AJFAND ONLINE
African Journal of Food, Agriculture, Nutrition and Development
Scholarly, Peer Reviewed
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

LIPOLYTIC ACTIVITY OF SOME FUNGAL SPECIES ON CASTOR OIL

Negedu A[1]*, Ameh JB[2], Umoh VJ[2] and SE Atawodi[3]

Anthony Negedu

*Corresponding author email: tonyneg2000@yahoo.com

[1] Raw Materials Research and Development Council, P.M.B. 232, Garki, Abuja,Nigeria.

[2] Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

[3]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

ABSTRACT

Castor seed is important because of the castor oil content (40 – 57%). The oil enjoys tremendous world demand in the pharmaceutical, paint, cosmetics, textile, leather, lubricant, chemical, plastic, fibre, automobile and engineering industries. Castor oil, aside being a renewable resource, it is biodegradable and eco-friendly. Agricultural products are natural habitats of fungi and these materials contain cellulose, hemi-cellulose, starch, oils and other complex organic compounds. The ability of the fungi to exist in these environments largely depends on their ability to produce enzymes capable of breaking down the various organic materials to provide their nutrients. Castor seeds and castor oil are good sources of nutrients for fungal growth and development. Fungi are capable of producing lipases which can hydrolyze fats or oils to fatty acids thereby increasing the free fatty acids of the produce and this is a deteriorating effect. Lipid degradation takes place when seeds or their oils are damaged by improper storage conditions or are exposed to certain microorganisms. Studies were carried out on the ability of nine fungal species namely: *Aspergillus tamarii Aspergillus chevallieri, Penicillium chrysogenum, Cephaliophora irregularis, Syncephalastrum racemosum, Aspergillus flavus, Aspergillus ruber, Aspergillus terreus* and *Aspergillus niger* to produce extracellular lipases in stationary liquid medium using quantitative and qualitative indices including, dry mycelia mass, free fatty acid and peroxide values. These studies showed that all the mould species exhibited lipolytic activity to varying levels as confirmed by formation of blue halo round the fungal colonies. The highest level of lipolytic activity (96.88% ±1.12%) was produced by *Aspergillus tamari*. which was also characterized by highest mycelia dry yield (2.54 mg/40ml ± 0.20 mg/40ml), while *Cephaliphora irregularis* produced the least lipolytic activity (3.10 % ±0.18%). The highest peroxide value (38.1 meq/kg ± 1.17meq/kg) was obtained in the medium inoculated with *Aspergillus tamarii* while the least (6.20 meq/kg±0.58)meq/kg was with *Aspergillus terreus* after 30 days of storage. The study showed that *Aspergillus tamarii* and *Aspergillus flavus* grew well and readily caused lipolytic activity when cultured in medium containing castor oil as the only carbon source, suggesting that these fungal species contributed significantly to castor oil bio-deterioration.

Key words: Castor, Seeds, Oil, Fungi, Deterioration

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

INTRODUCTION

Castor oil is globally important oil and had been used in the production of over 300 industrial products [1]. However, like most vegetable oils, lipid deteriorating effect occurs when seeds or their oils are damaged by improper storage conditions or are exposed to certain microorganisms such as fungi [2]. Fungi are capable of producing lipase; a glycerol ester hydrolase, a principal enzyme involved in the hydrolysis of seed lipids (triacylglycerol) to free fatty acids and glycerol [3].

Due to their high extracellular complex enzyme production, fungi have been implicated in the quality deterioration of many vegetable oils and oil seeds [4]. Some workers earlier reported the presence and activity of fungal lipases in the deterioration of grains and oil seeds and their associated oils. For instance, [5] reported the involvement of a number of mesophilic fungi and three thermophilic ones in the deterioration of oil palm fruits and palm oil. [6] Reported the deterioration of cotton seeds, soya beans and melon seeds and their oils by mesophilic moulds. In the same vein, [7] reported that fungal lipases of *Aspergillus* and *Penicillium* species were responsible for the lipid hydrolysis of wheat grains, while [8] reported the lipolytic activity of some moulds on wild mango (*Irvingia gabonensis*). For instance, the involvement of a number of mesophilic fungi and three thermophilic ones in the deterioration of oil palm fruits and palm oil as well as the deterioration of cotton seeds, soya beans and melon seeds and their oils by mesophilic moulds were reported [9, 10]. However, little appears to be known on the lipase activity of these mesophilic fungi on castor seeds in storage.

