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## RESEARCH ARTICLE

# INFLUENCE OF PHYSICAL FACTORS ON EXTRACELLULAR LIPASE PRODUCTION IN STORAGE SEED- BORNE FUNGI OF SOME OIL SEEDS

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### ABSTRACT

Fungal association in deterioration of seeds can better be correlated with the production of extra cellular hydrolytic enzymes. Therefore, dominant seed borne fungi of groundnut, sunflower and soybean were screened for their ability to produce extracellular lipase enzyme. Present study deals with the impact of physical factors such as temperature, pH and incubation period on extracellular lipase production in *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A. parasiticus*, *Fusarium moniliformae*, *F.oxysporum*, *Alternaria alternata*, *Curvularia lunata* and *Penecillium chrysogenum*. The lipase enzyme assay was carried out by cup plate method. The degree of enzyme production was found to be variable among the mycoflora.

#### Key words:

Extracellular lipase,  
physical factors, oilseeds,  
seed-borne fungi.

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## INTRODUCTION

In agriculture, seeds play very important role for the production of healthy crop. Oilseeds are the main source of raw material for vegetable oils. They are essential component of human diet and are rich source of energy and fat soluble vitamins. The fat and oils are also the major source of raw material of industrial products such as cosmetic, soaps, surface coatings, lubricants, pharmaceuticals etc. and also used for animal feed. The damage to agricultural produce that occurs during storage is mainly due to rodents, insects and microorganisms, which play an important role in affecting the quality of seed. Environmental factors influencing the life span of seeds such as relative humidity, temperature, and initial moisture content of seed. (Hartmann & Kesler, 1993).

Fungi are the major cause of deterioration during storage. Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively, which is due to chemical breakdown of proteins, oil, and fatty acids by the seed -borne microbes (Welbaum, 2006). Fungi associated with seed surface as well as internal mycoflora (Dutta & Roy, 1987). Fungal association in deterioration of seeds can better be correlated with the production of extracellular hydrolytic enzymes (Agrawal & Kharlukhi, 1987). Many seed-borne fungi hydrolyze lipids by their lipase activity, which results in an increase of free fatty acids (Christensen & Kaufmann 1974). Studies on the lipase activity of a few fungi have been studied (Somkuti & Babel 1968). Major commercially

important lipase producing fungi are: *Rhizopus arrhizus*, *Rhizopus japonicus*, *Rhizopus niveus*, *Mucor miehei*, *Candida arugosa*, *Aspergillus niger* and *Aspergillus terreus* (Elibol & Ozer 2000; Tweddell *et.al.*, 1998; Patti *et al.*, 2000; Benjamin & Pandey, 1998; Jamil & Omar 1992; Gulati *et al.*, 1999). Filamentous fungi are sources for lipase production; they produce extracellular lipases (Carvalho *et al.*, 2005). Most extracellular fungal lipases are influenced by nutritional and physical factors such as carbon sources, pH and temperature (Gunstone, 1999; Immanuel *et al.*, 2008; Kiran *et al.*, 2008). Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition besides physical factors such as temperature, pH, and incubation period.

## MATERIALS AND METHODS

### Collection of seed samples

Seeds of Groundnut (*Arachis hypogea* L.), Sunflower (*Helianthus annuus* L.) and Soybean (*Glycin max* L.) were collected from local market of Nanded in pre-sterilized polythene cotton bags, brought to the laboratory and a composite sample was prepared.

### Isolation of Lipase Producing Fungi

The isolation of seed-borne fungi was carried out by standard blotter paper method and agar plate method described by ISTA (1966). Their identification was made on the basis of their morphological and reproductive characters by using Labo made

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binocular research microscope and confirmed with standard literature (Barnett 1960; Ellies, 1971; Mukadam et al., 2006).

### Lipase Production

Lipase activity was studied by growing the fungi on liquid medium at pH 5.6 containing Oil- 10.0 ml, KNO<sub>3</sub> -2.5g, KH<sub>2</sub>PO<sub>4</sub>- 1.0g, MgSO<sub>4</sub> – 0.5g and distilled water 1000 ml. Treatments of different physical factors such as temperature, pH, and incubation period was given on inoculation. 25ml of the medium was poured in 100 ml conical flasks and autoclaved at 15 lbs pressure for 30 minutes and then on cooling, the flasks were inoculated separately with 1.0 ml spore suspension of the test fungi. In case of temperature and pH on 7<sup>th</sup> day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in pre-sterilized culture filtrate bottles and used as crude lipase.

### Lipase Activity Assay

Lipase activity was determined by cup-plate method (Sierra, 1957). The medium contains Difco peptone-10.0 g, NaCl-5.0 g, CaCl<sub>2</sub>.2H<sub>2</sub>O-1.0g, Agar 20.0 g and 10 ml lipid substrate Serbitan mono laurate (Tween-20) (Pre-sterilized), distilled water- 1000 ml and pH of the medium was adjusted to 6.0. Medium was poured in presterilized petridishes and allowed to solidify. In the centre of the medium, a cup cavity of 8 mm diameter was made with sterilized cork borer and was filled with 0.1ml culture filtrate (crude enzyme). The plates were incubated at 28°C for 24 hours; a clear circular zone was measured (mm) as lipase activity. Clear zones which formed around the colonies indicated degradation of substrate due to lipase activity.

## RESULT AND DISCUSSION

Seed mycoflora of groundnut, sunflower and soybean was isolated by standard blotter paper method and agar plate method. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A.parasiticus*, *F.moniliformae*, *F.oxysporum*, *A.alternata*, *C.lunata*, *P.chrysogenum* were selected to study their lipase activity. All these dominant fungi were able to metabolize lipid substrate to varying physical factors for lipase production. Such work earlier is supported by Neergaard, 1973; Agrawal, 1976; Chavan and Kakde, 2009.

