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EFFECT OF PHYSICAL FACTORS ON LIPASE ENZYME PRODUCTION BY SEED BORNE FUNGI OF GROUNDNUT

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ABSTRACT

During present investigation total eleven seed borne fungi were isolated from five different varieties of groundnut by moist blotter plate and agar plate method of which six dominant seed borne fungi were screened to study effect of physical factors such as incubation period, pH and temperature on lipase production. From the results it is evident that lipase activity of seed borne fungi was optimum at 9th day of incubation period in most of the test fungi. *Rhizopus nigricans* showed maximum lipase production while *Sclerotium rolfsii* produced least lipase. Maximum lipase production was observed in *Rhizopus nigricans* and *Fusarium oxysporum* in Groundnut variety TAG-24 while in SB-XI maximum lipase production was observed in *Rhizopus nigricans* and *Macrophomina phaseolina* at pH 6.5. It was also observed that as pH increased, the lipase production decreased. Similarly, it was noticed that all fungal isolates produced more lipase at 30°C temperature.

KEYWORDS: Lipase, pH, Temperature, Incubation period, TAG-24, SB-XI.

INTRODUCTION

Seeds of various crops are known to carry variety of fungi mainly the species of *Alternaria, Aspergillus, Curvularia, Chaetomium, Cladosporium, Drechslera, Fusarium, Macrophomina, Mucor, Penicillium, Rhizoctonia, Rhizopus, Syncephalastrum, Torulla,* etc. These during their association with seeds in field as well as during storage cause various types of harmful effects to the seeds. The whole process is termed as seed biodeterioration. It is estimated that about 4% of the world's grains are lost due to biodeteriorations caused by microorganisms (Clerke, 1966). It is clear from the literature that the degree of biodeterioration has been found to be directly related with efficiency of seed moulds to produce hydrolytic enzymes like amylase, protease and lipase.

Many researchers working on biodeterioration of oil seeds stated that loss in seed weight as well as oil contents may have relationship with that of the lipolytic nature of seed mycoflora.Lipase enzyme is abundantly produced in oilseeds during storage which breaks down the lipid into free fatty acid (M. Ameer Junaithal Begum,2014).

Seed biodeterioration of Groundnut has been attributed mainly due to lipase activity in addition to other hydrolytic enzymes of the seed borne fungi associated with the Groundnut seeds. Therefore extensive studies were carried out to find out effect of physical factors such as Incubation period, pH and Temperature on lipase production in seed borne fungi of Groundnut.

MATERIALS AND METHOD

A. Studies on Seed-Borne Fungi of Groundnut

I. Detection of seed mycoflora of different varieties of Groundnut

During the present studies the seed samples of different varieties of Groundnut were collected for the detection of seed mycoflora by the methods described by Paul Neergaard, (1977).

In order to study varietal variations in the seed mycoflora, seeds of five different Groundnut cultivars (varieties) namely LGN-169, LGN-189, TAG-24, SB-XI and VG- 9816 were collected separately and their seed mycoflora was detected by moist blotter plate and agar plate method.

i. Per cent incidence of seed mycoflora of different varieties of Groundnut by moist blotter plate method

A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized glass Petri plates of 10 cm diameter. Ten seeds were placed at equal distance on the moist blotter paper. Four hundred seeds were employed in every experiment. The plates were incubated at room temperature for seven

days. On eighth day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The percent incidence of mycoflora was recorded. The seed-borne fungi found on each and every seed were isolated and identified. The common and dominant seed borne-fungi were brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants for further studies.

ii. Per cent incidence of seed mycoflora of different varieties of Groundnut by agar plate method

In this method, pre-sterilized borosil glass petri-plates of 10 cm diameter were poured with 20 ml of autoclaved PDA (Potato Dextrose Agar) medium of pH 5.6. On cooling the medium, ten seeds per plate were equispaced aseptically. Four hundred seeds were employed in every experiment. The plates were incubated at room temperature for seven days. On eighth day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The percent incidence of mycoflora was recorded. The seed-borne fungi found on each and every seed were isolated and identified. The common and dominant seed borne-fungi were brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants for further studies.

