Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Special Issue 1, 2015, pp. 1919-1925
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Research Paper

ISOLATION OF FUNGI FROM STORED PULSES

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Abstract

For the present a sample of three pulses were taken, which are widely uses in India**.** In this investigation we stored pulses Urad (black gram), Arhar (red gram), Masoor (massor)) in different conditions. Three Pulses are stored in jute bag, polythene and clay jar for 3 months then isolate the fungi. After the storage of pulses 10 different types of fungi were isolated *Aspergilus niger, Aspergilus flavus, Fusarium* sp. (1), *Penicillium* sp.*, Cladosporium* sp.*, Fusarium* sp. (2), *Microsporium* sps.*, Sarcinella* sps.*.* and *Aspergilus* sp*.* Highest numbers of fungi were isolated in Black gram and Red Gram. In different condition Jute Bag show a large number of fungi and in polythene show lowest fungal infection. In Black gram highest number of fungi isolated (12). In Red Gram (11) and Massor (9). Highest fungi are isolated from Jute Bag. Enzymatic assay of isolated fungi were carried out. All *Aspergilus* sp. produce amylase, cellulase and gelatinase enzyme.
Key words: Fungi, Storage Pulses, Enzymatic assay, Food spoilage, Microbial spoilage.

INTRODUCTION

In India is one of the ancient countries in the world growing wide range of pulse crops as prime source of protein. India is leading country in pulse cultivation area contribute 25 to 27% of the world production and consumption respectively but also the largest importer of the pulses with the contribution of 34%of the global food use (FAOSTAT 2008). Everywhere in the world stored products are attacked by a number of storage enemies. Three major groups of storage enemies are fungi, insects, rats and mites. These organisms can damage a considerable part of the stored product. In many cases small improvements in storage methods may already lead to much better protection of your storage product and thus to less losses. However, good storage practices combined with good hygiene, adequate drying and all other safety measures will not always be effective in preventing storage losses there are many ways of protecting local storage products (Wageningen, 2004). Fungi are widely distributed in nature, grow over an extremely wide range of nutrients, temperature, pH, etc. and contaminate food products by many ways. They are considered a major factor in the spoilage of foodstuffs, leading to great economic loss and a major public health hazard by producing a wide variety of mycotoxins (Dwivedi *et al.,* 1984). Mycotoxigenic moulds produce mycotoxins, which are secondary metabolites frequently, produced in grain crops, cereals, pulses, dried fruits, feeds, nuts and other commodities. Although a wide variety of moulds is known to produce mycotoxins, only a few genera, Aspergillus, Penicillium, Fusarium, Alternaria and Cleviceps are considered important in foods. Mycotoxins are metabolites from moulds that are toxic to humans and domestic animals associated with food, animal feeds including wild birds and raw materials (Moss *et al.,* 2002). In

this study we stored a three pulse in three different types of storage bag. Storage pattern are also effect the spoilage of pulses.

METHODS AND MATERIALS

Three Pulses are stored in jute bag, polythene and clay jar for 3 months then isolate the fungi.

Isolation and Identification of Fungi from Pulses

Serial Dilution Method is done by Two Method Direct Shake and Crushed method of three Pulses (Black gram, Red gram & Massor). Fungal microscopic studies in the laboratory slide were prepared in different type of stain according to nature of fungus. After incubation distinct colonies were counted and identified. The cultures were identified on the basis of Macroscopic (Colonial Morphology, Color, Texture, Shape, Diameter and Appearance of colony) and Microscopic characteristics (for fungus Spore bearing fruiting body, Spore size, Growth rate of hyphae, Septation in mycelium, Presence of specific reproductive structures, Shape and structure of conidia and Presence of sterile mycelium. Lactophenol cotton blue mounting were used for staining of fungi. Pure cultures of fungi isolates were identified with the help of literature (Chupp, 1953; Ellis, 1976; Barnett *et al*., 1979; Domsch *et al.,* 1980; Ellis and Ellis 1985; Barnett & Hunter, 1999).

