Isolation of Fungi from Degraded Peanut and their Enzyme Activity

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Abstract

Eleven strains of fungi were isolated from samples collected from Dagon University. Fungi were isolated using direct plating methods on Potato Dextrose Agar (PDA), Cellulose agar and Czapek agar. The fungi were identified using the Literature of Barnet, H.L, (1969) and Davise H. Larone (1995). Eleven fungi belonging to 6 possible genuses were identified. These include *Aspergillus*, *Rhizoctonia*, *Paecilomyces*, *Coccidiodies*, *Bdelospora* and *Trichoderma*. The isolated strains exhibited potent enzymatic activity.Some of them show starch hydrolysis on wheat, rice, sticky and tapioca.

Keywords : Isolation degraded peanut fungi, their enzyme activity

Introduction

Peanut is an important leguminous agricultural plant in Myanmar. It is cultural for its seeds as a source of oil, for direct human consumption as a protein and vitamins A, B and some members of B_2 group supplement in humans and animals food (Purseglove, 1984). The byproduct, derived from the seeds after draw out of the oil could serve as an essential ingredient of poultry and animal feed; and could be made into groundnut cake popularly called 'kwulikwuli' in the Hausa language, and for manufacturing coarse boards, and cork substitute. The kernels could be eaten raw, roasted or sweetened. The oil could also be used for soap making, manufacturing of cosmetics and lubricants etc.

As reported by Nwokolo, (1996), peanuts are a good source of niacin, and thus contribute to brain health and blood flow, associated with reduced cardiovascular disease and reduce cancer risks; accelerate the growth of male and female hormones; are a source of co-enzyme along with oil fish, beef, soybean and spinach, and could course allergies. **Fungus,** plural fungi, any of about 80,000 known species of organisms of the kingdom Fungi, which consists the yeasts, rusts, smuts, mildews, molds, mushrooms, and toadstools.

Humans have been indirectly aware of fungi since the first loaf of leavened bread was baked and the first tub of grape must was turned into wine. Ancient peoples were familiar with the ravages of fungi in agriculture but attributed these diseases to the wrath of the gods..

Fungi are everywhere in very large numbers—in the soil and the air, in lakes, rivers, and seas, on and within plants and animals, in food and clothing, and in the human body. Together with bacteria, fungi are authority for breaking down organic matter and releasing carbon, oxygen, nitrogen, and phosphorus into the soil and the atmosphere. Fungi are tremendously important to human society and the planet we live on. Yet, despite their extraordinary impacts on our lives, both directly and indirectly, there is still relatively little known about them.

The Kingdom Fungi includes some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material, they continue the cycle of nutrients through ecosystems. In addition, most vascular plants could not grow without the symbiotic fungi, or mycorrhizae, that inhabit their roots and supply essential nutrients. Other fungi provide numerous drugs

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(such as penicillin and other antibiotics), foods like mushrooms, truffles and morels, and the bubbles in bread, champagne, and beer.

Peanut kernels are usually contaminated by a range of fungi. Several fungi are capable of infecting growing nuts and causing damage to hulls and kernels (Denizel *et.al.,* 2006). Contamination of nut seeds by fungi occurs during growth, harvesting and storage, where climatic conditions and agricultural and storage practices especially when nuts are store under adverse conditions of temperature and relative humidity are conducive to fungal growth and toxin production (Njobeh *et al.,* 2009 ; Bhattacharya and Raha, 2002; Bankole *et al.,* 2005; Abdel-Gawad and Zohri , 1993) . The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage in these nut seeds (Ayyasam and Baskaran, 2005). The fungi that produce mycotoxins are various species of *Aspergillus, Penicillium* and *Fusarium* but mycotoxin production is restricted to only a few species.

