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Production and Characterization of Lipolytic Enzymes by Seed Borne Fungi in Soybean

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Seedborne fungi in soybean causes seed deterioration by altering the quality of the seed content especially the protein and oil content. The oil content and dry weight of the seeds of soybean were found to be greatly affected. The deterioration of soybean seeds rich in oil was correlated with extracellular production of lipolytic enzymes by seed borne fungi.Three dominant fungi viz. *Aspergillus niger, Rhizopus stolonifer* and *Fusarium moniliforme* were studied for lipolytic enzyme synthesis. These fungi synthesized lipolytic enzymes in Czapek medium supplemented with soybean seed meal medium and Czapek medium supplemented with soybean oil separately. The fungi synthesized more enzymes in Czapek medium supplemented with soybean oil than in soybean seed meal medium. The enzyme production was affected by the pH and temperature. The optimum pH was found to be in the range of 5.5 and 40°C was optimum temperature.

Key words: Soybean, Seed borne fungi, Lipolytic enzymes.

Soybean (*Glycine max* Linn.) is an important oil seed crop cultivated over several parts of the world, both in the tropics and subtropics. Marathwada region of Maharashtra is a leading region in the hectarage and production. Soybean is affected by number of diseases caused by fungi, bacteria etc.

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Fungal diseases are more prevalent and most of the diseases are seed borne in nature. The presence of seed borne fungi imparts unpleasant flavour and rancidity to the oil. This can be attributed to the ability of seed borne fungi to synthesize lipolytic enzymes.

Lipolytic activity of seed-borne fungi was reported by Nagel and Semonik[1]. The oil content of the seed may or may not be affected, depending upon the lipolytic activity of the fungus[2]. Clark and Snyder[3] studied the influence of fungi on seed and oil quality of stored groundnut. *A. flavus*, *A. niger*, *A. tamarii*, and more destructive. *A. tamarii* caused the maximum loss of oil within 60 days and was attributed to synthesis of lipolytic nature of fungi. Saharan & Gupta[4] studied the biosynthesis of lipolytic enzymes by a culture of *Aspergillus sps.* on media providing different sources of nitrogen. The seed borne fungi in groundnut reduces the oil content and cause a

change in its colour, induce an unpleasant odour and lead to hydrolytic rancidity [5].

Changes in lipids during seed storage of groundnuts, rape, soybeans, sunflowers, under cool, dry conditions were examined using differential scanning calorimetry. It has been shown that there are changes in the lipid components of seeds that are associated with seed deterioration [6].

Storage of soybean (*Glycine max*) seed under tropical conditions can lead to deterioration that affects products taste and colour. Implications of these findings were discussed in view of the role of lipid peroxidation and the effect of its by products [7].

MATERIAL AND METHODS

Isolation of fungi

Untreated seeds were obtained from various sources – breeders, retailer, farmers etc. these seeds were assessed for presence of fungi using standard blotter method, agar plate method etc. as recommended by International Seed Testing Association[8-10].

Culture and Lipolytic enzymes production medium

The associated fungi were maintained on Czapek medium-broth supplemented with soybean seed meal instead of sucrose as carbon sources. Czapek medium-broth supplemented with 1% soybean powder or soybean oil was used as enzyme production medium. The pH was adjusted to 6.0 by adding dilute 0.1N NaOH /HCl [11].

Preparation of enzymes

Enzymes preparation medium was used for assessing synthesis of lipolytic enzymes by seed fungi. Czapek medium supplemented with soybean oil or soybean seed meal instead of sucrose as carbon source was used for protease production. The preparation of enzymes was done as described earlier[12].

Measurement of Lipolytic enzymes activity synthesized by seed fungi

Triacylglycerol acyihydrolase (EC 3.1.1.3) was determined by method given by Jayaraman [13]. One unit of enzyme is defined as the quantity of fatty acid released in unit time, measured by the quantity NaOH required to maintain pH constant. The milliequivalent of alkali consumed was taken as a measure of the activity of the enzyme.