**Table1** Effect of temperature on extracellular lipase production in some dominant seed- borne fungi of some oil seeds.

Temp.(°C)	Test Fungi									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
10	10	10	-	-	09	11	10	09	-	10
20	16	14	16	14	12	16	15	15	12	16
30	20	19	21	17	16	19	20	18	15	21
40	22	20	22	19	18	15	16	15	13	17
50	12	13	16	12	11	11	10	11	12	09
60	10	10	12	09	09	-	-	-	10	-

### Effect of temperature

To determine the effect of temperature on lipase activity, the experiment was carried out at different temperatures ranging from 10 to 60 °C. At 10°C *A. fumigatus*, *A. terreus*, *C. lunata* did not produce lipase activity, while at 20°C all test fungi showed the lipase activity. *F. moniliformae*, *F. oxysporum*, *A. alternata*, *C. lunata*, *P. chrysogenum* showed maximum lipase

production at 30°C. Moataza et al., (2004) reported that *F.oxysporum* produce maximum lipase at 30°C. Similar results were reported by Kakde and Chavan (2011). They found that *F. oxysporum*, *C. lunata*, *P. chrysogenum* produce maximum lipase at 30°C. *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A.parasiticus* showed maximum lipase activity at 40°C. Falony et al., (2006) recorded maximum lipase activity of *A. niger* at 40°C. Pera et al., (2006) reported that an optimum temperature for the lipase activity obtained from *A. niger* at 37°C.

**Table 2** Effect of pH on extracellular lipase production in some dominant seed- borne fungi of some oil seeds.

pH	Test fungi									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
3.5	10	10	12	09	09	10	11	09	10	12
4.5	15	13	14	14	11	12	13	10	12	18
5.5	18	16	17	15	16	14	15	12	13	19
6.5	21	20	22	19	20	18	18	15	16	18
7.5	20	18	19	17	18	22	23	18	18	22
8.5	10	09	12	10	11	-	-	-	-	15

### Effect of pH

To study the effect of pH, the lipase activity was measured at various pH ranging from 3.5- 8.5. All test fungi showed maximum lipase production in the pH between 6.5-7.5. *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A.parasiticus* showed maximum lipase production at pH 6.5. Kamini et al. (1998) have reported that, optimum pH for the lipase activity in *A. niger* was pH 7.0. In addition; Adham and Ahmed (2009) obtained optimum pH for lipase from *A.niger* at pH near 7.2. *F. moniliformae*, *F. oxysporum*, *A. alternata*, *C. lunata*, *P. chrysogenum* showed maximum lipase production at pH 7.5. At pH 8.5 *F. moniliformae*, *F. oxysporum*, *A. alternata*, *C. lunata* did not produce lipase. Similar results were obtained by Moataza et al., (2004) they found that, *F. oxysporum* did not produce lipase at pH 8.5. Kakde and Chavan (2011) found that *C. lunata* did not produce lipase at pH 8.5.

**Table3** Effect of incubation period on lipase production in some dominant seed-borne fungi of some oil seeds.

Incubation period	Test fungi									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
05	10	10	11	09	09	10	11	-	-	10
10	21	20	19	18	18	19	20	19	15	21
15	22	20	22	19	22	20	21	19	16	21
20	24	21	23	21	22	17	19	16	18	19

F1- *Aspergillus niger* F2- *A. flavus* F3- *A. fumigatus* F4- *A. terreus*  
 F5- *A.parasiticus* F6- *F.moniliformae* F7- *F.oxysporum* F8- *Alternaria alternata*  
 F9- *Curvularia lunata* F10- *P. chrysogenum*

### Incubation Period

It is clear from the result (Table 3) lipase production was increased with incubation period, and after reaching the optimum, there was gradual decrease. At 5<sup>th</sup> day of incubation period *A. alternata*, *C. lunata* did not produce lipase. Similar results were reported by Kakde & Chavan (2011). They found that, *C. lunata* did not produce lipase at 5<sup>th</sup> day. Moderate lipase activity was found at 10<sup>th</sup> day. At 15<sup>th</sup> day *F. moniliformae*, *F. oxysporum*, *A. alternata*, *P. chrysogenum* showed maximum lipase enzyme production while at 20<sup>th</sup> day *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A. parasiticus*, *C. lunata* showed maximum lipase production. Lipase from *A. niger* showed the greatest activity at pH 6-8 and temperature 40-55°C (Shu et al., 2007). Maryam et al., (2011) reported

that *A. niger* showed maximum lipase activity at pH value 7.0 and temperature 30°C. Licia *et al.*, (2006) tested the effect of pH on the lipase enzyme activity of *A. niger* at pH range of 2.0–8.0, temperature range of 4–55°C and incubation period range of 1–6 days and they found that *A. niger* showed maximum lipase activity at pH 6.5, temperature at 37°C and incubation period at 4th day. Vaidehi and Jagdamba (1984) found that maximum lipase activity of *A. niger* and *A.flavus* at 6th day and pH 6 and *F. oxysporum* and *Rhizoctonia solani* showed maximum lipase activity at 8th day and pH 8. Brooks and Asamudo (2011) recorded lipase from *A. niger* and *A. fumigatus* were most active at pH 6.5 and optimum temperature for lipase activity was 40°C for *A. niger* and 35°C for *A. fumigatus*. The lipase produced by *F. oxysporum*, *Fusarium* sp. and *F. oxysporum* f. sp. *vasinfectum*, showed optimum activity at temperature 42 and 45°C, respectively (Hoshino *et al.*, 1992; Pimentel *et al.*, 1994). Lipase from *P. chrysogenum* showed the greatest activity at pH 7.9–8.1 and 45 °C ( Ferrer *et al.*, 2000).

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