Aspergillus flavus are common contaminant of peanuts is a serious problem as it has adverse effects on human health (Hedayati *et al.,* 2007*;* Peraica and Domijan, 2001) Some 300 to 400 compounds are now known as mycotoxins of which approximately a dozen groups regularly receive attention as threat to human and animal health. Species of the *Aspergillus flavus*produce highly carcinogenic aflatoxins that can cause hepatomas in human (Wogan, 1966). Studies indicated that aflatoxin have been shown to increase the incidence of human liver cancer by acting synergistically with hepatitis (Mishra and Das. 2003).Hence , there nuts production and consumption are predominant .It has also been estimated that 25% of the world's crops are affected by mould or fungal growth (Bryden, 2007). The economic loss resulting from fungal contamination of nuts is difficult to estimate. They had been infected during or after harvesting, storage and transition (Bruce et al., 2003).

In the present work, fungi are screened from contaminate peanut on three selected media such as Potato Dextrose Agar (PDA), Cellulose agar and Czapek agar. The isolated eleven fungi are carried out into pure culture by using selected media. All the isolated fungi were subjected in the study of cultural and morphological characters, enzymatic activities, hydrogen sulfide tests.

Material and Methods

Collection of Sample

The samples were collected from Dagon University.

Isolation of Fungi strains from Sample

The growing fungi on contaminated peanut were photographed using camera and were isolated on plate agar media potato Dextrose Agar (PDA), Cellulose Agar and Czapek Agar. They were incubated for 3-5 days at room temperature.

Identification of Isolated Fungi

Potato Dextrose Agar and Agar media were used for fungal isolations. The plates were incubated at room temperature for 3 days. Cultural characters were assessed by eye and microscopic examination. Morphological identification of isolated fungi was made using imperfect fungi (Barnett, 1969) and medically important fungi (Larone, 1995), Starch Hydrolysis, Hydrogen Sulfide Production (Cruickshank 1968).

Detection of Antimicrobial Activity of Fungal Strains

The study of Antimicrobial activity was performed by paper well diffusion method. 20 ml of the boiled nutrient medium was poured into the flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the flasks were cooled down to 30-35°C and poured into each sterilized Petri dish and 0.001 ml of test organisms were also added into the Petri-dishes. The nine different types of test organisms were also to prepare the basal Petri-dishes and subjected in the antimicrobial activity test. Petri dishes were made 0.9 mm in diameter of well, then they were filled with 5 day old culture of fungi broth (0.02 ml) were incubated for 24 hours at room temperature. After, the size of clear zone around agar well diffusion was measured and recorded as the antimicrobial activity against nine test organisms. (Harley Prescott, 2002).

Results

Collection of sample

The samples were collected from Dagon University.

Fig. (1) The growing fungi on contaminated Peanut

Screening of Fungi

In the present work, altogether eleven isolated fungi were maintained into pure culture and designated into 1 to 11.

Culture Media	Designated Strains
Medium I	1,2,3,9,11
Medium II	4,5,10,8
Medium III	6,7

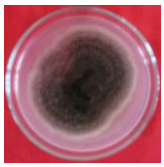
Purification of Fungi

Isolated fungi growing on selective media plates were isolated and purified. The isolated pure fungi were preserved on agar slants for further studies.

Fig. (2) The pure culture of eleven fungi isolated from Peanut

Cultural, morphological and microscopic character of isolated fungi from Peanut

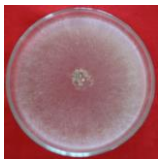
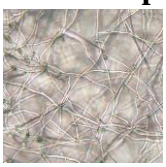
Cultural Character **Strain-1 Microscopic character**




Division - Ascomycota
Class - Eurotiomycetes
Order - Eurotiales
Family - Trichocomaceae
Genus - *Paecilomyces*

Conidiophores mostly arising from aerial hyphae; phialides in a loose verticillate group on the conidiophores; basal portion of phialide nearly cylindrical, tapering gradually; conidia produced successively (basipetally) and held together in chains, 1-celled, hyaline. The conidia are elliptical or oblong and occur in long, unbranched chains.

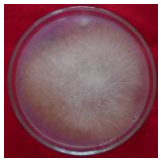
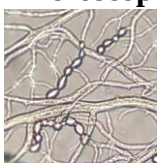
Cultural Character **Strain-2 Microscopic character**

Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales
Family - Ceratobasidiaceae
Genus - *Rhizoctonia*

Asexual fruit bodies and spores lacking; sclerotia brown on black, variable in form, frequently small and loosely formed, formed among and connected by mycelia threads; hyphae with long cells, septa of branch set off from main hypha.