The present study was, therefore, designed to confirm and provide more information on the lipolytic activity of nine fungal species (*Aspergillus tamarii, Aspergillus chevallieri, Penicillium chrysogenum, Cephaliophora irregularis, Syncephalastrum racemosum, Aspergillus flavus, Aspergillus ruber, Aspergillus terreus* and *Aspergillus niger)* isolated from naturally infected stored castor seeds.

MATERIALS AND METHODS

Sample Collection

Wild castor (*Ricinus communis L.*) seeds were collected from stores in four locations based on the segmentation used by Kogi state Agricultural development programme (Kabba, Ankpa, Ogaminana and Ajaka), Nigeria.

Two hundred gramme seed lots from each location were collected in triplicates from 100kg-sacks of seeds using a grain probe and stored in sterile polyethylene bags. In all, 12 stores in Kogi State were visited and 36 samples were collected. The samples weighed 200 – 400g and were conveyed to the levorotary of the Department of Microbiology of the Ahmadu Bello University, Zaria until required. The temperature of the stores at the point of sample collection was recorded using a thermometer (0 – 100^0C). The storage temperature after collection was atmospheric room temperature (27 ± 1^0C).

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

Isolation of Fungi from the Seeds

Ten grammes (approx 24 seeds) from each seed sample were taken as sub-samples for the isolation of mycoflora. The sub-samples were surface-sterilized by immersion in 1% sodium hypochlorite (NaOCl) solution for thirty seconds and rinsed three series of sterile distilled water.

Based on 6 seeds per dish, the surface-sterilized seed were directly plated on SDA that contained chloramphenicol (100ml/L) in accordance with the procedures of the International Seed Testing Association (ISTA, 1976). The plates were then kept at room temperature (27 + 1^0C) for 7 – 12 days for e examination. Examination for the presence of fungi on the seeds was carried daily using a dissection microscope and compound light microscope (Magnification X 100). Fungal isolates were obtained directly from fungal colonies on the seeds by plating fungal spores or mycelium on Sabouroud Dextrose Agar (SDA) complemented with chloramphenicol (100mg/L). Percentage frequency of each fungus was calculated as total number of seeds on which a particular fungus appeared per dish/total number of feeds per dish. Pure cultures of the fungal isolates were transferred on to fresh SDA slants and stored at room temperature for identification.

Identification of Fungal Isolates

Identification of the isolated fungi was carried out at the crop protection department of the Ahmadu Bello University, Zaria with reference to mycological texts [11] and confirmed by the Global Plant Clinic of the Common Wealth Agricultural Bureau International (CABI), London

Quantitative Determination of the Lipolytic Activity of the Fungi

The lipolytic ability of the mould species was determined as described by Oso [3]. A basal medium was prepared as described by OSO, 1974, using castor oil as sole carbon and energy source. The pH of the medium was adjusted to 7.0 with 0.2M NaOH, and was dispensed into 250ml conical flasks at 20ml per flask. To the liquid medium in each flask, 20ml of castor oil (Integrated Oil Mills ltd, Trikania, Kaduna State, Nigeria. Batch No. 0660216), was added and autoclaved at 121^0C for 15minutes.

Preparation of the Inoculum

A pure culture of each of the nine isolates was grown for five days on Sabouraud Dextrose Agar (SDA) complimented with chloramphenicol (100mg/L to inhibit the growth of bacteria) in Petri-dishes. One disk (5mm diameter) of agar and mycelium of each isolate were asceptically introduced into the flasks using a sterile cork borer. An uninoculated mixture in the flask served as control. Each complete treatment was replicated three times and was incubated at room temperature (27 ± 1^oC) conditions for 30 days.

At intervals of 15 days, one complete set of the treatment was withdrawn and the percentage free fatty acids, peroxide values and dry weight of mycelium were determined. The dry weight of mycelia, free fatty acids and peroxide values were determined.