II. Isolation and Identification of seed mycoflora of Groundnut

Infected Groundnut seeds were selected for isolation of seed borne fungi of Groundnutby using the procedure published by ISTA (2003). Infected seeds were first disinfected with 0.1 % $HgCl_2$ for 2 to 3 minutes and then washed with sterile distilled water 2 to 3 times. Disinfected seeds were then aseptically transferred to the moist chamber plate. The plate was then incubated at room temperature for 7 day and also watered regularly with sterile distilled water. After 7 days incubation, the mycelial growth with spores was removed aseptically and transferred to PDA plates and slants.

The fungi were identified by observing colony morphology and microscopic characteristic. Pure cultures of the isolated fungi were transferred to PDA slants and kept in refrigerator at 4°C for further use.

The fungus was identified by observing colony morphology and microscopic characteristic.

Identification was confirmed with the help of latest manuals, Subramanian, (1971), Neergaard and Mathur, (1980), Jha, (1993) and Mukadam, (1997). Pure culture of the identified fungi were prepared and maintained on PDA (Potato Dextrose Agar) slants.

B) Production of Lipase

Lipase production was studied by using liquid medium containing 10 mloil (specific oils used for fungi harvested from respective oilseeds), KNO_3(2.5 g), KH_2PO_4(1.0 g) and $MgSO_4$(0.5 g), distilled water 1000 ml and pH 5.5. Treatments of different physical factors such as temperature, pH and incubation period were given to above basal medium. 25 ml of the medium was poured in 100 ml conical flask, sterilized for 30 min. at 15 lbs pressure and inoculated separately with 1 ml spore/mycelial suspension of test fungus (grown for seven days on PDA). The flasks were incubated for 6 days at 23ºC ±1 ºC with diurnal periodicity of light. Flasks were harvested by filtering through Whatman No.1 filter paper and the filtrates collected in pre sterilized bottles were termed as crude lipase (Umatale, 1995 & Kakade,2011).

Enzyme assay: (Titration)

Enzyme assay was worked out as described by Ure*et al.*, (1962).

The reaction mixture contained 2 ml of Glycerol triacetate (Triacetin), 5 ml of 0.2 M citrate phosphate buffer at pH 8.0 and 2 ml of enzyme source, incubated at 36 ºC ± 1 ºC for three hours. The reaction was terminated by adding 10 ml of absolute alcohol. The amount of acids produced by the activity of enzyme was estimated by titrating against 0.05 N NaOH using 1 % phenolphthalein (1 ml) as an indicator till the development of pink colouration. Reaction mixture soon after the addition of enzyme served the blank. The enzyme activity was expressed in units; one unit is defined as 0.1 ml of 0.05 N NaOH required to neutralize the fatty acids liberated during incubation. Constitutive enzyme production was done by substituting starch with glucose (1%) in the above medium.

C) Biostatic analysis

Data obtained was statistically analyzed for Critical difference (C.D.) by following Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

A. Studies on Seed-Borne Fungi of Groundnut

I. Detection of seed mycoflora of different varieties of Groundnut

In order to study varietal variations in the seed mycoflora, seeds of different varieties of Groundnut i.e. LGN-169, LGN-189, VG-9816, TAG-24 and SB-XI were plated separately on moist blotters and agar plates and incubated for seven days at room temperature. The percent incidence of seed-borne fungi was recorded and presented in table-1 and table-2.

From the results presented in table-1 and table-2, it was observed that among the seeds of five different varieties of Groundnut, there was variation in the degree of incidence of mycoflora in both moist blotter plate method and agar plate method. In all agar plate method yielded more per cent incidence of seed borne fungi as compared to moist blotter plate method.

From the results presented in table-1 it was noticed that total ten different fungi were isolated from the seeds of all the test varieties of Groundnut by moist blotter plate

method. The seeds of variety TAG-24 showed maximum seed mycoflora (ten fungi) while seeds of variety SB-XI showed presence of only one fungus. The seeds of all the test varieties invariably showed presence of *Aspergillus niger*. Similarly, *Aspergillus candidus* appeared only on seeds of LGN-189 and TAG-24. *Alternaria alternata* and *Aspergillus fumigatus* appeared only on LGN-169 and TAG-24. *Aspergillus terreus* appeared only on LGN-189 and TAG-24 while *Sclerotium rolfsii* appeared on LGN-189 and TAG- 24. *Macrophomina phaseolina* appeared on VG-9816 and TAG-24 by moist blotter plate method.