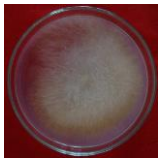
Cultural Character **Strain-3 Microscopic character**

Division - Ascomycota
Class - Euascomycetes
Order - Onygenales
Family - Onygenaceae
Genus - *Coccidioides*

Mycelium septate, branched hyphae that produce thick-walled, barrel-shaped arthroconidia that alternate with empty cells break and are characteristically present on either end of the free conidia

Cultural Character **Strain-4 Microscopic character**




Division - Zygomycota
Class - Zycomycetes
Order - Zoopagales
Family - Zoopagaceae
Genus - *Bdellospora*

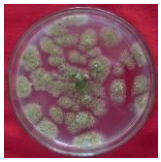
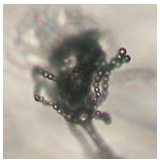
Aerial hyphae slender, non-septate; haustoria short, branched; conidia 1-celled, hyaline, elongate, catenulate.

Cultural Character **Strain-5 Microscopic character**


Division - Ascomycota
Class - Eurotiomycetes
Order - Eurotiales
Family - Trichocomaceae
Genus - *Paecilomyces*

Conidiophores mostly arising from aerial hyphae; phialides in a loose verticillate group on the conidiophores; basal portion of phialide nearly cylindrical, tapering gradually; conidia produced successively (basipetally) and held together in chains, 1-celled, hyaline. The conidia are elliptical or oblong and occur in long, unbranched chains.

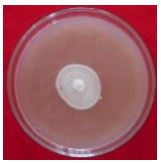
Cultural Character **Strain-6 Microscopic character**

Division - Ascomycota
Class - Ascomycetes
Order - Aspergillales
Family - Aspergillaceae
Genus - *Aspergillus*

Conidiophores upright, simple, terminating in a globose, bearing phialides at the apex or radiating from the entire surface; conidia 1-celled, globose, often variously colored in mass, catenulate, produced basipetally.

Cultural Character **Strain-7 Microscopic character**




Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Hypocreaceae
Genus - *Trichoderma*

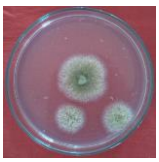
Conidiophores' hyaline, upright, much branched not verticillate; phialides single or in groups; conidia hyaline, 1-celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches or cushions of conidia.

Cultural Character **Strain-8 Microscopic character**


Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales
Family - Ceratobasidiaceae
Genus - *Rhizoctonia*

Asexual fruit bodies and spores lacking; sclerotia brown on black, variable in form, frequently small and loosely formed, formed among and connected by mycelia threads; hyphae with long cells, septa of branch set off from main hypha.

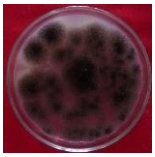
Cultural Character **Strain-9 Microscopic character**




Division - Ascomycota
Class - Ascomycetes
Order - Aspergillales
Family - Aspergillaceae
Genus - *Aspergillus*

Conidiophores upright, simple, terminating in a globose, bearing, phialides at the apex or radiating from the entire surface; conidia 1-celled, globose, often variously colored in mass, catenulate, produced basipetally.

Cultural Character **Strain-10 Microscopic character**




Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales
Family - Ceratobasidiaceae
Genus - *Rhizoctonia*

Asexual fruit bodies and spores lacking; sclerotia brown on black, variable in form, frequently small and loosely formed, formed among and connected by mycelia threads; hyphae with long cells, septa of branch set off from main hypha.

Cultural Character **Strain-11 Microscopic character**


Division - Ascomycota
Class - Ascomycetes
Order - Aspergillales
Family - Aspergillaceae
Genus - *Aspergillus*

Conidiophores upright, simple, terminating in a globose, bearing, phialides at the apex or radiating from the entire surface; conidia 1-celled, globose, often variously colored in mass, catenulate, produced basipetally.