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

Qualitative Determination of Lipolytic Activity of the Fungi

The lipolytic ability of the isolates was tested using the method as described by [3]. The medium contained castor oil (Integrated Oil Mills Limited No.17 Tricania, Kaduna, Kaduna state.) 100ml, 0.1% aqueous Nile blue sulphate solution 50ml, Bactotryptone 5g, agar 15g and distilled water to make up 1 liter. The pH was adjusted to 7.0 with acetate buffer solution and autoclaved at 1.05cm^2 at 121°C for 15 minutes, cooled and dispensed into Petri-dishes. Before pouring into the Petri-dishes, the medium was vigorously shaken to ensure that the oil appeared in form of tiny globules well dispersed in the orange-pink medium. The inoculum was a pure culture of each of the nine isolates grown for five days on Sabouraud Dextrose Agar (SDA) complemented with chloramphenicol (100mg/L) in Petri-dishes. Each plate was centrally and asceptically inoculated with a disk (5mm diameter) of agar and mycelia of the isolates. For each fungus, three plates were inoculated and an uninoculated plate containing the medium served as control and all the plates were incubated at room temperature (28 ± 1°C) and examined daily for a blue halo formation around each fungal colony for two weeks.

Dry Weight of Mycelia

Growth was determined by the dry weight of mycelia produced on the medium as adopted by [9] .The dry weight of mycelia was obtained by harvesting the mycelia on the growth medium by filtration through a dried and pre-weighed No. 1 Whatman filter paper (9cm diameter) in a Buckner funnel. The mycelia were washed with chloroform to remove any adhering oil and the filter paper together with mycelia were dried in an oven at 80°C for 24 hours, cooled in a desiccator and weighed using analytical balance. The difference in weight gives the mycelia dry weight.

Free Fatty Acid Value.

The free fatty acid value was determined using method [10]. A solvent mixture of 25ml ethanol (95%) and 25ml diethyl ether was neutralized with 0.1M ethanolic potassium hydroxide (KOH) using phenolphthalein as indicator. Two grammes of oil sample was dissolved in the neutralized solvent mixture and titrated with 0.1M KOH solution until a faint pink colour appeared in the supernatant alcohol for 15 seconds.
The acid value was calculated. Thus:

$$\text{Acid Value} = \frac{56.1 \times T \times V}{M}$$

Where T = Molarity of ethanolic acid

V = Volume of KOH used

M = Mass in grams of test sample

The free fatty acid was calculated thus:

$$\%\ \text{Free Fatty Acid} = \frac{\text{Acid Value}}{2}$$

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
Published by African Scholarly Science Communications Trust

Peroxide Value

The peroxide value was determined using method [10]. Two grammes of the extracted castor oil was weighed into a 200ml conical flask already containing of 20ml acetic acid – chloroform solution (2 parts of acetic acid to 1 part of chloroform) and 1ml of saturated KI solution (4 parts of KI in 3 parts of distilled water) was added and allowed to stand in the dark for exactly one minute. Thirty milliliters of distilled water was added and titrated with 0.002M solution of sodium thiosulphate using 1ml of 1% starch solution as indicator (the indicator was prepared by dissolving 1 g of soluble starch powder in enough cold water to form a thin paste and adding 100ml of boiling water and boil with stirring). A blank was run simultaneously but the volume of sodium thiosulphate used for the blank is usually negligible.

The peroxide value was calculated thus:

$$PV = \frac{V \times T}{M} \times 100$$

Where: V = Volume of $Na_2S_2O_3$ used

T = Molarity of $Na_2S_2O_3$

M = Mass of the test sample in grammes

RESULTS

Table 1, presents the mean values of the free fatty acids liberated from oil by the hydrolysis of castor oil by the isolates (triplicates of same sample of castor seeds). All the isolates exhibited lipolytic activity but to varying levels. *Aspergillus tamarii* had the highest lipolytic activity (96.88±1.12%) and followed by *Aspergillus flavus* with 89.74±0.39 %, while *Cephaliophora irregularis* had the least lipolytic activity (3.10± 0.18%).