From the results presented in table-2 it was noticed that total eleven different fungi were isolated from the seeds of all the test varieties of Groundnut by agar plate method. The seeds of variety LGN-169 showed maximum seed mycoflora (eleven fungi) while seeds of variety SB-XI showed presence of three seed borne fungi. The seeds of variety TAG-24 showed presence of ten seed borne fungi while the Groundnut variety LGN-189 showed incidence of total seven seed borne fungi and variety VG-9816 showed incidence of total four seed borne fungi. In all seed borne fungi per cent incidence of *Rhizopus nigricans* was found to be highest in all the test varieties while per cent incidence of *Sclerotium rolfsii* was found to be lowest in all test varieties by agar plate method. It was also noticed that agar plate method yielded more seed borne fungi than moist blotter plate method.

Table-1: Per cent incidence of seed mycoflora of different varieties of Groundnut by moist blotter plate method.

Sr. No.	Seed borne Fungi	Per cent incidence of seed-borne fungi on Groundnut varieties				
		LGN-169	LGN-189	VG-9816	TAG-24	SB-XI
1	*Alternaria alternata* (Fr.) Keissler	10	00	00	10	00
2	*Aspergillus candidus* Link	00	10	00	10	00
3	*Aspergillus flavus* Link ex Fr.	05	15	10	20	00
4	*Aspergillus fumigatus* Fresenius	15	00	00	10	00
5	*Aspergillus niger* van Tieghem	25	20	30	40	25
6	*Aspergillus terreus* Thom.	00	05	00	10	00
7	*Fusarium oxysporum* Schlechtend emend Sny. & Hans.	00	05	00	10	00
8	*Macrophomina phaseolina* (Tassi) Goldanich	00	00	10	10	00
9	*Rhizopus nigricans* Ehrenb.	40	00	45	20	00
10	*Sclerotium rolfsii* Sacc.	00	05	00	05	00
	S.E. ±	1.47	0.64	1.69	2.62	0.91
	C.D. at 0.05%	4.36	1.90	5.01	7.77	2.71

Table- 2: Per cent incidence of seed mycoflora of different varieties of Groundnut by agar plate method.

Sr. No.	Seed borne Fungi	Per cent incidence of seed-borne fungi on Groundnut varieties				
		LGN-169	LGN-189	VG-9816	TAG-24	SB-XI
1	*Alternaria alternata* (Fr.) Keissler	15	00	00	20	00
2	*Aspergillus candidus* Link	05	10	00	15	00
3	*Aspergillus flavus* Link ex Fr.	10	15	10	30	20
4	*Aspergillus fumigatus* Fresenius	20	00	00	10	00
5	*Aspergillus niger* van Tieghem	25	20	30	50	25
6	*Aspergillus terreus* Thom.	05	05	00	20	00
7	*Fusarium oxysporum* Schlechtend emend Sny. & Hans.	05	05	00	20	00
8	*Macrophomina phaseolina* (Tassi) Goldanich	05	00	10	10	00
9	*Rhizopus nigricans* Ehrenb.	55	30	50	60	20
10	*Rhizopusstolenifer*	20	00	00	25	00
11	*Sclerotium rolfsii* Sacc.	05	10	00	00	00
	S.E. ±	1.56	2.40	1.80	2.88	1.56
	C.D. at 0.05%	4.59	7.07	5.32	8.50	4.59

II. Isolation and Identification of seed mycoflora of Groundnut

Infected Groundnut seeds were selected for isolation of seed borne fungi of Groundnutby using the procedure published by ISTA (2003).The fungus was identified by observing colony morphology and microscopic characteristic Table 3.

Table 3: Microscopic characteristics used for the identification of Seed borne fungi of Groundnut.