Characteristic of Isolated Fungi

Many fungi produce enzymes called hydrolases. Hydrolyase means the splitting of organic molecules into smaller molecules in the presence of water.The starch molecule consists of two constituent amylase (an unbranched) and amylopectin. Both amylopectin and amylase are rapidly hydrolyzed by certain bacteria using their α-amylase to yield dextrin, maltose and glucose.

Table (1) Characteristic of isolated fungi from Peanut

Strains	Wheat	Tapioca	Sticky Rice	Rice	H_2S
1	++	+	+	+	+
2	+	+	+	+	-
3	+	+	+	+	-
4	+	+	+	+	-
5	++	+	+	+	-
6	++	+	+	++	-
7	+	-	-	-	-
8	++	++	++	-	-
9	+	++	++	+	++
10	+	+	-	+	+
11	+	+	+	+	-

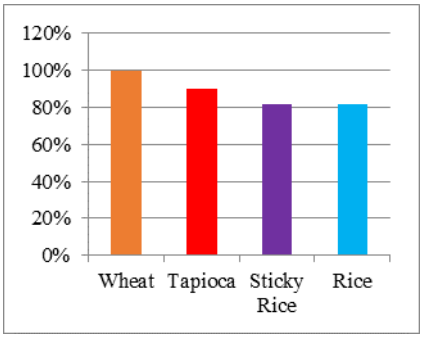


Fig. (3) Starch hydrolysis of isolated fungi from Peanut

Starch Hydrolysis of Isolated Fungi

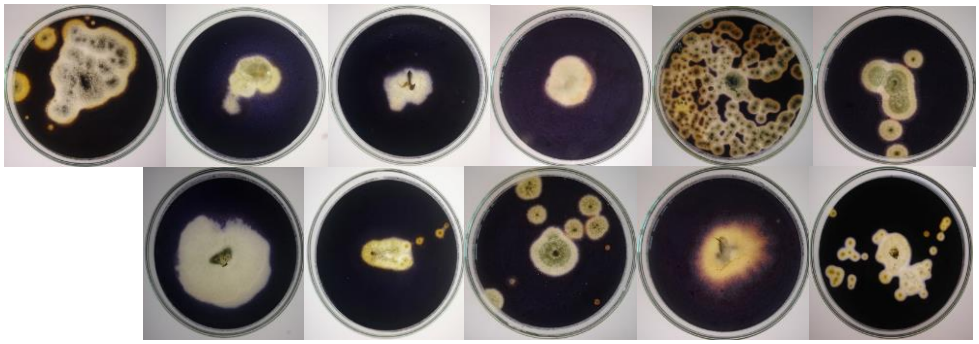


Fig. (4)Wheat hydrolysis for isolated fungi

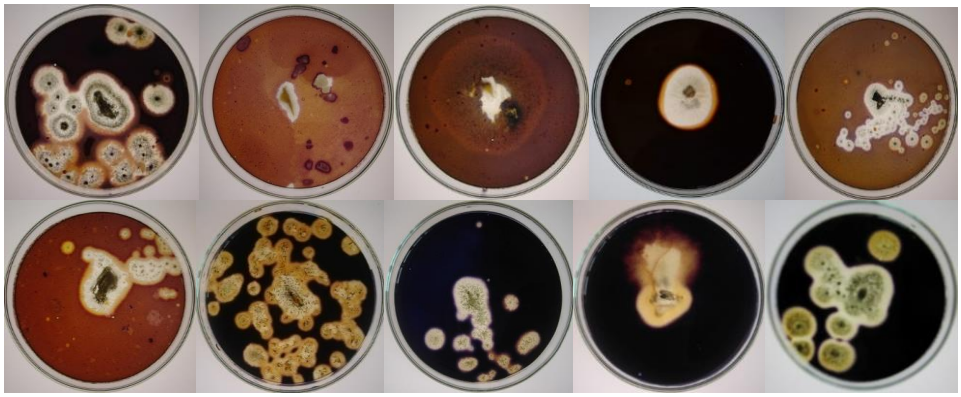


Fig.(5)Tapioca hydrolysis for isolated fungi

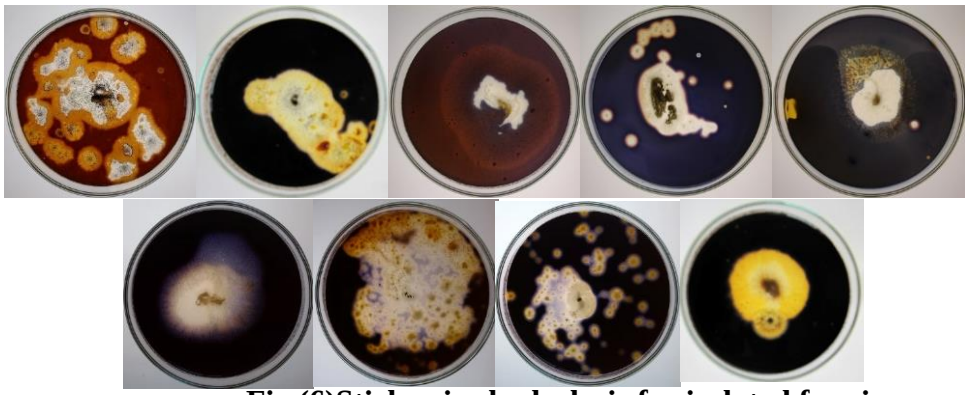


Fig.(6)Sticky rice hydrolysis for isolated fungi

Fig.(7)Rice hydrolysis for isolated fungi

Hydrolysis of Wheat shown by all isolated fungus. Hydrolysis of Tapioca demonstrates by all isolated fungus except 7.Hydrolysis of Sticky Rice illustrated by all isolated fungus except 7, 10.Condition of Rice Hydrolysis given by all isolated fungus except 7, 8.Therefore, based on the result , eleven isolated fungi are recorded to have enzymatic activity (amylase).

Hydrogen Sulfide Test

Fig.(8) Hydrogen sulphide test for isolated fungi from Peanut

Among all isolated fungi No. 1, 9 and 10 were exhibited hydrogen sulfide.

Discussion and Conclusion

In the present study include *Aspergillus, Rhizoctonia, Paecilomyces, Bdelospora* and *Trichoderma were obtained. Aspergillus* and *Penicillum* are the six possible genera common genera of fungi isolated from each samples. They can cause weight decrease, seed discoloration, heating and mustiness, and production of mycotoxins, especially aflatoxins. Butler, 1974 was reported the latter are toxic metabolic substance