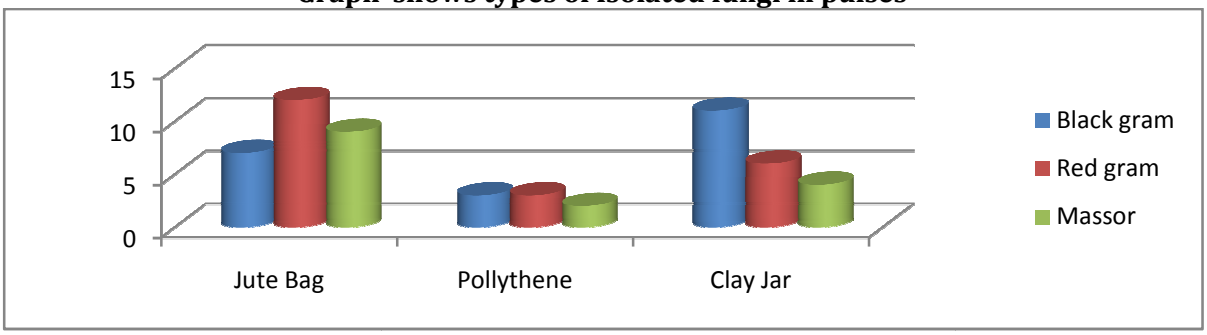
Enzyme Assay: - Enzymatic Assay (Amylase, Cellulase and Gelatinase Production) were done with the Isolated Fungi.

Amylase Production Test: - Melt the starch agar medium, cool to 45ºC and pour into sterile petri dishes. Allow it to solidify. Label each of the starch agar plate with the name of the organism to be inoculated. Using sterile technique, make a single streak inoculation of each organism into the centre of its appropriately labelled plate. Incubate the bacterial inoculated plates for 48 hours at 37ºC and fungal inoculated plates for 72-96 hours at 25ºC in an inverted position. Flood the surface of the plates with iodine solution with a dropper for 30 second. Pour off the excess iodine solution.

Cellulose Production Test: - Pour the autoclave CMC medium cooled to 45-45ºC into sterile petri plates. Allow the medium to solidify. Label the plates each with the organism to be inoculated. Inoculate the appropriately labelled plates with the respective organism. Incubate inoculated plates at 35ºC in an inverted position for 2-5 days. Flood the plates with 1% aqueous solution of hexadecyltrimethyl ammonium bromide.

Production of Gelatinase: - Melt the gelatin-agar medium, cool to 40-45ºC and pour into four sterile Petri dishes (approx. 15 ml in each) and allow in solidifying. Label each of nutrients – gelatin deep tubes and gelatin agar medium plates with the name of bacterial isolate to be inoculated. Using inoculating loop, make a stab inoculation (i.e. puncture of the agar column from top to bottom with withdrawal of the needle through the same) from each culture into its appropriately labelled deep tube of nutrient gelatin. Uninoculated deep tube should be used as a control. Make a single streak inoculation from each culture into its appropriately labelled petri plate across the surface of the medium. Incubate all the inoculated tubes, uninoculated deep tube and plates at 37ºC for 4 to 7 days. After incubation, place the tubes into a refrigerator at 4ºC for 15 minutes. Flood the incubated agar plates with mercuric chloride solution and allow the plates to stand for 5 to 10 minute.

Graph shows types of Isolated fungi in pulses



RESULTS AND DISSCUSSION

Isolation of Fungi from Pulses:- Isolation of fungi from pulses (Black gram (6), Red gram (6) & Massor (5)) shown in Table 1.

Types. of fungi isolated from Pulses- Types of fungi were isolated in Black gram (6), Red gram (6) and Massor (5) shown in Table 1(a).

Table 1 (a) Types of fungi isolated from Pulses

S.N.	Pulses	Types of Isolated fungi in pulses
1	Black gram	**6**
2	Red gram	**6**
3	Massor	**5**

Total number of fungi isolated from pulses in different condition: - Highest numbers of fungi are isolated in Black gram and Red Gram. In different condition Jute Bag show a large number of fungi and in polythene show lowest fungi infection. In Black gram highest number of fungi isolated (11). In Red Gram (12) and Massor (9). Highest fungi are isolated from Jute Bag.

Table1(b) Total No. of fungi isolated from pulses in different condition

S.N	Pulses	N0. of Isolated fungi		
		Jute Bag	Polythene	Clay Jar
1	Black gram	**7**	**3**	**11**
2	Red gram	**12**	**3**	**6**
3	Massor	**9**	**2**	**4**

2. Black Gram: - Black Gram stored in 3(Jute Bag, Polythene & Clay Jar) condition by two methods and Fungi isolated in two medium (PDA & Sabourd) are shown in Table 2 (a).

