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LIPOLYTIC ACTIVITY OF FUNGI ASSOCIATED WITH SOME OILY-SEEDS AT ASSIUT GOVERNORATE, EGYPT

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A total of 27 fungal species and one variety related to 9 genera were recovered from sesame (21+1 & 7), peanut (12 & 5) and soybean (19+1 & 6) gathered from different stores at Assiut Governorate. *Aspergillus* was the most prevalent genus whereas, the most common species were *A. niger*, and *A. flavus*. Out of 134 fungal isolates tested for lipase production, only 39 isolates have lipolytic properties and *A. niger* 49sp and 38sp recovered from soybean seeds were the highest producers giving each 1.07 cm for lipase activity. Using 1% NaCl solution, 1% NaCl + 1% tween 80, and phosphate buffer (pH 7.0) for extracting lipase enzyme from sesame, groundnut and soybean seeds. It was proved the high efficiency of 1% NaCl + 1% tween 80 in the extraction with four concentrations through the three seeds. The current study proved the high efficiency of oilseeds borne fungi for lipase production and the important of using the right extraction solvent. Also, this study is the precedent for further trials, where it is intended to improve the production of the enzyme lipase to be purified and commercialized.

Key words: lipase, oilseeds, fungi, enzymes, solvents.

INTRODUCTION

Oil seeds are a global leader in providing high-quality vegetable oils to the nutritious products, and natural foods. Corn, peanut, cotton, sesame, soybean, sunflower, coconut, olives, and almond are oil-producing crops. However, corn, soybean, sunflower, sesame, and olives are majorities of oilseeds that grown in most of the world countries [1]. Small grains have low oil content ranged from 1 to 2%, whereas oilseeds have higher oil content ranging from 20 to 40% [2]. Soybean, corn, sunflower, cotton, and olives are the main sources of edible oils around the world [1]. They do not include a significant amount of carbohydrate,

but they do have high vitamin content; B vitamins, thiamine, vitamin A and nicotinic acid and providing high-quality protein content [3].

Sesame (*Sesamum indicum* L.), also known as sesamum or benniseed, is a member of the Pedaliaceae family and one of the oldest oilseed crops known to humans. Sesame is a vital component of human diet. The majority of sesame seeds are utilized for oil extraction, while the remainder is consumed [4]. It is grown in Asia, Africa, South and North America, Europe, and Australia [5]. Sesame is grown in Egypt's Governorates of Ismailia, Sharkiya, Fayoum, and Sohag, and is considered a food crop rather than an oil seed crop [6]. Soybean (*Glycine max* L.) originates from East Asia, and soybean seed is one of the most important protein sources for human and livestock all over the world. Soybeans are rich with high quality protein (43 %) and rich with high oil content (20 %) with unsaturated fatty acids and no cholesterol [1, 7]. Peanut (*Arachis hypogaea* L.) is self-pollinating allotetraploid legume crop belonging to the Fabaceae family and called as King of the oilseeds. peanutt seeds are a rich source of oil (35–56%), protein (25–30%), carbohydrates (9.5–19.0%), minerals (P, Ca, Mg and K) and vitamins (E, K and B) [1,8].

Seed borne fungi of oily plants represent a significant group of fungi that produce high amount of various enzymes especially lipase enzyme for its high oil content [9]. The most common fungal genera recovered as oilseed borne fungi were *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., and *Fusarium* sp.[10,11,12]. Lipases (E.C. 3.1.1.3) are hydrolases and water soluble enzyme(s) that catalyse the hydrolysis of insoluble triacylglycerols to produce free fatty acids, diacylglycerols, monoacylglycerols, and glycerols (Figure 1). They catalyse a wide range of processes, including hydrolysis, transesterification, and interesterification of other esters, as well as ester synthesis, and have a variety of regio-, enantio-, and stereo-selective transformation characteristics [13,14].

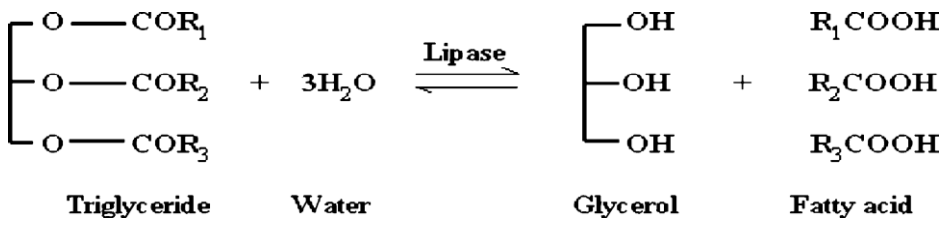

Figure 1: Hydrolysis of triglyceride by lipase

Lipase-producing microorganisms can be found in a wide variety of environments, including waste from the vegetable oil and dairy product industries, soil polluted with oils, seeds, and spoiled food [14]. The most common fungal species recovered as oilseed borne fungi and produce lipase enzyme are *Alternaria* sp., *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium moniliforme, Penicillium* sp., and *Rhizopus stolonifer* [10, 15, 16, 17, 18, 19, 20, 21].

The aim of this work is to estimate the seed borne fungi of three oil seeds sesame (*Sesamum indicum* L.), soybean (*Glycine max* L.), and peanut (*Arachis hypogaea* L.) on general medium and lipase specific medium. Lipolytic abilities of the isolated fungi and optimizing the extraction solutions of lipase enzyme using solid state fermentation by common fungal species (*Aspergillus niger* ASU49) were also assessed.

MATERIALS AND METHODS

1. Collection of samples

Thirty samples of sesame (*Sesamum indicum* L.), peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) seeds (10 samples each) were collected from local stores in Assiut Governorate. The samples were placed in clean bags, transported to the mycological laboratory, and directly utilized for the isolation of oilseed-borne fungi.

2. Media used for isolation of fungi

Two media were used for isolation of seed-borne fungi associated with oilseeds. General isolation medium; Czapek's dextrose agar (CzDA) medium that contained (g/L): glucose, 10.0; $NaNO_3$, 3.0; KCl, 0.5; KH_2PO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; $FeSO_4$, 0.07; agar, 15.0 and 1000 ml

distilled water (Pitt 1979). Lipase specific isolation medium; Czapek's Oil agar medium (CzOA) containing (g/L): 10.0; $NaNO_3$, 3.0; KCl, 0.5; KH_2PO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; $FeSO_4$, 0.07; agar, 15.0 and 1000 ml distilled water was also embloyed. The media were autoclaved at 121ºC for 20 min., and then supplemented with chloramphenicol (250 mg/ml) as bactericidal agents and 10 g/l tween 80 sterilized separately [22].

3. Isolation of seed-borne fungi

For isolation of fungi, direct plating [23] and dilution plating techniques [24] were used. In direct plating method, 15 seeds (5 per each plate) from each type were used for each medium. In dilution plate method, one gram of seeds was diluted in sterilized water to appropriate concentration from which, 1 ml was transferred to each Petri dish. Three plates were used for each medium. Plates were then incubated at 28±2 ºC for 7 days. The developing fungal colonies were counted and calculated as colony forming unit (CFU) per 15 seeds for each medium used in direct plating method, and per 1 g seeds in dilution plate method.

4. Phenotypic identification of fungi

The identification of fungal genera and species was based on macroscopic and microscopic features following the keys and descriptions of Ellis [25,26] for Dematiaceous Hyphomycetes; Pitt [27] for *Penicillium* and its teleomorphs; Raper and Fennell [28] for *Aspergillus* species; Booth [29], Leslie and Summerell [30] for *Fusarium* species; Moubasher [31] and Domsch *et al.* [32] for fungi in general.

