	1.0	0.7	2.3	1.5	-	1.8	2.5	0.1

found to be very good at 20°C in all tested Fusarium species.The Protease Production was very poor in *F. oxysporium* at 40°C temperature. The Continuous light showed strong support for Protease Production in all tested Fusarium species.The alternative Light showed a low response in all the three Fusarium species.

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