secreted by *A.flavus*and *A.parasiticus*and are known to be carcinogenic agent. Sultan Y and N. Magan 2010 were stated that fungi of the genera *Rhizopus, Asperigillus, Fusarium* and *Penicillum*are commonly present in peanut seed. Horn (2005) proposed that soil is a source of primary inoculums for *Aspergillusflavu s*and *A.parasiticus* that produce highly carcinogenic aflatoxins in peanuts. Aflatoxigenic fungi commonly invade peanut seeds during maturation and the highest concentrations of aflatoxins are found in damaged seed. The test a protects the highly nutritious seed contents, but it is clear from observation and experiments that at least some seeds of some species are susceptible to attack by microorganisms. Small seeds may be more vulnerable to pathogens (Crist *et al*., 1993).

In the present work, fungi are screened from contaminate peanut on three selected media such as Potato Dextrose Agar (PDA), Cellulose agar and Czapek agar. The isolated eleven fungi are carried out into pure culture by using selected media. All the isolated fungi were subjected in the study of cultural and morphological cultures, enzymatic activities and hydrogen sulfide tests were investigated.

Besides, all isolated fungi are found to possess four kinds of starch hydrolysis. Strain 1, 2, 3, 5, 6, and 9 were able to hydrolyze four different types of starch source (wheat, tapioca, sticky rice and rice) .Moreover isolated fungi 4, 8,10and 11 were revealed hydrolysis on three different types of starch source .Therefore, based on the results, eleven isolated fungi are recorded to have enzymatic activity (amylase).These strain can be applied in the manufacture of amylase enzyme.

Therefore, the prevention of contamination with toxigenic fungi of foods during harvest, processing and storage is the best way to control aflatoxin formation.

In addition, the mold growth and toxin formation may significantly be limited by packaging, removing of damaged and moldy fruit and mechanical drying prior to storage.

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