5. Qualitative determination of lipase activity

It was evaluated on the medium suggested by Ullman and Blasins [33] with some modifications, which contains (g/L): $NaNO_3$, 3; KCl, 0.5; K_2HPO_4,1;$FeSO_4$, 0.07; $MgSO_4.7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 80, 10 ml; and agar, 15. The medium was sterilized by autoclaving for 20 minutes at 121°C. Tween 80 was autoclaved separately before being introduced to the sterile basal medium. In 15 cm test tubes (10 ml/tube), the medium was dispensed aseptically. A 50 µL of fungal spore suspension was used to inoculate test tubes on the surface of agar, and

incubated for 10 days at 25 °C. The lipolytic capacity of fungi was seen as a visible precipitate formed by crystals of calcium polysorbates released by the enzyme. The depth of each visible precipitate (in mm) was measured (**Figure 2)**.

.6. Optimization of lipase extraction from different oily seeds

a. Culture conditions

Sesame, peanut, and soybean seeds were ground to a fine powder. In a 250-ml Erlenmeyer conical flask, 10, 20, 30 and 40 g of each seed type was individually transferred, along with 10 ml of oil-free Czapek's broth. The medium contained (g/l): $NaNO_3$, 2.0; KCl, 0.5; K_2HPO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01 and $CuSO_4$, 0.005. After autoclaving at 121 C for 20 min., the flasks were inoculated each with 2 ml of spore suspension obtained from 7-day-old culture of *Aspergillus niger* (ASU49). The flasks were then incubated at 28 °C in a static condition for 7 days.

b. Extraction and assay of lipase enzyme

Following the incubation, the contents of each flask were well mixed, 2 g were collected and homogenized separately in 20 ml of 1% NaCl solution or 1% NaCl + 1% tween 80 or phosphate buffer (pH 7.0). Filtration with filter paper No. 1 and centrifugation at 10,000 xg for 10 minutes yielded the cell-free supernatant. Two ml of the supernatant was mixed with 2 ml of tween 80 and 6 ml of phosphate buffer (pH 8.0) for the lipase assay, and the reaction mixture was incubated in a water bath at 35 °C for 3 hours. The reaction was stopped by adding 25 ml of 95 % ethanol. Two drops of 0.2 % phenolphthaleine solution (made in ethanol/water 1: 1) were added, and the flask content was titrated against 50 mM sodium hydroxide solution until a purple colour was observed, then calculated as mg/g oilseed [34].

RESULTS

1. Fungi isolated from sesame seeds:

The current results revealed that, a total of 21 species and one variety belonging to 7 genera were recovered from the 10 sesame seed samples on Czapek's dextrose agar (CzDA) and Czapek's oil agar (CzOA) media using the direct plating technique and dilution plating technique at 28 °C for 7 days.

Using the direct plating technique, the 10 samples of sesame seeds on CzDA at 28 °C yielded a total of 21 species and one variety belonging to 7 genera. *Aspergillus*, *Macrophomina*, and *Rhizopus* were the most common genera, of which *A. flavus*, *A. niger*, *A. oryzae*, *Macrophomina phaseolina* and *Rhizopus stolonifer* were the most common species. *Aspergillus* was obtained from all samples, accounting for 53.87% of the total fungi. There were 11 species and one variety identified from the genus, *A. niger*, *A. flavus*, and *A. oryzae* being the most common species giving 90%, 70%, and 70% of the samples, accounting for 32.68 %, 31.37 %, and 11.11 % of total *Aspergillus* and 17.60 %, 16.90 %, and 5.98 % of total fungi, respectively. The other *Aspergillus* species were seen in moderate and low occurrence; they constituted collectively around 13.37 % of total fungi (Table 1).

Rhizopus stolonifer and *Macrophomina phaseolina* ranked second to *Aspergillus*. They were isolated each from seven samples (out of ten) and matched 19.72 % and 7.74 % of the total fungi, respectively. *Fusarium* was attributed for the third highest incidence rate. It was appeared in 50 % of the samples, accounting for 2.82 % of the total fungi. Two species of the genus were reported, *F. oxysporum* and *F. solani*. They were isolated respectively from two and three samples containing approximately 2.82 % of total fungi. *Penicillium* came in fourth position in terms of the number of isolated fungi. It was recovered from four samples constituting 5.28 % of total fungi. Four species from the genus were found, accounting for approximately 5.28 % of total fungi. *Cladosporium sphaerospermum* and *Phoma glomerata* were identified in

low abundance, accounting for 1.40 % and 9.15 % of total fungi, respectively (Table1).

On CzOA, the direct plating technique at 28 °C produced 9 species and one variety belonging to 7 genera. *Aspergillus* was the most frequent genus emerged in 9 samples, accounting for 57.36 % of the total fungi. *Aspergillus was represented* by four species of which *A. niger* and *A. flavus* were the most common. They were found in around 70 % and 50 % of the samples, accounting for 47.85 % and 40.16 % of total *Aspergillus* and 27.45 % and 23.04 % of the total recoveredbfungi, respectively. The remaining *Aspergillus* species were seen quite infrequently. It supplied around 6.86 % of total fungi. *Macrophomina phaseolina* came in second place, after *Aspergillus*. It was isolated from six samples (out of ten) and matched 18.63 % of the total fungi. *Rhizopus stolonifer* had the third highest incidence. It was found in 30 % of the samples, accounting for 10.29 % of the total fungi. *Fusarium solani* and *Phoma glomerata* came in fourth place in terms of isolations. They were recovered from two samples containing 11.27 % and 18.63 % of total fungi, respectively. *Cladosporium* (represented by *C. sphaerospermum*) was identified in low numbers and recovered from only one sample, accounting for 0.49 % of all fungi (Table 1).

By using the dilution- plate technique, the CzDA yielded five species and one variety linked to one genus (*Aspergillus)* from the all studied samples. It appeared in ten samples on the applied isolation medium, accounting 100 % of total fungi. Five species and one variety namely, *A. flavus*, *A. niger*, *A. oryzae*, *A. flavus* var. *columnaris*, *A. parasiticus*, and *A. terreus* were detected. They were found in 50.59 %, 28.24 %, 9.41 %, 5.88 %, 3.53 %, and 2.35 % of total fungi, respectively. While on CzOA, four species and one variety belonging to two genera were identified using the dilution plate method. The most prevalent genus was *Aspergillus*. It appeared in 9 of the 10 analyzed samples, accounting for 94.73 % of the total fungi. Three species and one variety were recognized within the genus, namely *A. flavus*, *A. flavus* var. *columnaris*, *A. niger*, and *A. parasiticus*. They represented in 40.35 %, 12.28 %, 40.35 %, and 1.75 % of total fungi, respectively. *Rhizopus stolonifer* was

isolated on a rare occasion. It was isolated from a single sample that matched 5.26 % of the total fungi (Table1).

Table 1: Total counts (TC calculated per 15 seed for each sample), percentage of total count (TC%), number of cases of isolation (NCI, out of 10 samples) and occurrence remarks (OR) of seed-borne fungi isolated from 10 samples of sesame seeds on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA) using direct-plating technique & dilution- plate method at 28±1°C.