RESULTS AND DISCUSSION

Fungi are known for their capacities to synthesize a variety of enzyme depending upon availability of substrate. A series of experiments were undertaken to assess the ability of fungi to degrade lipids present in the soybean seed by secretion of lipolytic enzymes.

Aspergillus niger, *Rhizopus stolonifer* and *Fusarium moniliforme* were isolated, selected

Table 1. Production of lipolytic enzymes by seedborne fungi on soybean oil containing medium

Age of culture filtrate (Days)	Lipolytic Enzymes activity (U/ml)		
	Aspergillus niger	*Fusarium moniliforme*	*Rhizopus stolonifer*
1	0.00	0.00	0.00
2	0.00	0.02	0.00
3	0.00	0.06	0.00
4	0.02	0.06	0.05
5	0.04	0.08	0.06
6	0.07	0.12	0.10
7	0.10	0.18	0.12
8	0.13	0.24	0.13
9	0.15	0.23	0.12
10	0.10	0.23	0.12

Table 2. Production of lipolytic enzymes by seedborne fungi on lipolytic enzymes production medium

Age of culture filtrate (Days)	Lipolytic Enzymes activity (U/ml)		
	Aspergillus niger	*Fusarium moniliforme*	*Rhizopus stolonifer*
1	0.00	0.00	0.00
2	0.01	0.00	0.00
3	0.02	0.02	0.01
4	0.03	0.08	0.02
5	0.06	0.12	0.04
6	0.08	0.15	0.08
7	0.10	0.20	0.11
8	0.12	0.24	0.16
9	0.18	0.25	0.12
10	0.18	0.22	0.10

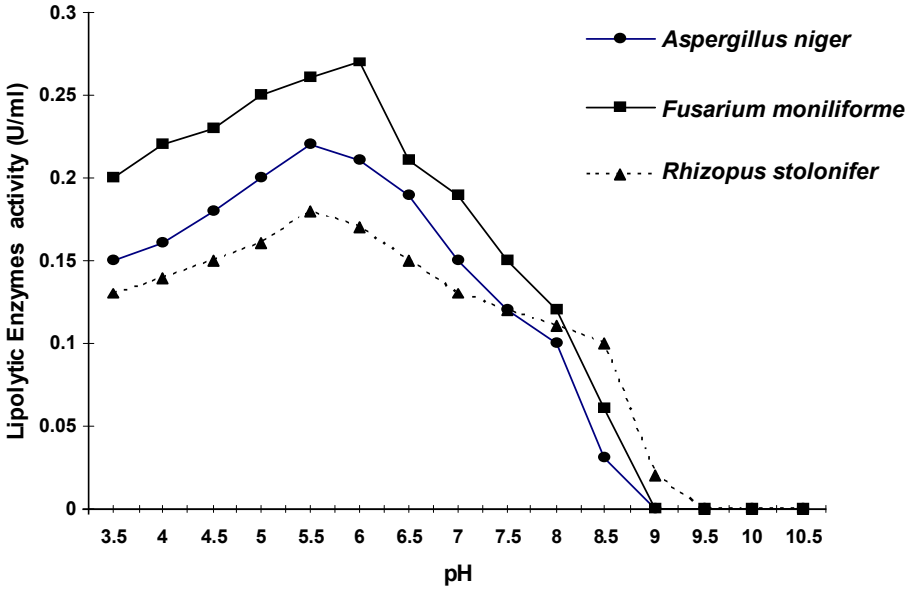

Fig. 1. Effect of pH on produciton of Lipolytic enzymes by seedbome fungi on soybean oil containing medium