Table 2 shows the peroxide value of the castor oil upon hydrolysis by the isolates. Peroxide value is a measure of the extent of oxidation of the oil. The free fatty acids released upon hydrolysis were oxidized to form hydroperoxides. The highest peroxide value was obtained in the medium inoculated with *Aspergillus tamarii* (38.10± meq/kg) and followed by that inoculated with *Syncephalastrum racemosum* (35.40± meq/kg). The medium inoculated with *Aspergillus terreus* had the least peroxide value (6.20± meq/kg).

Table 3 presents the biomass of the isolates grown on castor oil, *Aspergillus tamarii* had the highest dry weight of mycelia (2.54±mg/40ml) and followed by *Aspergillus flavus* (2.00±0.43 mg/40ml) and *Syncephalastrum racemosum* (1.90±0.30 mg/40ml), while *Aspergillus niger* had the least (0.63±0.34 mg/40ml).

All the isolates grew well on the castor oil /nile blue sulphate medium forming a blue halo round the colony of each fungus (Table 4). The formation of a blue halo

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

indicated that the isolate was able to break down the oil to liberate free fatty acids which subsequently reacted with the orange-pink colour of the oil/nile blue sulphate medium to produce the blue halo.

DISCUSSION

This study showed that all the nine fungi caused increase in the free fatty acids (FFA) of the oil in the liquid medium to varying levels. The highest level of lipolytic activity was significantly ($P<0.05$) produced by *Aspergillus tamarii* (Table 1), which was also characterized by highest mycelia dry yield (Table 3), followed by Aspergillus flavus (Table 1) both in the same taxonomic group, while *Cephaliphora irregularis* produced the least lipolytic activity (Table 1). The highest oxidative value (peroxide value) was obtained in the medium inoculated with *Aspergillus tamarii* (Table 2), while *Aspergillus terreus* had the least peroxide value (Table 2) after 30 days of storage. Except *Cephaliphora irregularis* and *Penicilium chrysogenum,* all the fungal species caused increase in free fatty acid above 20%, the value after which rancidity of oil becomes noticed [3].

The varying lipolytic activity of these fungi agreed with [11], who reported similar observation on melon seed oil. In the same name Ward and Diener made similar report on fungi associated with peanut oil and [8], on wild mango (*Irvingia gabonensis*). The increased level of free fatty acid content could be attributed to the activity of extracellular lipases from the infecting fungi and the slight increase in the (FFA) content of the control experiment after 4 weeks might be due to auto – oxidation of the triglycerides [2]. This reason is supported by Kuku (1980) and Ogundero (1981) who reported similar results for melon seeds and groundnuts respectively.
The differences in the lipolytic activity may be attributed to differences in their lipase enzyme production capacity which could be due to differences in their lipase enzyme production capacity which relates to the rate at which they carried out the hydrolysis of the triglycerides to release FFA and glycerol. This reason is supported by Negedu et al., (2011); Bhattacharya and Raha, (2004); Bankole and Joda, (2004) [14, 15, 16] who reported that the ability of a fungus to exist on an organic matter would depend on its ability to produce the necessary enzymes and also the capacity of the extracellular enzyme production in the substrate.

In addition, the lipolytic activity showed by these fungi might be as a result of individual species showing specificity and preferences in the utilization of component fatty acids as carbon sources [17]. The fact that *Aspergillus tamarii* had the highest dry weight of mycelium on the growth medium could be due to its faster rate of hydroysis and consumption of the products of the lipolysis, free fatty acids and glycerol [17, 18].

It has been reported that very high amount of lipase in products can lead to extensive breakdown of fats with concomitant release of low molecular weight fatty acids that may result in the development of undesirable rancid flavours usually associated with deteriorating and deteriorated oilseeds, oils and oil-containing products [12, 13,14].

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 6
October 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

Increase in the FFA content of oil hampers bleaching, deodorization and increased rancidity, resulting in higher cost of processing, as well as low quality products [10]. Therefore, limits are set at 5% for the level of FFA which can be tolerated in product [19].

CONCLUSION

From this study it has been shown that castor oil, which is the economic end product of castor seed is negatively affected by the lipolytic activity of associated fungi and ultimately reducing the commercial value in the market, therefore the presence of any fungus in such oil is not desirable and effort should be geared towards prevention, since any percentage increase in the free fatty acid beyond the allowable limit attracts penalty in price reduction in the market.