ngal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR
Aspergillus	**51.00**	**53.87**	**10H**	**39.01**	**57.36**	**9H**	**283.33**	**100**	**10H**	**180.00**	**94.73**	**9H**
A. awamori Nakazawa	0.67	0.70	1L	4.00	5.88	1L	0.0	0.0	-	0.0	0.0	-
A. carbonarius (Bainier)Thom	2.00	2.11	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. flavus Link	16.00	16.90	7H	15.67	23.04	5M	143.33	50.59	4M	76.67	40.35	4M
A. flavus var. *columnaris* Raper & Fennell	1.00	1.06	1L	0.67	0.98	1L	16.67	5.88	1L	23.33	12.28	1L
A. fumigatus Fresenius	1.67	1.76	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. niger van Tieghum	16.67	17.61	9H	18.67	27.45	7H	80.00	28.24	4M	76.67	40.35	3M
A. oryzae (Ahlb.) Cohn.	5.67	5.99	7H	0.0	0.0	-	26.67	9.41	2L	0.0	0.0	-
A. parasiticus Speare	2.99	3.17	3M	0.0	0.0	-	10.00	3.53	1L	3.33	1.75	1L
A. sydowii (Bain.&Sart.) Thom & Church	1.67	1.76	3M	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. tamarii Kita	1.00	1.06	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. terreus Thom	1.33	1.41	1L	0.0	0.0	-	6.67	2.35	1L	0.0	0.0	-
A. versicolor (Vuill.) Tirab.	0.33	0.35	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Cladosporium sphaerospermum Penz	**1.33**	**1.41**	**2L**	**0.33**	**0.49**	**1L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
Fusarium	**2.67**	**2.82**	**5M**	**1.00**	**1.47**	**2L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
F. oxysporum Schltdl.	1.00	1.06	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Macrophomina phaseolina (Tassi.) Goid.	**7.33**	**7.75**	**7H**	**7.67**	**18.63**	**6M**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
Penicillium	**5.00**	**5.28**	**4M**	**0.33**	**10.29**	**1L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR
P. chrysogenum Thom	3.00	3.17	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
P. glabrum (Wehmer) Westling	0.67	0.70	1L	0.33	0.49	1L	0.0	0.0	-	0.0	0.0	-
P. purpurogenum Stoll	0.33	0.35	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
p. roquefortii Thom	1.00	1.06	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Phoma glomerata (Corda) Wollenw. & Hochapfel	**8.67**	**9.15**	**2L**	**12.67**	**18.63**	**2L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
Rhizopus stolonifer (Ehrenb.) Vuill.	**18.67**	**19.72**	**7H**	**7.00**	**10.29**	**3L**	**0.0**	**0.0**	**-**	**10.00**	**5.26**	**1L**
Total counts	**94.67**	**100.00**		**68.00**	**100.00**		**283.33**	**100.00**		**190.00**	**100.00**	
No. of genera (7)	**7**			**7**			**1**			**2**		
No. of species & varieties (21+1)	**21+1**			**9+1**			**5+1**			**4+1**		

OR; occurrence remarks, H; High occurrence isolated from>5 samples, M; Moderate occurrence isolated from 3-5 samples, L; Low occurrence isolated from<3 samples.

2. Fungi recovered from peanut seeds:

By using the direct- plating technique for isolation, the CzDA yielded a total of 12 species assigned to 5 genera from the ten samples of peanut seeds on at 28 °C. *Aspergillus*, *Rhizopus*, *Penicillium* and *Fusarium* were the the highest incidence and contributed the highest species spectra. *Aspergillus* was isolated from all samples consisting of 75.67 % of the total fungi. Seven species of the genus were discovered, among which *A. flavus* and *A. niger* were the most frequent. They appeared on 100% of the samples each, representing 27.72 % and 41.58 % of total *Aspergillus*, and 20.97 % and 31.46 % of total fungi, respectively. The remaining *Aspergillus* species were collectively gave 23.22 % of all fungi (Table 2). *Rhizopus stolonifer* was occupied second

place and recovered from 6 samples out of ten representing 17.98 % of the total fungi. *Penicillium* had the third highest occurrence rate. It was appeared in 40 % of the samples, which accounted for 3.37 % of total fungi. From the genus two species were recovered each from two samples called *P. glabrum* and *P. oxalicum*, each given 3.37 % of the total fungi in low incidence. In the number of instances of isolation, *Fusarium oxysporum* placed fourth comprising 2.25 % of total fungi, while *Cladosporium sphaerospermum* counting 0.75 % of the total fungi (Table 2).

From CzOA medium six species relating to four genera were isolated. The most common genera were *Aspergillus, Fusarium* and *Rhizopus*. The first genus of incidence was *Aspergillus* which recovered from all samples containing 39.0 % of the total fungi. *Aspergillus flavus* and *A. niger* were the most frequent species. Each of these was reported as having occurred on 70 % of the samples, representing 41.87 % and 53.0 %, and 33.11 % and 41.89 %, of total *Aspergillus* and total fungi, respectively. At low frequency, the remaining species of *Aspergillus* was occurred comprising 4.05 % of all fungi. Fusarium *oxysporum* came behind *Aspergillus*. It was isolated from 3 (out of 10) samples, corresponding to 12.16 % of the total fungi. *Rhizopus stolonifer* had the third highest incidence rate. It was occurred in 20 % of the total examined samples, which accounted for 8.11 % of the total fungi. In the number of cases of isolation, *Penicillium glabrum* placed fourth by recovering from one sample with 0.68 % of the total fungi (Table 2).

While by using the dilution-plate method for isolation, the incidence of fungal genera and species was found to be very low on both isolation media. On CzDA, four species were related to 2 genera were isolated accounting 236.67 CFUs. *Aspergillus* was the most common genus. It was occurred in 10 samples examined constituting 92.95 % of total fungi. From the genus 3 species were identified namely *A. flavus, A. niger,* and *A. ochraceus*. They were occurred in 70.42%, 21.12 % and 1.40 % of total fungi, respectively. *Rhizopus stolonifer* was isolated in rare frequency of occurrence appearing in only one sample matching 7.04 % of the total recovered fungi. On CzOA, one species belonging to one

genus was recovered accounting 113.33 CFUs. *A. flavus* was isolated only on CzOA matching 70% of the samples comprising 100% of total fungi. Some species were isolated by using direct-plating technique and not encountered in dilution plate method such as: *A. awamori*, *A. fumigatus*, *A. terreus*, *A. ustus*, *Cladosporium sphaerospermum*, *Fusarium oxysporum*, *P. glabrum* and *P. oxalicum.* Also, some species were isolated on CzDA and not isolated on CzOA such as: *A. awamori*, *A. ochraceus*, *A. terreus*, *A. ustus*, *Cladosporium sphaerospermum* and *P. oxalicum* (Table 2).

Table 2: Total counts (TC calculated per 15 seed for each sample), percentage of total count (TC%), number of cases of isolation (NCI, out of 10 samples) and occurrence remarks (OR) of seed-borne fungi isolated from 10 samples of peanut seeds on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA) using direct-plating technique & dilution- plate method at 28±1°C.

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR	TC	%TC	NCI &OR
Aspergillus	**67.34**	**75.67**	**10H**	**39.00**	**79.0**	**10H**	**220.00**	**92.95**	**10H**	**113.33**	**100**	**7H**
A. awamori	0.33	0.37	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. flavus	18.67	20.97	10H	16.33	33.11	7H	166.67	70.42	9H	113.33	100	7H
A. fumigatus	16.00	17.98	2L	2.00	4.05	1L	0.0	0.0	-	0.0	0.0	-
A. niger	28.00	31.46	10H	20.67	41.89	7H	50.00	21.13	2L	0.0	0.0	-
A. ochraceus G. Wilh.	0.67	0.75	1L	0.0	0.0	-	3.33	1.41	1L	0.0	0.0	-
A. terreus	1.00	1.12	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. ustus (Bain.) Thom & Church	2.67	3.00	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Cladosporium sphaerospermum	**0.67**	**0.75**	**1L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
Fusarium oxysporum	1.99	2.25	3M	6.00	12.16	3M	0.0	0.0	-	0.0	0.0	-
Penicillium	**3.00**	**3.37**	**4M**	**0.33**	**0.68**	**1L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
P. glabrum	2.33	2.62	2L	0.33	0.68	1L	0.0	0.0	-	0.0	0.0	-

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	%TC	NCI&OR	TC	%TC	NCI&OR	TC	%TC	NCI&OR	TC	%TC	NCI&OR
P. oxalicum Currie & Thom	0.67	0.75	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Rhizopus stolonifer	**16.00**	**17.98**	**6M**	**4.00**	**8.11**	**2L**	**16.67**	**7.04**	**1L**	0.0	0.0	-
Total counts	**88.99**	**100.00**		**49.33**	**100.00**		**236.67**	**100.00**		**113.33**	**100**	
No. of genera (5)	**5**			**4**			**2**			**1**		
No. of species (12)	**12**			**6**			**4**			**1**		

OR; occurrence remarks, H; High occurrence isolated from>5 samples, M; Moderate occurrence isolated from 3-5 samples, L; Low occurrence isolated from<3 samples.