2 (a) Jute Bag: - In Black Gram containing in Jute Bag *Aspergilus niger***,** *Aspergilus flavus* (2), *Fusarium* sp. (1) and *Penicillin* sp. were found.

Table 2(a) Jute Bag

S.N	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus flavus*	*Aspergilus flavus*	-	*Aspergilus flavus*
2.	*Fusarium* sp.(1)	-	-	*Fusarium* sp.(1)
3.	*Penicillium* sp.	-	-	*Aspergilus niger*

2 (b) Polythene: - In Black Gram containing in polythene *Aspergilus flavus* (2) and *Aspergilus* niger were found.

Table 2 (b) Polythene

S.N	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus niger*	-	*Aspergilus flavus*	*Aspergilus flavus*

2 (c) Clay Jar: - In Black Gram containing in Clay Jar *Aspergilus* sp. (1)**,** *Aspergilus flavus* (2), *Fusarium* sp. (1), *Aspergilus* niger, *Penicillium* sp. and *Aspergillus ustus* were found.

Table 2 (c) Clay Jar

S.N	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus* sps.*(1)*	*Aspergilus flavus*	*Aspergilus flavus*	*Aspergilus flavus*
2.	-	*Aspergilus niger*	*Aspergilus sps..(1)*	*Fusarium sps..(1)*
3.	-	*Penicillium* sp	*Fusarium sps..(1)*	*Aspergilus ustus*
4.		-	-	*Aspergilus niger*

3.Red Gram: - Red Gram stored in 3(Jute Bag, Polythene & Clay Jar) condition by two methods and Fungi isolated in two medium (PDA & Sabourd).

3 (a) Jute Bag: - In Red Gram containing in Jute Bag *Aspergilus* sp. (1)**,** *Aspergilus ustus* , *Fusarium* sp. (2) and *Cladosporium* sp*.* were found.

Table 3 (a) Jute Bag

S.N	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus sps..(1)*	*Aspergilus sps..(1)*	*Aspergilus ustus*	*Aspergilus sps..(1)*
2.	*Cladosporium sps..*	*Cladosporium sps..*	-	*Cladosporium sps..*
3.	*Fusarium sps. (2)*	*Fusarium* sps. *(2)*	-	*Fusarium sps. (2)*
4.	-	*Aspergilus ustus*	-	*Aspergilus ustus*

3(b) Polythene: - In Red Gram containing in polythene *Aspergilus* sp. (1)**,** *Fusarium* sp. (2) and *Aspergilus* niger were found.

Table 3(b) Polythene

S.N	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus sps. (1)*	*Fusarium sps. (2)*	-	*Aspergilus niger*

3(c) Clay Jar: - In Red Gram containing in Clay Jar *Aspergilus* sp. (1)**,** *Fusarium* sp. (2), *Aspergilus* niger and *Cladosporium* sp*.* were found.

Table 3(c) Clay Jar

S.N.	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	-	-	*Aspergilus sps..(1)*	*Cladosporium sps..*
2.	-	-	*Cladosporium sps..*	*Fusarium sps. (2)*
3.	-	-	*Fusarium sps. (2)*	-
4.	-	-	*Aspergilus niger*	-

4.Massor: - Massor stored in 3(Jute Bag, Polythene & Clay Jar) condition by two methods and Fungi isolated in two medium (PDA & Sabourd).

4(a) Bora: - In Massor containing in Jute Bag *Aspergilus* sp. (1)**,** *Aspergilus ustus, Fusarium* sp. (2), *Sarcinella* sp. and *Microsporium* sp. were found.

Table 4 (a) Jute Bag

S.N.	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	-	*Fusarium sps. (2)*	*Fusarium sps. (2)*	*Fusarium sps. (2)*
2.		*Aspergilus ustus*		*Aspergilus ustus*
3.		*Microsporium sps..*		*Microsporium sps..*
4.		*Sarcinella sps..*		*Sarcinella sps..*

4(b) Polythene: - In Massor containing in polythene *Aspergilus ustus, Fusarium* sp. (2) were found.

Table 4 (b) Polythene

S.N.	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	-	*Fusarium sps. (2)*	-	*Aspergilus ustus*

4(c) Clay Jar: - In Massor containing in Clay Jar *Aspergilus* sp. (1)**,** *Fusarium* sp. (3), *Sarcinella* sp. and *Microsporium* sp. were found.