In addition, the production of hydroperoxides, which are themselves unstable and further oxidation or decomposed to by-products, such as epoxides, aldehydes, ketones, hydrogen sulphide that are responsible for the beany and off flavours of deteriorating oils or oil-containing products. Other negative impacts may also be associated with fungal growth in oil seeds and their oils which may require some studies.

AJFAND ONLINE Scholarly, Peer Reviewed
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

Table 1: Free fatty acid liberated at 30 days of growth on castor oil medium (with castor oil as sole carbon source)

Name of isolate	Lipolytic activity as % Free fatty acid	
	15 days	30 days
Aspergillus chevallieri	21.5 ±1.18	80.89 ±1.50
Aspergillus tamari	34.64 ±1.13	96.88 ±1.12
Penicillium chrysogenum	6.53 ±0.35	13.80 ±0.15
Cephaliophora irregularis	1.88 ±0.09	3.10 ±0.18
Syncephalastrum racemosum	8.93 ±0.97	87.03 ±2.99
Aspergillus flavus	22.09 ±1.72	89.27 ±0.39
Aspergillus rubber	15.60 ±0.13	72.14 ±0.96
Aspergillus terreus	18.05 ±0.15	55.51 ±0.86
Aspergillus niger	63.17 ±1.39	59.94 ±27.56
Control (un-inoculated medium)	9.30 ±0.41	11.32 ±0.41

Values are means of triplicate determinations

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
Published by ASSCAT African Scholarly Science Communications Trust

Table 2: Quantitative determination Peroxide value (meg/kg)

s/n	Isolates	Peroxide value (meq/kg)	
		15 days	30 days
1.	*Aspergillus chevallieri*	5.02 ±0.17	20.24 ±1.11
2.	*Aspergillus tamari*	7.35 ±0.75	38.12 ±1.17
3.	*Penicillium chrysogenum*	2.16 ±0.18	10.49 ±1.41
4.	*Cephaliophora irregularis*	2.31 ±0.62	10.17 ±0.87
5.	*Syncephalastrum racemosum*	5.77 ±0.52	35.44 ±1.39
6.	*Aspergillus flavus*	4.60 ±0.87	26.52 ±1.34
7.	*Aspergillus ruber*	2.36 ±0.47	25.21 ±1.49
8.	*Aspergillus terreus*	1.42 ±0.26	6.20 ±0.58
9.	*Aspergillus niger*	2.01 ±0.01	27.88 ±1.92
	Control (un-inoculated medium)	2.18 ±0.21	5.05 ±0.18

Values are means of triplicate determinations

AJFAND ONLINE
African Journal of Food, Agriculture, Nutrition and Development
Scholarly, Peer Reviewed
Volume 12 No. 6
October 2012
ISSN 1684 5374
Published by African Scholarly Science Communications Trust

Table 3: Dry weight of mycelia of the isolates

Isolates	Dry weight at 15 days (mg/40ml)	Dry weight at 30 days (mg/40ml)
Aspergillus tamari	1.33 ± 0.66	2.54 ±0.20
Aspergillus chevallieri	0.70 ±0.34	1.52 ±0.70
Penicillium chrysogenum	0.73 ±0.13	1.43 ±0.74
Cephaliophora irregularis	0.83 ±0.29	1.34 ±0.59
Syncephalastrum racemosum	1.20 ±0.08	1.90 ±0.30
Aspergillus flavus	0.97 ±0.68	2.00 ±0.43
Aspergillus ruber	0.74 ±0.45	1.20 ± 0.02
Aspergillus terreus	0.75 ±0.17	1.28 ±0.44
Aspergillus niger	0.60 ±0.16	0.63 ± 0.34
Control(un-inoculated medium)		

Values are means of triplicate determinations

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
African Journal of Food, Agriculture, Nutrition and Development
Volume 12 No. 6
October 2012
Published by African Scholarly Science Communications Trust

Table 4: Qualitative lipolytic activity of the isolates on castor oil/nile blue sulphate medium