3. Fungi recovered from soybean seeds:

Using the direct-plating technique for isolation, the CzDA produced a total of 19 species and one variety belonging to 6 genera were isolated and identified from 10 samples of soybean seeds. *Aspergillus*, *Fusarium, Penicillium* and *Rhizopus* exhibited the highest frequency of occurrence and the highest species diversity. *Aspergillus* was the highest incidence genus. It was recovered from all samples comprising 82.33 % of total fungi. *Aspergillus* was represented by 12 species and one variety, of which *A. flavus* and *A. niger* were the most prevalent. They occurred in 90 %, and 70 % of the total tested samples constituting 30.76 % and 27.12 % of total *Aspergillus* and 24.43 % and 21.54 % of total fungi, respectively. *A. oryzae*, *A. tamarii*, *A. parasiticus*, *A. ochraceus*, *and A. versicolor* were isolated in moderate occurrence from 6, 5, 4, 3, and 3 samples out of 10, giving 8.04%, 6.11%, 2.89%, 3.54%, and 3.22% of the total fungi, respectively (Table 3). The second rank behind *Aspergillus* was *Fusarium oxysporum* (5 samples, out of 10) representing 9.0 % of the total fungi. *Rhizopus stolonifer* had the third highest incidence rate and was isolated from 40 % of samples accounting 5.79 % of total fungi. Concerning the number of cases of isolation, *Penicillium* occupied the

fourth place (3 samples, out of 10) containing 3.54 % of the total fungi. Three species namley, *P. chrysogenum, P. purpurogenum* and *P. roquefortii,* have been recovered from 3.54 % of total fungi. In low occurrences *Stachybotrys chartarum* and *Trichoderma harzianum* were isolated comprising 1.28 % and 0.96 % of total fungi, respectively (Table 3).

From 10 samples of soybean seeds, fourteen species and one variety related to four genera were identified on CzOA at 28 °C using the direct-plating technique for isolation. The most common genera were *Aspergillus*, and *Fusarium*. *Aspergillus* had the initial occurrence of all samples retrieved and accounted for 83.65 % of the entire fungi. Ten species of *Aspergillus* and one variety were recognized, of which *A. flavus, A. niger,* and *A. ochraceus* were recorded in 80 %, 50 %, and 30% of total samples making up 42.44 %, 25.36 %, and 5.85% of the entire *Aspergillus*, and 35.51 %, 21.22 %, and 4.9% of the whole fungi, respectively. The remaining species of *Aspergillus* comprising 26.92 % (Table 3). *Fusarium* oxysporum took the second spot Aafter *Aspergillus*. It was found in three (out of ten) samples to match 8.98 % of the total fungi. *Penicillium* (*P. purpurogenum, P. roquefortii*) and *Rhizopus stolonifer* had the low incidence rate in the third group. They appeared each in 20 % of samples, accounting for 1.63 % and 5.71 % of total fungi, respectively. *P. purpurogenum* and *P. roquefortii* were found to be two *Penicillium* species. They were all taken from a single sample, totaling roughly 1.63 % of all fungi (Table 3).

The findings of utilizing the dilution-plate method for isolation indicated that the incidence of fungal genera and species on the two isolation medium was very low. The examined samples on CzDA and CzOA yielded 7 and 2 species related to 2 and 1 genera and encountered a total of 223.33 and 86.67 CFUs, respectively, *Aspergillus* was the most prevalent genus found in 9 and 7 samples, respectively, accounting for 100 percent of total fungus on the two isolation media. Six *Aspergillus* species and one variety were identified on CzDA namely *A. flavus, A. flavus* var. *columnaris, A. niger, A. ochraceus, A. oryzae* and *A. sydowii*, and *A. ochraceus*. They comprised 1.43 %, 72.86 %, 2.86 %, 7.14 %, 10.0

% and 1.43 % of total fungi, respectively. *Fusarium oxysporum* was isolated from two samples (out of 10) matching 4.29 % of total fungi on CzDA only. While on CzOA, only *A. flavus* var. *columnaris* and *A. ochraceus* were isolated from 70 % and 10 % of total samples comprising 84.62 % and 15.38 % of total fungi, respectively (Table 3). Some species were isolated by using direct-plating technique only, these namely *A. flavus* var. *columnaris, A. fumigatus, A. japonicus, A. parasiticus, A. tamarii, A. terreus, A. versicolor, P. chrysogenum, P. purpurogenum, P. roquefortii, Rhizopus stolonifer, Stachybotrys chartarum, Trichoderma harzianum.* Conversely, some others were isolated on CzDA only namely *A. fumigatus, A. terreus, P. chrysogenum, Stachybotrys chartarum, Trichoderma harzianum* (Table 3).

Some species were isolated from sesame seeds and not on peanut and soybean seeds such as *A.carbonaris, F.solani, Macrophomina phaseolina, Phoma glomerata.* Some species were isolated from peanut seeds and not on sesame and soybean seeds such as *A.ustus, P.oxalicum*. Some species were isolated from soybean seeds and not on sesame and peanut seeds such as *A.jabonicus, Stachybotrys chartarum, Trichoderma harzianum.*

Table 3: Total counts (TC calculated per 15 seed for each sample), percentage of total count (TC%), number of cases of isolation (NCI, out of 10 samples) and occurrence remarks (OR) of seed-borne fungi isolated from 10 samples of soybean on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA) using direct-plating technique & dilution- plate method at 28±1°C.

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR
Aspergillus	**82.33**	**79.41**	**10H**	**68.32**	**83.65**	**10H**	**223.33**	**100**	**9H**	**86.67**	**100**	**7H**
A. awamori	2.67	2.57	1L	2.00	2.45	1L	3.33	1.43	1L	0.0	0.0	-
A. flavus	25.33	24.44	9H	29.00	35.51	8H	170.00	72.86	8H	73.33	84.62	7H
A. flavus var. *columnaris*	1.33	1.29	2L	1.00	1.22	1L	0.0	0.0	-	0.0	0.0	-
A. fumigatus	1.33	1.29	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
A. japonicas Saito	0.33	0.32	1L	2.33	2.86	1L	0.0	0.0	-	0.0	0.0	-
A. niger	22.33	21.54	7H	17.33	21.22	5M	6.67	2.86	1L	0.0	0.0	-
A. ochraceus	3.67	3.54	3M	4.00	4.90	3M	16.67	7.14	1L	13.33	15.38	1L
A. oryzae	8.33	8.04	6M	5.33	6.53	2L	23.33	10.00	1L	0.0	0.0	-
A. parasiticus	3.00	2.89	4M	2.33	2.86	1L	0.0	0.0	-	0.0	0.0	-
A. sydowii	3.00	2.89	2L	0.67	0.82	1L	3.33	1.43	1L	0.0	0.0	-
A. tamarii	6.33	6.11	5M	3.00	3.67	1L	0.0	0.0	-	0.0	0.0	-
A. terreus	1.33	1.29	2L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR
A. versicolor	3.33	3.22	3M	1.33	1.63	1L	0.0	0.0	-	0.0	0.0	-
Fusarium oxysporum	**9.33**	**9.00**	**5M**	**7.33**	**8.98**	**3M**	**10.00**	**4.29**	**2L**	**0.0**	**0.0**	**-**
Penicillium	**3.67**	**3.54**	**3L**	**1.33**	**1.63**	**2L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**
P. chrysogenum	1.67	1.61	1L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
P. purpurogenum	1.33	1.29	1L	1.00	1.22	1L	0.0	0.0	-	0.0	0.0	-
P. roqueforti	0.67	0.64	1L	0.33	0.41	1L	0.0	0.0	-	0.0	0.0	-
Rhizopus stolonifer	**6.00**	**5.79**	**4M**	**4.67**	**5.71**	**2L**	**0.0**	**0.0**	**-**	**0.0**	**0.0**	**-**

Table 3: Continued

Fungal species	Direct plating technique						Dilution plate technique					
	CzDA			CzOA			CzDA			CzOA		
	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR	TC	% TC	NCI & OR
Stachybotrys chartarum (Ehrenb.) S. Hughes	1.33	1.29	1 L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Trichoderma harzianum Rifai	1.00	0.96	1 L	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Total counts	103.67	100.00		81.67	100.00		233.33	100.00		86.67	100.00	
No. of genera (6)	6			4			2			1		
No. of species & varieties (19+1)	19 +1			14 +1			7			2		

OR; occurrence remarks, H; High occurrence isolated from>5 samples, M; Moderate occurrence isolated from 3-5 samples, L; Low occurrence isolated from<3 samples.