Sr. No.	Name of Fungi	Microscopic characteristics
1	*Alternaria alternata* (Fr.) Keissler	The mycelium is profusely branched, brownish and septate. Conidiophores: Developed singly or in small groups, branched or unbranched. Conidia: In long chains (often branched), oval to ellipsoidal, with 2-7 transverse and 1-4 longitudinal or oblique septae, tapering end to form a short beak at the apex. The number of conidia in a chain varied from 2-8.
2	*Aspergillus candidus* Link.	Colony pale yellow, the fungus produces white as well as globose conidia-producing bodies which produce globose and subglobose conidia. These conidia were smooth, thin-walled and revealed to be about 2.5–3.5 µm in diameter. Vesicles spherical to subspherical which were entirely covered with metulae.
3	*Aspergillus flavus* Link ex Fr.	The mycelium is found to be submerged in the seed coat and forms a white to grey, tough mass. Conidiophores erect, simple, unbranched, hyaline, transparent and smooth. The apex of the conidiophores was inflated into a vesicle upon which radiating phialides are formed. Conidial heads were biseriate, globose to radiate often columnar, very light to deep yellow green, olive brown often brown. Conidia were found to be hyaline, single celled and produced in chains. They were globose to subglobose, often elliptical to pyriform and conspicuously echinulate.
4	*Aspergillus fumigatus* Fresenius	The mycelium produced blue-dull green colony on PDA medium, conidial heads being light green to dull blue green, vesicles uniseriate, pyriform, conidial head columnar, compact, densely crowded, Conidia globose to subglobose, green in mass 2-3 µm in diameter.
5	*Aspergillus niger* van Tieghem	Mycelium was found to be often scanty, hyaline to white or light yellow. Conidiophores were found to be developed directly from the seed coat They were hyaline to light brown, long, thin, unbranched, erect, brittle and terminating in to an inflated apex. Conidial heads appeared globose, but subsequently split into a few to several irregular or well-defined divergent columns of conidial chains. They were black, globose or radiate. Conidia found to be in chains on the sterigmata. They were single celled, pale to dark brown, more or less globose, with low to prominent ridges surfaces.
6	*Aspergillus terreus* Thom.	Mycelium found to be rapidly growing with variable colony appearance ranging from heavily sporulating colonies to fluffy, poorly sporulating colonies. The conidiophores were long, columnar, hyaline and smooth giving rise to sub-spherical biseriate vesicles. Conidia were found to be smooth walled, globose to slightly elliptical and striate.
7	*Fusarium oxysporum* Schlechtend emend Sny. & Hans.	The mycelium found to be white to light pink, aerial, unbranched or branched, very short monophialides bearing microconidia on false heads. The microconidia produced on micrconidiophores were abundant, hyaline, single celled, oval or elliptical. Macroconidia were found to be produced on the pale orange sporodochia. Macroconidia produced on macroconidiophores were hyaline, often 3 to 5 septate, falcate to almost straight, thin walled, with a curved apical cell and slightly foot-shaped basal cell. Chlamydospores were found to be terminal and intercalary, irregular in shape, thick walled with smooth surfaces.
8	*Macrophomina phaseolina* (Tassi) Goldanich	The mycelium found to be with thick hyphae. They were gray to brown or dark brown to black or dull white to light brown. Pycnidia were larger than the sclerotia, dark brown to black and scattered throughout the surface. They were found to be separate or confluent, rough, globose or irregular, beaked and ostiolate. Mature pycnidia were found to be dehisced and ooze conidia in a dull white, gelatinous mass. Conidia were aseptate, hyaline, ellipsoid to obovoid. Sclerotia were black, shiny, irregularly shaped.
9	*Rhizopus nigricans* Ehrenb.	The colonies were found to be whitish, with aerial mycelium and black spots of sporangia and dark sporangiophores. Rhizoids were well developed. Sporangiophores (on stolons) were found to be brown, in groups of 1-3. Sporangia were blackish, powdery in appearance. Columellae were conical and mouse-grey. Sporangiospores angular-globose-ellipsoidal and distinctly striate.
10	*Sclerotium rolfsii* Sacc.	The fungus was found to be produced white, dense, radiating mycelial growth on potato dextrose agar medium. In the early stages, the fungus was found to be produced white mycelium and gradually lost its luster and became some what dull in appearance. Aerial hypae were not uniformly distributed. Initiation of sclerotial bodies were observed from fifth day onwards after inoculation. In the beginning, the sclerotial

		bodies were white which gradually turned to buffbrwoncolour and then to chocolate brown at maturity. The fully matured sclerotia were spherical, ellipsoidal.