Isolate	Lipolytic activity
Aspergillus chevallieri.	+
Aspergillus flavus	+
Aspergillus niger	+
Aspergillus ruber	+
Aspergillus tamarii.	++
Aspergillus terreus	+
Cephaliophora irregularis	+
Penicilium chrysogenum	+
Syncephalastrum racemosum	+

+ = Lipolytic activity

++ = High lipolytic activity

REFERENCES

1. **Roetheli JC**, **Glaser LK** and **RD Brigham** *Castor:* Assessing the feasibility of U.S. Production. Workshop Summary, Plain view, Tx, Sept. 18-19, 1990. 1991

2. **Adekunle AA** and **NU Uma** Lipase activity of fourteen fungi on *Cucummeropsis manni* seeds. *Nigerian Journal of Botany*; 1996; **9**:35-40.

3. **Oso BA** The lipase activity of *Talaromyces emersoni*. *Canadian Journal of Botany*, 1978; **56**: 1840-1843.

4. **Njoku OU**, **Ononogbu IC, Nwaneri VO** and **JO Ugwuanyi** Lipase activity in Hura-crepitan seed endosperm during germination. *Nigerian Journal of Botany*, 1996; **9**: 21-26.

5. **Coursey DG** and **HOW Eggins** Microorganisms de L'alteration de huile palme pendant de stockage. *Oleagineux*, 1961; **16**:227-233

6. **Kuku FO** Fungal deterioration of Nigerian melon seeds (*Citrulus vulgaris* Schrad). Report of Nigerian Stored Product Research Institute. *Technical Report*, 1980; **7**: 63-70.

7. **Dirks BM, Bayer PD** and **WF Geddes** Some properties of fungal lipases and their significance in stored grains. *Cereal Chemistry*, 1955; **32**: 363-373

8. **Ataga AE and G Ota-Ibe** Seed-borne fungi of the wild mango (ogbono) [*Irvingia gabonensis* (Aubry-Leconte ex. Rorke) Bail] and their effects on food composition. *Nigerian Journal of Botany,* 2006; **19**: 54-60.

9. **Oso BA** Thermophilic fungi and the deterioration of Nigerian oil palm Kernels. *Economic Botany,* 1979; **33** (**1**): 58-62.

10. **International Union of Pure and Applied Chemistry (IUPAC**) *Standard Methods of Analysis of Oils and Fats and their Derivatives.* Pergamon Press, Oxford. 1979

11. **Negedu A, Dapiya SH, Wartu JR and HH Migap** Biodeterioration of Soya bean oil by mesophilic moulds. *Biological and Environmental sciences Journal for the Tropics*, 2010; **7** (3): 113 – 118.

12. **Ward HS and UL Diener** Biochemical changes in shelled peanuts caused by storage fungi. 1. Effect of *Aspergillus tamarii*, four species of *Aspergillus glaucus* group and *Penicillium citrinum*. *Phyptopathology,* 1961; **51**: 244-250.

13. **Ogundero VW** Isolation of thermophilic and thermotelorant fungi from stored groundnuts and determination of their lipolytic activity. *International Biodeterioration Bulletin,* 1981; **17**: 51-56.

14. **Negedu A, Ameh JB**, **Umoh VJ and SE Atawodi** Mycoflora associated with stored wild castor (*Ricinus communis* L.) seeds in storage. *International Journal of Pure and Applied Microbiology,* Accepted. 2011.

15. **Bhattacharya K and S Raha** Deteriorative changes of maize, groundnut and soyabean seeds by fungi in storage. *Mycopathologia,* 2004; **155**: 135-141.

16. **Bankole SA and AO Joda** Effect of lemon grass (*Cynbopogun citratus* Stapf.) Powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (C*olosynthis citrulus* L.) *African Journal of Biotechnology,* 2004; **3**: 52-59.

17. **Chakrabarti DK** A review of oilseeds with special reference to India. *International Biodeterioration,* 1987; **23**: 137- 157.

18. **Singh BK and T Prasad** Effect of seed-borne fungi on the phytochemical properties of S*esamum* seed oil. *Journal of Indian Botanical Society* 1983; **62** : 43-53.

19. **Majunder SK** Nutritional implication of recently developed techniques of storage and pest control. Retrieved from file://:/nutrional implication.htm on 2007; 13/12/2007.