2. Screening for lipolytic production by the isolated fungi

In this experiment, out of 54 isolates identified (related to 5 genera, 18 species, and one variety) from sesame seed only 12 isolates demonstrated lipolytic activity on basic agar medium supplemented with 1% tween 80 as a substrate **(Figure 2)**. These lipolytic isolates were all related to the genus *Aspergillus*, with 7 being strong lipase producers, 4 moderate, and one low lipase producers. The highest producers were; *A. niger* 6G, *A. carbonairus* 8G, *A. flavus* 1, 8, 10G, *A. fumigatus* 2G, and *A. parasiticus* 4G giving 1.03, 0.87, 0.6, 0.73, 0.73, 0.6, and 0.53 cm, respectively. The moderate producers were; *A. flavus* 6, 9G, *A. flavus* var. *columnaris* 5G, and *A. awamori* 3G giving 0.4, 0.33, 0.4, and 0.37 cm, respectively. While, *A. flavus* 2G give low enzyme index 0.17 cm **(Table 4)**.

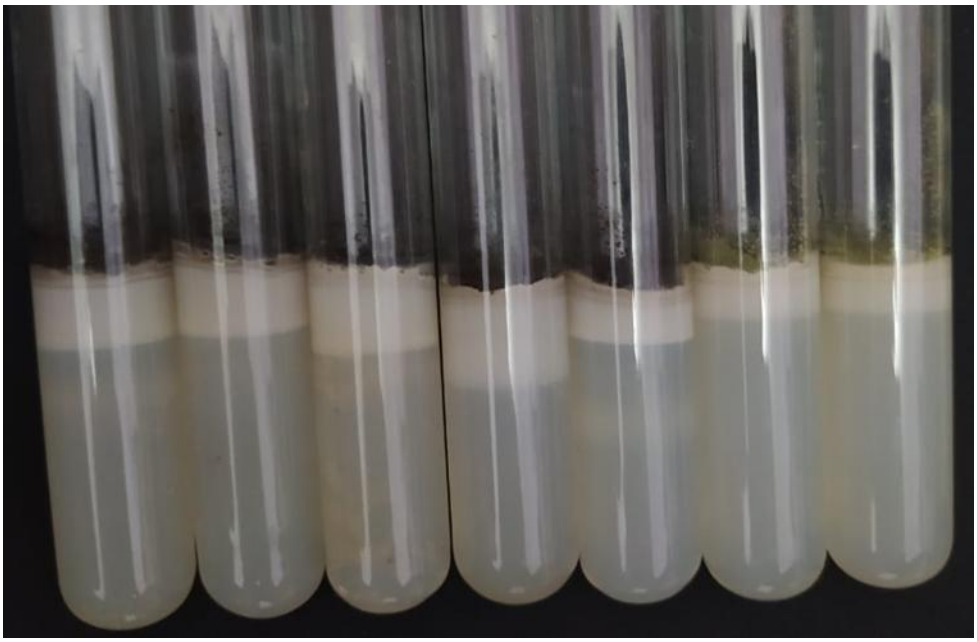

Figure 2: Varied levels of lipolytic activities of oilseed borne fungi represented by the white precipitate.

Table 4: Screening of lipolytic activity of oilseed borne fungi isolated from sesame seeds on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA)

Fungal species	No of isolate	Type of media	Enzyme activity (cm) &standard deviation (± SD)	Enzyme index
Aspergillus awamori	3G	CzDA	0.37±0.07	M
A. carbonairus	2G	CzDA	0	-
A. carbonairus	8G	CzDA	0	-
A. carbonairus	8G	CzDA	0.87±0.15	H
A. flavus	8G	CzDA	0.73±0.06	H
A. flavus	3G	CzDA	0	-
A. flavus	3sp	CzOA	0	-
A. flavus	1G	CzDA	0.6±0.1	H
A. flavus	6G	CzDA	0.4±0.1	M
A. flavus	2G	CzDA	0.17±0.06	L
A. flavus	9G	CzDA	0.33±0.058	M
A. flavus	10G	CzDA	0.73±0.12	H
A. flavus	6sp	CzOA	0	-
A. flavus var. *columnaris*	5G	CzDA	0.4±0.1	M
A. flavus var. *columnaris*	5sp	CzOA	0	-
A. fumigatus	2G	CzDA	0.6±0.26	H
A. niger	1sp	CzOA	0	-
A. niger	2sp	CzOA	0	-
A. niger	8G	CzDA	0	-
A. niger	1G	CzDA	0	-
A. niger	9sp	CzOA	0	-

Table 4: Continued

Fungal species	No of isolate	Type of media	Enzyme activity (cm) &standard deviation (± SD)	Enzyme index
A. niger	6G	CzDA	1.03±0.058	H
A. niger	7G	CzDA	0	-
A. niger	4G	CzDA	0	-
A. niger	10G	CzDA	0	-
A. niger	5G	CzDA	0	-
A. oryzae	1G	CzDA	0	-
A. oryzae	4G	CzDA	0	-
A. oryzae	6G	CzDA	0	-
A. oryzae	5G	CzDA	0	-
A. oryzae	9G	CzDA	0	-
A. oryzae	2G	CzDA	0	-
A. parasiticus	1G	CzDA	0	-
A. parasiticus	4G	CzDA	0.53±0.06	H
A. tamarii	6G	CzDA	0	-
A. terreus	6G	CzDA	0	-
A. versicolor	7G	CzDA	0	-
Cladosporum sphaerospermum	4G	CzDA	0	-
Fusarium solani	10sp	CzOA	0	-
F. solani	10G	CzDA	0	-
F. solani	3G	CzDA	0	-
F. solani	5sp	CzOA	0	-
F. solani	5G	CzDA	0	-

Table 4: Continued

Fungal species	No of isolate	Type of media	Enzyme activity (cm) &standard deviation (± SD)	Enzyme index
F. oxysporum	**4G**	**CzDA**	**0**	**-**
F. oxysporum	**7G**	**CzDA**	**0**	**-**
Penicillium chrysogenum	**10G**	**CzDA**	**0**	**-**
P. glabrum	***8sp***	**CzOA**	**0**	**-**
P. purpurogenum	***3G***	**CzDA**	**0**	**-**
P. roquefortii	***3G***	**CzDA**	**0**	**-**
P. roquefortii	**4G**	**CzDA**	**0**	**-**
Rhizopus stolonifer	**7sp**	**CzOA**	**0**	**-**
R. stolonifer	**8G**	**CzDA**	**0**	**-**
R. stolonifer	**5G**	**CzDA**	**0**	**-**
R. stolonifer	**2G**	**CzDA**	**0**	**-**

G; general medium, Sp; specific medium, H; high enzyme activity>0.5 cm , M; moderate enzyme activity 0.3-0.5 cm, L; low enzyme activity <0.3 cm.