III. Effect of incubation period on lipase production in seed borne fungi of Groundnut var. TAG-24 and SB-XI

Lipase production in six seed borne fungi of Groundnut was analyzed at six different incubation periods and the results are presented in table-4 and 5.

From the results presented in table-4 and 5, it was noticed that as incubation period increased, lipase enzyme production also increased. All six seed borne fungi of Groundnut var. TAG-24 and SB-XI produced detectable amount of lipase at fifth day of incubation and it increased gradually up to ninth day. Lipase production remained constant after ninth day of incubation period in most of the test fungi. Among the test seed borne fungi, *Rhizopus nigricans* showed maximum lipase production while *Sclerotium rolfsii* produced least lipase.

IV. Effect of pH on lipase production in seed borne fungi of Groundnut var. TAG-24 and SB-XI

From table 6 and 7, it was observed that there was no enzyme production by test fungi in case of seeds of both the Groundnut var. TAG-24 and SB-XI at pH 3.5 and pH 4.5. Maximum lipase production was observed at pH 6.5 in both the varieties of Groundnut. It was also observed that as pH increased, the lipase production decreased.

Maximum lipase production was observed in *Rhizopus nigricans* and *Fusarium oxysporum* in Groundnut variety TAG-24 while in SB-XI maximum lipase production was observed in *Rhizopus nigricans* and *Macrophomina phaseolina* at pH 6.5.

V. Effect of temperature on lipase production in seed borne fungi of Groundnut var. TAG-24 and SB-XI

The seed borne fungi of Groundnut var. TAG-24 and SB-XI were grown at eight different temperatures and the effect of lipase production was noted. The results are mentioned in table-8 and 9.

It was observed that at 10 °C there was no lipase production by all the test fungi. It was noticed that lipase production was initiated at 15 °C temperature and increased at 20 °C, 25 °C and 30°C in both the varieties of Groundnut. It was also noticed that all fungal isolates produced more lipase at 30 °C. It was observed that lipase production was decreased at 40 °C and 45 °C temperature except in *Rhizopus nigricans*. In *Rhizopus nigricans* maximum lipase production was observed at 45 °C.

Table 4: Effect of incubation period on lipase production in seed borne fungi of Groundnut var. TAG-24. (pH 6.5, at room temperature).

Seed borne fungi	Enzyme Activity (Units)* Incubation Period (Days)					
	1	3	5	7	9	11
Aspergillus flavus Link ex Fr.	00	00	06	08	10	10
Aspergillus niger van Tieghem	00	00	08	08	10	10
Fusarium oxysporum Schl. emend Sny. & Hans.	00	00	06	07	08	08
Macrophomina phaseolina (Tassi) Goldanich	00	00	07	06	10	10
Rhizopus nigricans Ehrenb.	00	00	08	10	12	12
Sclerotium rolfsii Sacc.	00	00	04	05	06	06
S.E. ±	00	00	0.43	0.58	0.75	0.77
C.D. at 0.05%	00	00	1.37	1.82	2.37	2.41

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

Table 5: Effect of incubation period on lipase production in seed borne fungi of Groundnut var. SB-XI. (pH 6.5, at room temperature).

Seed borne fungi	Enzyme Activity (Units)* Incubation Period (Days)					
	1	3	5	7	9	11
Aspergillus flavus Link ex Fr.	00	00	06	08	10	10
Aspergillus niger van Tieghem	00	00	05	06	09	09
Fusarium oxysporum Schl. emend Sny. & Hans.	00	00	04	06	08	08
Macrophomina phaseolina (Tassi) Goldanich	00	00	07	08	09	09
Rhizopus nigricans Ehrenb.	00	00	09	10	13	13
Sclerotium rolfsii Sacc.	00	00	04	06	06	06
S.E. ±	00	00	0.43	0.51	0.67	0.57
C.D. at 0.05%	00	00	1.36	1.62	2.12	1.78

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

Table 6: Effect of pH on Lipase production in seed borne fungi of Groundnut var. TAG-24 on 9th day of incubation and at room temperature.