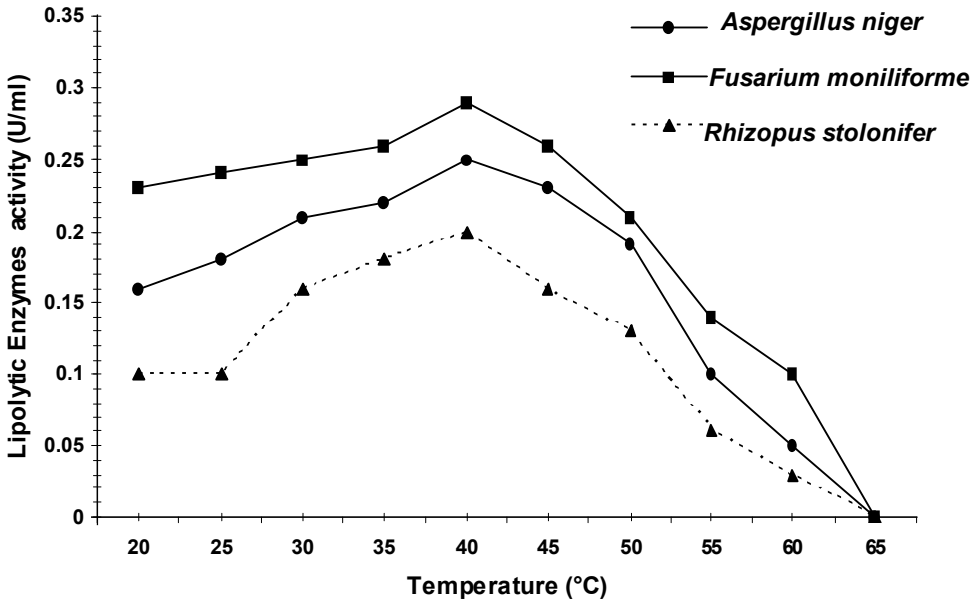

Fig. 2. Effect of Temperature on produciton of Lipolytic enzymes by seedbome fungi on soybean oil containing medium

and grown on soybean seed meal medium as well as lipolytic enzymes production medium. The Eight days old culture filtrate was used as crude enzyme source.

All the three fungi synthesize lipolytic enzymes in both the media. The synthesis increased with increase in time of incubation in both the media, however the amount of enzymes varied. Maximum enzymes were secreted in lipolytic enzymes production medium (Table 1) followed by soybean seed meal medium (Table 2). The quantity of enzyme secreted in lipolytic enzymes production medium increased during the study period, the maximum amount (0.18 U/ml, 0.25 U/ml and 0.12U/ml) was detected in 9 days. In the study of effect of temperature and pH on the synthesis of lipolytic enzymes, it was found that 5.5 (Fig. 1) and 40°C (Fig. 2) was optimum pH and temperature respectively.

Aspergillus flavus removes oil from the seeds and the cotyledons turn brittle and yellow [5]. The fungus synthesises enzymes and uses hydrolysis products of lipid proteins & carbohydrates. The study of the dominant fungi for synthesis of lipolytic enzymes revealed that they were ardent producers of lipolytic enzymes. So the deterioration of oil can be attributed to the lipolytic ability of fungi. The main chemical changes that are brought about in the groundnut seed are loss of organic matter, degradation of sucrose, decrease in total oil and increase in fatty acids. Lipolytic activity differed in different seed borne fungi [14]. Seed lipolytic enzymes and lipolytic enzymes produced by these fungi appear to have synergestic action which accounts for rapid decrease in oil content.

Rhizopus stolonifer is a secondary invader and probably uses free fatty acids released by the action of pioneers or by seed. It is quite likely that seed secretes substances inhibitory for *R. stolonifer* lipolytic enzymes. Phycomycetes usually lack enzymes for fat degradation being mainly saccharolytic. It appears thus the fungi exhibit different trends of production of lipolytic enzymes in relation to growth on different seeds. Maximum lipolytic enzymes production by *A. niger* and *A. flavus* after sporulation have been reported earlier also[15].

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