From peanut seeds, out of 36 isolates (related to 9 species and 4 genera) 7 isolates demonstrated lipolytic activity on basic agar medium supplemented with 1% tween 80 as a substrate. These isolates were also all from the genus *Aspergillus*, with 1 being strong lipase producers, 3 moderate, and 3 low lipase producers **(Table 5).** The highest producers was *A. flavus* 25G gives 0.73 cm, while the moderate producers were; *A. niger* 13G, *A. flavus* 20G, *26*G giving 0.5, 0.33, and 0.37 cm, respectively. The low producers were;, *A. flavus* 19, 22, 24G giving 0.17, 0.17, and 0.2 cm, respectively **(Table 5)**.

Table 5: Screening of lipolytic activity of oilseed borne fungi isolated from peanut seeds on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA)

Fungal species	No of isolate	Type of media	Enzyme activity (cm) &standard deviation (± SD)	Enzyme index
Aspergiilus flavus	18G	CzDA	0	-
A. flavus	19G	CzDA	0.17±0.058	L
A. flavus	23G	CzDA	0	-
A. flavus	21G	CzDA	0	-
A. flavus	13sp	CzOA	0	-
A. flavus	24G	CzDA	0.2±0.1	L
A. flavus	25G	CzDA	0.73±0.06	H
A. flavus	26G	CzDA	0.37±0.058	M
A. flavus	20G	CzDA	0.33±0.06	M
A. flavus	22G	CzDA	0.17±0.058	L
A. flavus	26sp	CzOA	0	-
A. flavus	20sp	CzOA	0	-
A. fumigatus	18sp	CzOA	0	-
A. fumigatus	13G	CzDA	0	-
A. niger	13G	CzDA	0.5±0.1	M
A. niger	24G	CzDA	0	-
A. niger	13G	CzDA	0	-

Table 5: continud

Fungal species	No of isolate	Type of media	Enzyme activity (cm) &standard deviation (± SD)	Enzyme index
A. niger	**25sp**	**CzOA**	**0**	**-**
A. niger	**26G**	**CzDA**	**0**	**-**
A. niger	**26sp**	**CzOA**	**0**	**-**
A. niger	**18G**	**CzDA**	**0**	**-**
A. niger	**21sp**	**CzOA**	**0**	**-**
A. niger	**19sp**	**CzOA**	**0**	**-**
A. niger	**22sp**	**CzOA**	**0**	**-**
A. niger	**23sp**	**CzOA**	**0**	**-**
A. niger	**20G**	**CzDA**	**0**	**-**
A. ochraceus	**13G**	**CzDA**	**0**	**-**
A. ustus	**13G**	**CzDA**	**0**	**-**
A. ustus	**20G**	**CzDA**	**0**	**-**
Fusarium oxysporum	**21G**	**CzDA**	**0**	**-**
F. oxysporum	**25sp**	**CzOA**	**0**	**-**
F. oxysporum	**24G**	**CzDA**	**0**	**-**
Penicillium glabrum	***24sp***	**CzOA**	**0**	**-**
P. glabrum	***19G***	**CzDA**	**0**	**-**
P. oxalicum	***22G***	**CzDA**	**0**	**-**
Rhizopus stolonifer	**23sp**	**CzOA**	**0**	**-**

G; general medium, Sp; specific medium, H; high enzyme activity>0.5 cm , M; moderate enzyme activity 0.3-0.5 cm, L; low enzyme activity <0.3 cm.

From soybean seeds, 44 isolates (13 species and 3 genera) were investigated. From the result 20 isolates demonstrated lipolytic activity on basic agar medium supplemented with 1% tween 80 as a substrate. These isolates were all from the genus *Aspergillus*, with 10 being strong lipase producers, 7 moderate, and 3 low lipase producers **(Table 6).** highest producers were; *A. niger* 38, 49sp, 37G, *A. awamori* 39sp, *A. jabonicus* 41sp, *A. flavus* 42, 47G, 49sp, *A. ochraceus* 42sp and *A. oryzae* 56G giving 1.07, 1.07, 0.73, 0.93, 0.77, 0.7, 0.73, 0.57, 0.63 and 0.57 cm, respectively. The moderate producers were; *A. flavus* 39, 41, 48, 49G, 38, 39sp and *A. fumigatus* 41G giving 0.5, 0.37, 0.43, 0.37, 0.47, 0.5, and 0.43 cm, respectively. While, *A. flavus* 37G, *38G, and* 42sp gives low enzyme index 0.1, 0.2, and 0.17 cm, respectively **(Table 6)**.

Table 6: Screening of lipolytic activity of oilseed borne fungi isolated from soybean seeds on Czapek's dextrose agar medium (CzDA) & Czapek's oil agar medium (CzOA)

Fungal species	No of isolate	Type of media	Enzyme activity (cm)&standard deviation (± SD)	Enzyme index
Aspergillus awamori	**39sp**	**CzOA**	**0.93±0.28**	**H**
A. flavus	**38G**	**CzDA**	**0.2±0.1**	**L**
A. flavus	**39G**	**CzDA**	**0.5±0.1**	**M**
A. flavus	**14G**	**CzDA**	**0**	**-**
A. flavus	**47G**	**CzDA**	**0.73±0.058**	**H**
A. flavus	**49G**	**CzDA**	**0.37±0.06**	**M**
A. flavus	**49sp**	**CzOA**	**0.57±0.058**	**H**
A. flavus	**42G**	**CzDA**	**0.7±0.1**	**H**
A. flavus	**42sp**	**CzOA**	**0.17±0.058**	**L**
A. flavus	**41G**	**CzDA**	**0.37±0.12**	**M**
A. flavus	**48G**	**CzDA**	**0.43±0.15**	**M**
A. flavus	**48sp**	**CzOA**	**0**	**-**
A. flavus	**47sp**	**CzOA**	**0**	**-**
A. flavus	**37sp**	**CzOA**	**0**	**-**
A. flavus	**39sp**	**CzOA**	**0.5±0.1**	**M**

Table 6:continud				
Fungal species	**No of isolate**	**Type of media**	**Enzyme activity (cm)&standard deviation (± SD)**	**Enzyme index**
A. flavus	**38sp**	**CzOA**	**0.47±0.058**	**M**
A. flavus	**37G**	**CzDA**	**0.1±0**	**L**
A. flavus* var. *columnaris	**42G**	**CzDA**	**0**	**-**
A. flavus* var. *columnaris	**39sp**	**CzOA**	**0**	**-**
A. fumigatus	**41G**	**CzDA**	**0.43±0.058**	**M**
A. jabonicus	**41sp**	**CzOA**	**0.77±0.06**	**H**
A. niger	**37sp**	**CzOA**	**0**	**-**
A. niger	**14G**	**CzDA**	**0**	**-**
A. niger	**48sp**	**CzOA**	**0**	**-**
A. niger	**49sp**	**CzOA**	**1.07±0.058**	**H**
A. niger	**49G**	**CzDA**	**0**	**-**
A. niger	**38sp**	**CzOA**	**1.07±0.12**	**H**
A. niger	**37G**	**CzDA**	**0.73±0.058**	**H**
A. ochraceus	**42sp**	**CzOA**	**0.63±0.058**	**H**
A. ochraceus	**41sp**	**CzOA**	**0**	**-**
A. oryzae	**42sp**	**CzOA**	**0**	**-**
A. oryzae	**39G**	**CzDA**	**0**	**-**
A. oryzae	**56G**	**CzDA**	**0.57±0.058**	**H**
A. tamarii	**56G**	**CzDA**	**0**	**-**
A. tamarii	**38G**	**CzDA**	**0**	**-**
A. terreus	**41G**	**CzDA**	**0**	**-**
A. versicolor	**42G**	**CzDA**	**0**	**-**
A. versicolor	**38G**	**CzDA**	**0**	**-**
Fusarium oxysporum	**39G**	**CzDA**	**0**	**-**
F. oxysporum	**37G**	**CzDA**	**0**	**-**
F. oxysporum	**38sp**	**CzOA**	**0**	**-**

Table 6:continud

Fungal species	No of isolate	Type of media	Enzyme activity (cm)&standard deviation (± SD)	Enzyme index
F. oxysporum	**41G**	**CzDA**	**0**	**-**
F. oxysporum	**48G**	**CzDA**	**0**	**-**
P. chrysogenum	**48G**	**CzDA**	**0**	**-**

G; general medium, Sp; specific medium, H; high enzyme activity>0.5 cm , M; moderate enzyme activity 0.3-0.5 cm, L; low enzyme activity <0.3 cm.