Seed borne fungi	Enzyme Activity (Units)* pH of Medium					
	3.5	4.5	5.5	6.5	7.5	8.5
Aspergillus flavus Link ex Fr.	00	00	06	12	05	03
Aspergillus niger van Tieghem	00	00	05	11	04	03
Fusarium oxysporum Schl. emend Sny. & Hans.	00	00	08	16	08	01
Macrophomina phaseolina (Tassi) Goldanich	00	00	06	14	04	03
Rhizopus nigricans Ehrenb.	00	00	07	16	08	05
Sclerotium rolfsii Sacc.	00	00	05	10	04	00
S.E. ±	00	00	0.56	0.51	0.43	0.51
C.D. at 0.05%	00	00	1.78	1.62	1.36	1.62

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

Table 7: Effect of pH on Lipase production in seed borne fungi of Groundnut var. SB-XI on 9th day of incubation and at room temperature.

Seed borne fungi	Enzyme Activity (Units)* pH of Medium					
	3.5	4.5	5.5	6.5	7.5	8.5
Aspergillus flavus Link ex Fr.	00	00	04	10	04	03
Aspergillus niger van Tieghem	00	00	05	12	05	04
Fusarium oxysporum Schl. emend Sny. & Hans.	00	00	05	12	06	03
Macrophomina phaseolina (Tassi) Goldanich	00	00	08	15	04	05
Rhizopus nigricans Ehrenb.	00	00	08	15	08	05
Sclerotium rolfsii Sacc.	00	00	04	08	06	00
S.E. ±	00	00	0.45	0.71	0.43	0.35
C.D. at 0.05%	00	00	1.40	2.22	1.37	1.09

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

Table 8: Effect of temperature on lipase production in seed borne fungi of Groundnut var. TAG-24 (pH 6.5, Incubation period 9 days).

Seed borne fungi	Enzyme Activity (Units)* Temperature (°C)							
	10	15	20	25	30	35	40	45
Aspergillus flavus Link ex Fr.	00	05	10	18	30	30	28	21
Aspergillus niger van Tieghem	00	06	11	20	32	32	28	20
Fusarium oxysporum Schl. emend Sny. & Hans.	00	08	16	22	35	33	26	20
Macrophomina phaseolina (Tassi) Goldanich	00	03	05	16	18	19	16	12
Rhizopus nigricans Ehrenb.	00	10	12	23	30	30	32	38
Sclerotium rolfsii Sacc.	00	05	10	26	30	30	22	12
S.E. ±	00	0.45	0.57	0.87	0.85	0.89	0.99	0.77
C.D. at 0.05%	00	1.40	1.78	2.73	2.67	2.79	3.12	2.41

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

Table 9: Effect of temperature on lipase production in seed borne fungi of Groundnut var. SB-XI (pH 6.5, Incubation period 9 days).

Seed born.e fungi	Enzyme Activity (Units)* Temperature (°C)							
	10	15	20	25	30	35	40	45
Aspergillus flavus Link ex Fr.	00	05	10	16	28	27	22	21
Aspergillus niger van Tieghem	00	06	11	20	30	29	28	20
Fusarium oxysporum Schl. emend Sny. & Hans.	00	08	16	25	35	33	26	20
Macrophomina phaseolina (Tassi) Goldanich	00	04	05	11	19	16	16	12

Rhizopus nigricans Ehrenb.	00	10	15	25	33	30	33	38
Sclerotium rolfsii Sacc.	00	05	10	19	29	26	20	12
S.E. ±	00	0.43	0.50	0.77	0.99	1.01	1.01	0.89
C.D. at 0.05%	00	1.36	1.59	2.41	3.13	3.20	3.20	2.81

** One unit is equivalent to 0.1 ml 0.05 N NaoH required to neutrilise fatty acids liberated.*

CONCLUSION

Agar plate method yielded more seed borne fungi than moist blotter plate method. From results it is evident that as incubation period increases, lipase enzyme production also increased. In case of pH, as pH increased lipase enzyme production decreased, it was also noted that there was no enzyme produced by test fungi in both groundnut varieties between pH 3.5 to 4.5. It was also noticed that at low temperatures i.e. up to 10°C there was no lipase enzyme production. Hence it can be concluded that by manipulating the physical factors, lipase production can be minimized which reduce loss caused due to biodeterioration of seeds. Thus by changing environmental conditions we can control seed borne pathogens and damage caused by them.

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