Table 4(c) Clay Jar

S.N.	Shake Method		Crushed method	
	PDA	SDA	PDA	SDA
1.	*Aspergilus sps. (1)*	-	*Fusarium sps. (3)*	*Sarcinella sps.*
				Microsporium sps.

5.Enzymatic Assay: - Enzymatic Assay of isolated fungi were carried out. All *Aspergilus* sp. produce the amylase, cellulase & gelatinase enzyme. Except Fusarium sp. (2) and *Sarcinella* sps.. all species show positive result for amylase production. Except *Fusarium* sp. (1) all sp. show positive result for cellulase enzyme production. Except *Aspergilus* sp all species show negetive result for gelatinase production.

Table 5 Show that enzymatic assay of isolated fungi.

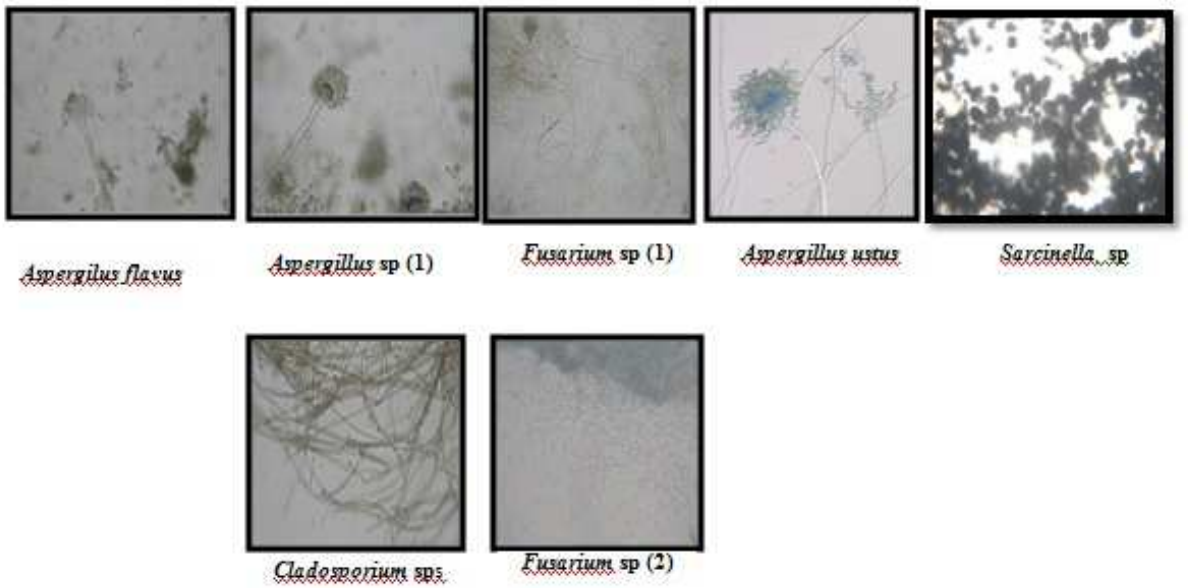
S.N.	Isolated Fungi	Amylase	Enzyme Assay	
			Cellulose	Gelatinase
1.	*Aspergilus niger*	+	+	+
2.	*Aspergilus flavus*	+	+	+
3.	*Fusarium* sp. (1)	+	-	+
4.	*Penicillium sp.*	+	+	
5.	*Aspergillus ustus*	+	+	+
6.	*Fusarium* sp. (2)	-	+	-
7.	*Cladosporium sp.*	+	+	-
8.	*Microsporium sps..*	+	+	-
9.	*Sarcinella sps..*	-	+	-
10.	*Aspergilus sp.*	+	+	+

After the storage of pulses isolates the 10 different types of Fungi are *Aspergilus niger, Aspergilus flavus, Fusarium* sp. (1), *Penicillin* sp., *Cladosporium* sp., *Fusarium* sp. (2), *Microsporium* sps., *Sarcinella* sps.. and *Aspergilus* sp. Highest numbers of fungi are isolated in Black gram and Red Gram. In different condition Jute Bag show a large number of fungi and in polythene show lowest fungi infection. In Black gram highest number of fungi isolated (12). In Red Gram (11) and Massor (9). Highest fungi are isolated from Jute Bag. Enzymatic Assay of isolated fungi is carried out. All *Aspergilus sp.* Produce the amylase, cellulase & gelatinase enzyme. Except *Fusarium* sp. (2) and *Sarcinella* sps.. all sp. show positive result for amylase production. Except *Fusarium* sp. (1) all sp. show positive result for cellulase production. Except *Aspergilus* sp all sp. show negetive result for gelatinase production.