5. Optimization of lipase extraction from different oily seeds and grains

Solid state fermentation using sesame, peanut, and soybean seeds with 10, 20, 30 and 40 g concentration and three extraction solutions (1 % NaCl, 1% NaCl + 1% tween 80, and phosphate buffer) was performed for optimizing the lipase extraction as illustrated in figures **3, 4, and 5.** For sesame seeds 1 % NaCl +tween 80 was the best extracting solution for 10, 30, 40 g seeds giving lipase activity 4.24, 4.78, and 5.25 U/g SSF, respectively. While, phosphate buffer was the best for concentration 20 g seeds and gives lipase activity 5.09 U/g SSF **(Figure 3)**. For peanut seeds 1 % NaCl +tween 80 was best extracting solution for all concentrations 10, 20, 30, 40 g seeds giving lipase activity 4.75, 4.84, 4.42, and 4.48 U/g SSF, respectively **(Figure 4)**. For soybean seeds 1 % NaCl +tween 80 was best extracting solution for 10, and 30 g seeds giving lipase activity 4.81 and 5.075 U/g SSF, respectively. While, phosphate buffer was the best for concentration 40 g seeds and gives lipase activity 4.63 U/g SSF, 1 % NaCl was the best for concentration 20 g seeds and gives lipase activity 4.87 U/g SSF **(Figure 5)**.

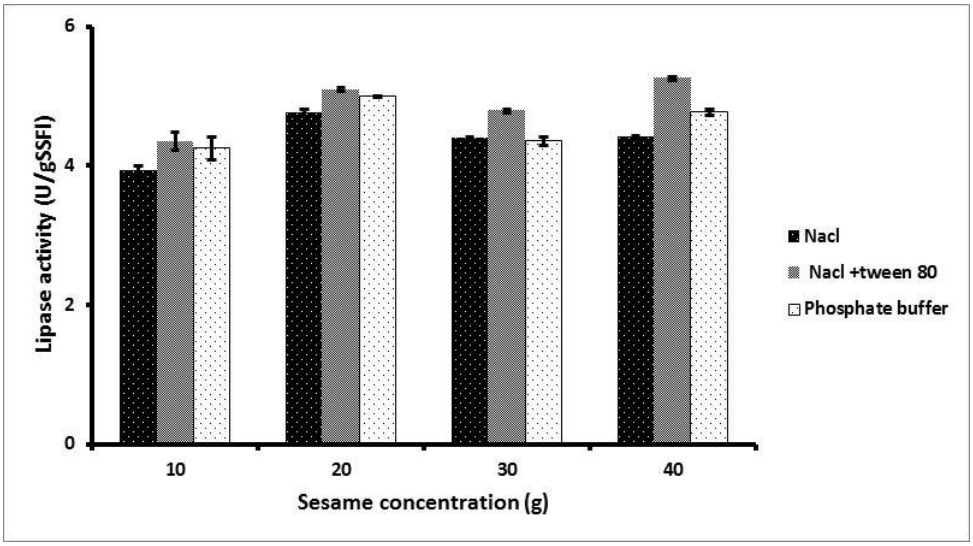

Figure 3: Lipase activity (U/g SSF) from sold state fermentation of sesame seeds using three extraction solutions (1 % NaCl, 1% NaCl + 1% tween 80, and phosphate buffer).

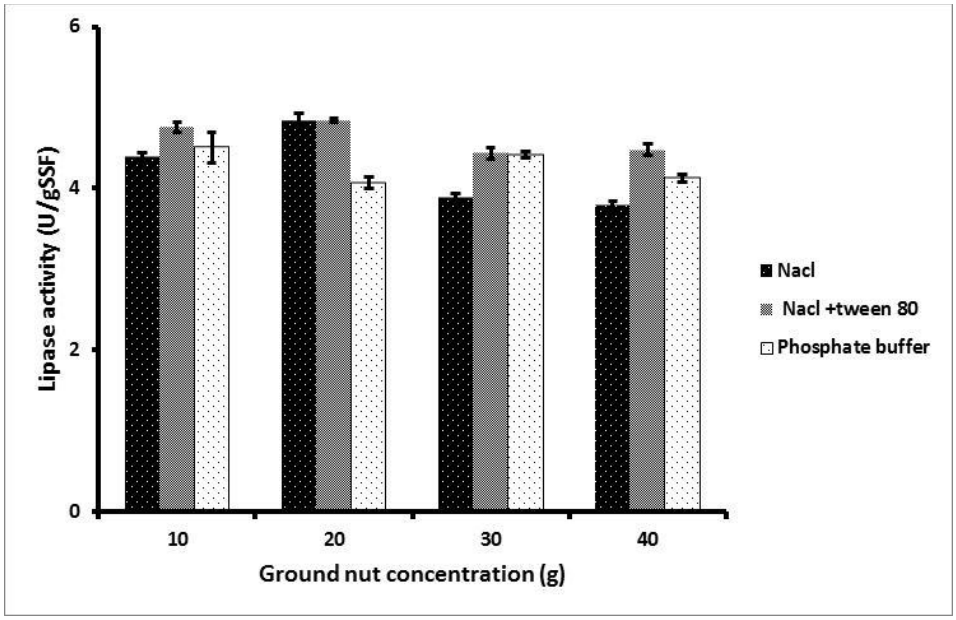

Figure 4: Lipase activity (U/g SSF) from sold state fermentation of peanut seeds using three extraction solutions (1 % NaCl, 1% NaCl + 1% tween 80, and phosphate buffer).

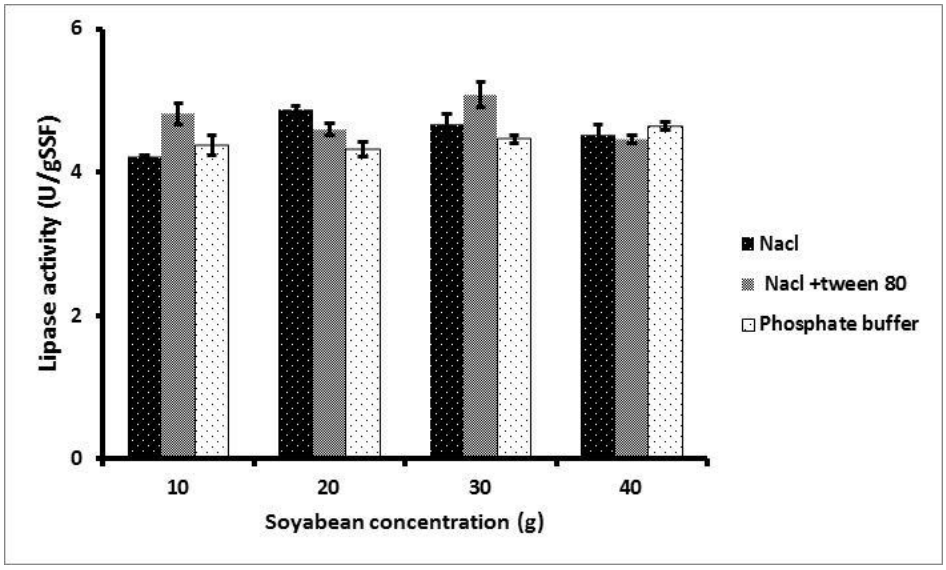

Figure 5: Lipase activity (U/g SSF) from sold state fermentation of soyabean seeds using three extraction solutions (1 % NaCl, 1% NaCl + 1% tween 80, and phosphate buffer).

DISCUSSION

The cost-effective quest of novel sources of lipases with varied catalytic properties has fueled the ongoing isolation and selection of new strains [35]. The current results revealed that, a total of 27 species and one variety belonging to 9 genera were recovered from the 10 sesame seed samples on Czapek's dextrose agar (CzDA) and Czapek's oil agar (CzOA) at 28 °C. In this regard, thirty four fungal species were previously isolated from sesame seeds, namely *Alternaria alternata*, *A. sesamicola*, *A. tenuis*, *Fusarium moniliforme*, *F. oxysporum*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *S. rolfsi*, *Cercospora sesami*, *Curvularia lunata*, *Macrophomina phaseolina*, *Cladosporium cladosporioides*, *C. herbarum*, *C. fulvum*, *C. chlorocephalum*, *Acremonium* sp., *Helminthosporium* sp., *Gliocladium roseum*, *Neurospora glabra*, *Cunninghamella elegans*, *Chaetomium globosum*, *Stachybotrys chartarum*, *S. atra*, *Pestalotia macrotricha*, *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *A. versicolor*, *A. terreus*, *A. candidus*, *Haplosporangium* sp., *Penicillium citrinum*, *Rhizopus nigricans*, and *R.*

stolonifer [36]. Ghosh *et al.* [37] reported that sesame seeds were highly infected with *Alternaria sesamicola, Corynespora cassiicola,* and *Drechslera sesame*. Twelve species belonging to 5 genera were recovered from the 10 peanut seed samples on Czapek's dextrose agar (CzDA) and Czapek's oil agar (CzOA) at 28 °C. It was found that *Rhizopus* sp., *Penicillium* sp., *Fusarium* sp., and *Sclerotium bataticola* were isolated from stored groundnut [38]. *Alternaria alternata*, *Alternaria citri*, *Aspergillus flavus, A. niger, Fusarium solani*, *F. oxysporum, F. moniliforme, Macrophomina phaseolina*, *Mucor* sp., and *Rhizoctonia solani* were found predominant on peanut [39]. Kakde [10] isolated *Alternaria tennuisima, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Penicillium notatum,* and *Rhizopus stolonifer* from groundnut. Ghosh et al [37] reported that *Macrophomina phaseolina, Aspergillus flavus* and *Aspergillus niger* were the common peanut seed borne fungi. Also, 19 species and one variety belonging to 6 genera were recovered from the 10 soybean seed samples on Czapek's dextrose agar (CzDA) and Czapek's oil agar (CzOA) at 28 °C. *Aspergillus niger, Fusarium moniliforme,* and *Rhizopus stolonifer* were isolated soyeabean [15]. *Alternaria* sp., *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium moniliforme, Penicillium* sp., and *Rhizopus stolonifer* [10].

When basic agar medium supplemented with 1% tween 80 was employed as a substrate, only 39 isolates (out of 134) exhibited lipolytic activity. These 39 isolates were all the genus *Aspergillus*, with 18 producing strong lipase, 14 producing intermediate lipase, and 7 producing low lipase. Venkatesagowda *et al.* [40] used Tween-20 on agar to test 1279 fungal isolates for lipolytic activity. Forty isolates exhibited high lipolytic activity and were morphologically identified as belonging to 19 taxa (*Alternaria*, *Aspergillus*, *Chalaropsis*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phyllosticta*, *Rhizopus*, *Sclerotinia*, *Stachybotrys* and *Trichoderma*Various microorganisms, including bacteria, filamentous fungi, and yeasts, are recognized extracellular lipase producers [41]. Microorganisms having the ability to produce lipases can be found in a variety of environments,

including waste vegetable oils, dairy products, oil polluted environments, seeds, and damaged food [14]. Indeed, because lipases are derived from enzyme classes commonly employed in biotechnological applications and organic chemistry, Nature offers a significant opportunity for finding new sources of lipases with unique characteristics [42]. Fungi are considered as one of the finest lipase sources among microbes [43]. Fungal lipases are gaining popularity in the industry due to their substrate specificity and stability under a variety of chemical and physical conditions.

Fungal lipases have advantages over bacterial lipases since modern technology favours batch fermentation and low-cost extraction techniques. *Rhizopus*, *Aspergillus*, *Penicillium*, *Mucor*, *Geotrichum*, *Beauveria*, *Humicola*, *Rhizomucor*, *Fusarium*, *Acremonium*, *Alternaria*, *Eurotium*, and *Ophiostoma* are some of the most important filamentous fungal genera [44]. Lipases are widely utilized in a variety of industries, including food, pharmaceutics, biofuels, oleochemical, textile, agro-chemical, paper production, cosmetics, and many more. Lipases can be employed in the food business as flavour modifiers via synthesis of short chain fatty acid esters and alcohols, as well as to generate goods with higher nutritional value by altering the triacylglycerol structure for inter- or transesterification [45]. Lipases are possible emulsifier replacements in the bakery [46], and they used to synthesize structural lipids, low calorie lipids and milk fat and in ripening cheese [47, 48, 49]. Several techniques for microbial screening based on the presence of extracellular lipases are available. The use of a solid medium in conjunction with inducer substrates such as vegetable oils, standard triglycerides, and Tween 80 [50, 51]. However, some of these substrates may be insufficient for lipase detection, making verification of the specificity of the lipase analysis critical [52]. The majority of lipases are inducible by oils [14], though other inducers also induce increased production of lipases, such as free fatty acids, hydrolysable esters, bile salts and glycerol [53]. Lipase producers normally grow in complex growth media, which include carbon sources (oils, sugars), nitrogen sources and salts. Compounds such as olive oil, oleic acid and Tween 80 are also important in enzyme synthesis.

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نشاط التحلل الدهنى للفطريات المصاحبة لبعض البذور الزيتية في محافظة اسيوط- مصر

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تعتبر البذور الزيتية من المحاصيل الهامة التي يتم استهلاكها بشكل كبير نظرأ لمحتواها من الزيوت. كما إنها تحمل كائنات دقيقة ذات نشاط عالي لتحلل الدهون. تم في هذه الدراسة عزل وتعريف ٢٧ نوعا فطريا بالإضافة الي صنف تنتمي الي ٩ أجناس وذلك من بذور السمسم (٢١،٧) ، الفول السودانى (١٢،٤) ، فول الصويا (٢٠،٦) على الأوساط وطرق العزل المختلفة. أظهرت النتائج أنا الأنواع التابعة لجنس الأسبرجيللس هى أكثر الأنواع شيوعا وتعدادا وكانت أنواع نيجر وفلافس هى أكثرها إنتشارا في البذور المختبرة. أوضحت النتائج أن ٣٩ عزلة فطرية من ١٣٤ عزلة لها القدرة على إنتاج إنزيم الليبيز وكانت فطرة أسبرجيللس نيجر رقم ٣٨ ، ٤٩ ذات كفاءة عالية لإنتاج الإنزيم حيث أظهرت منطقة تحلل ١,٠٧ سم.

بإستخدام أربعة أنواع من مذيبات الإستخلاص إتضح أن المذيب ١% كلوريد الصوديوم مع ١% تويين ٨٠ هو المذيب الأعلى كفاءة لإستخلاص الإنزيم مع كل التركيزات المستخدمة من الثلاث أنواع من البذور المختبرة . أثبتت الدراسة الحالية الكفاءة العالية للفطريات التى تحملها البذور الزيتية في إنتاج إنزيم الليبيز وكذلك أهمية إستخدام مذيبات إستخلاص مناسبة. أيضاً هذه الدراسة هى سابقة لمزيد من التجارب، حيث تهدف الى تحسين إنتاج إنزيم الليبيز ليتم تنقيته وتسويقه.