DISSCUSSION

Most of the households surveyed used bags to store pulses often with no use of chemical insecticides **(**Diop *et. al* 1996). In the present study three pulses Black gram, Red gram and Massor are tested. The pulses are stored in different conditions (Jute Bag, Polythene, and Clay Jar). Storage of pulses in Jute bag and Clay jar are impact on the quality pulses. In storage pulses 11 sp. of fungi are isolated *Alternaria alternata, Aspergilus niger, Aspergilus flavus, Fusarium* sp. (1), *Penicillium* sp., *Cladosporium* sp., *Fusarium* sp. (2), *Microsporium* sps.., *Sarcinella* sp. and *Aspergilus sp.* In 11 fungi *Alternaria alternate* are most abundant. *Alternaria alternata*, a post–harvest pathogen of many vegetables and fruits (Thomma 2003). *Alternaria alternata* was recovered with highest percentage followed by *A. niger* and *Penicillium* sp. Similar findings of mycoflora associated with rice grains were also reported by (Taligoola *et. al* 2010; 2011) also isolated toxigenic fungi from stored rice grains in Uganda. According to Agrios (1978), the most common storage fungi are *Aspergillus* and *Penicilium* species. Seed infestation by microorganisms is a common and widespread phenomenon which has been variously reported. Amadi and Oso (1996) had reported *Aspergillus* sps., *Alternaria longissima,* species in *Vigna*

unguiculata seeds in Ibadan, Nigeria. Amadi (2002) had also reported 11 fungi including *Alternaria, Aspergillus, Fusarium,* and *Penicillium,* species in *Saccharum officinarum* seeds.



Storage fungi are usually not present in large quantities before harvest but are widely distributed and almost always present. Contamination occurs through small quantities of spores contaminating the grain as it is going into storage from the harvest in handling and storage equipment or from spores already present in storage structures (IRRI, 2006). *A. niger* was found to be the most abundant fungal species associated with all the test pulse species with 3-50% frequency of occurrence. *A. niger* is worldwide in distribution and has been isolated from numerous habitats. It is generally regarded as a strict saprophyte and has been isolated from 37 genera of plants in USA (Farr *et al.,* 1989). It is also the major spoilage isolate on bakery products (Smith *et al.,* 1988). *A. niger* can cause the rotting of numerous fruits, vegetables, and other food products, thus causing substantial economic losses due to spoilage (Sharma and Vir, 1986, Prakash and Raoof, 1989). *A. niger* is regarded as an opportunistic pathogen, it can cause otomycosis in healthy, uncompromised persons (Austwick, 1965). When inhaled, *A. niger* can cause hypersensitivity reactions such as asthma and allergic alveolitis (Edwards and Al-Zubaidy, 1977). However, only a few instances of asthma induced by *A. niger* have been reported. Keeping in view the adverse effects of *A. niger* on human health, there is also a need to use fungus free pulses for dietary purposes. *Fu.sarium caused* ear diseases of cereals (also called head blight or scab) is caused primarily by *Fusarium culmorum* and *F. grumineurum* (Wiese, 1987). Both species can produce deoxynivalenol (and related trichothecenes), zearalenone and several other biological active metabolites in the grains (Gareis et al., 1989). Whereas the fusaria will be eliminated during food processing, a significant carry-over of toxins will be possible as they are resistant to cleaning of grains, milling, baking and other cooking processes. *Alternaria* toxin*s have* been detected infrequently in grains (Andrews, 1986; Champ et al., 1991; Cheikowski and Visconti, 1992). with *Cludosporium* spp. , *Alternuria* can cause discolouration of the grains by their abundant presence on the grain, called black (sooty) heads. And other sp. also play a key role in spoilage of pulses that a impact on their quality or nutrient level of pulses. In India pulses are eat as a main dietry food so that this study show that storage of pusles in Jute bag and Clay Jar are impact on decreasing the concentration of protein, total carbohydrate and fatty acid due fungi abundant.

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