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STORED PULSES-ISOLATION OF FUNGI IN SPECIAL REFERENCE WITH GREEN, RED, BLACK GRAMS AND MASSOR

E. Rajender Reddy

Assistant Director, Dr. Braou Regional Study Centre, SRR Government Degree College, Karimnagar, Karimnagar, Telangana, India

ABSTRACT

Four pulses red gram, black gram, green gram and massor are taken as sample to study Fungi isolation. As we know these are the pulses widely uses in India. Natural method of observation the technique of preservation of pulses at the homes of farmers has observed and the same method has applied for storage of pulses. For storage jute and polythene bags and clay jars are used to observe the growth of ten types fungi which commonly grows on pulses like Aspergillosis niger, and flavus, Fusarium sp (1), Penicillicum sp, Cladosporium sp, (2), Microsporium sp, Sarcinella sp and Aspergillus sp. Majority number of fungi in green and black gram. The different techniques of pulses preservation has shown a clear indication depend on the method of preservation. Enzymatic assay of isolated fungi are carried out. All Aspergillus sp. produce amylase, cellulase and gelatinase enzyme.

KEYWORDS: *Fungi, Storage, Pulses, Enzymatic Assay, Method, Isolation*

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INTRODUCTION

A wide range of pulses grow throughout India which are the richest sources of protein. Our country is the leading country in its cultivation with an area of 26 to 27 percent of the world's pulses production and as well as the biggest consumption and a largest importer of it with 34.67 percent of the global food usage (FAOSTAT 2008). Naturally there are several enemies to storage food throughout the world. It is also in the case of the pulses too. We can come to a conclusion on it that is there are threats while storage like fungi, insects, rats and mites. These can damage a considerable source of the stored food which results in failing germination of the seed or unable to use it as dietary one. In several cases a very few improvements in storage methods have already lead good results and optimise the loss or reduce the spoilage, but good preservative techniques mixing with good hygiene, adequate drying and all the safety measures may not effective in storage losses. There are several ways of protecting local storage products (Wageningen 2004).

Fungi are extensively distributed in the nature, grow everywhere of the majority of the nutrients, temperature, pH, etc. And it communicates in several other ways to the food products. A great economic loss and spoilage of public health is due to fungi in food products because of mycotoxins (Dwivedi et al., 1984). These are produced by mycotoxigenic moulds. These are secondary metabolites commonly produced in grain crops, cereals, pulses, dried fruits, feeds, and nuts. Several variety of moulds produce mycotoxins, only a few like Aspergillus, Penicillicum, Fusarium, Alternaria and Cleviceps are

considered prominent in foods. Mould's metabolites are mycotoxins. These are toxic to human and domestic animals which are associated with food, animal feeds including wild birds and raw materials (Moss et al., 2002).

RESEARCH METHOD

For this study a case study has take by visiting the farmers' houses who store pulses by applying different techniques with natural methods amalgamation. The select four pulses are stored in three different types of storage bags, in some of the cases storage pattern also affect preservation of pulses. These four pulses are stored in jute, polythene and clay jar for a period of three months then isolate fungi.

Isolation and Identification of Fungi from Pulses

A method of Serial Dilution Method is happen by two method direct shake and crushed method of four pulses (Green gram, Black gram, Red gram and Massor). The study of fungal microscopic in the laboratory slides are prepared in different types of staining according to the fungi's nature. After incubation specific colonies are counted by identifying. The cultures are identified with the help of a macroscopic (Colonial Morphology, Texture, Colour, Shape, Diameter and colony appearance) and microscopic characters 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 are used for straining fungi. Pure cultures of fungi isolates are identified with the help of literature (Chupp, 1953, Barnett and Hunter, 1999).

ENZYME ASSAY

Amylase, Cellulase and Gelatinase production Enzymatic Assay are done with the isolated fungi.

Amylase Production Test

Melt the starch agar medium, cool to 104^0 F and pour into Petri dishes of sterile and allow it to solidify. Label each of the starch agar plate with me of inoculated organism. Using sterile technique and make a single streak inoculation of each organism into its centre to the labelled plate. Incubate the bacterial inoculated plates for two days (48 Hours) at 98.6^0 F and fungal inoculated plates for three to four days at 77^0 F in inverted position. Clean the surface of the plates with iodine solution with a dropper for half minute exactly, and off the excess iodine solution.

Test of Cellulose Production

Culture Medium Colony on autoclave cooled at plus or minus of 104^0 F into sterile Petri plates, then allow the medium to solidify. Label the plates all with the organism to inoculate appropriately labelled plates with the respective organism. Incubate inoculated plates at 95^0 F in inverted position for 48 to 120 hours. Pour on the plates with one percent aqueous solution of hexadecyltrimethyl ammonium bromide.

Gelatinase Production

Melt the gelatin-agar medium, cool to 104^0 F to 113^0 F, pour into four sterile Petri dishes (15 Ml) and allow in solidifying. Label all the nutrients gelatin deep tubes and gelatin agar medium plates with the name bacterial isolate to inoculate. Using the loop of inoculation make a stab inoculation fr tubes of each culture into its appropriate labelled deep tube of nutrient gelatin. A deep uninoculated tube is used as a control. Make a single streak inoculation from each culture into appropriate labelled Petri plate across the surface of the medium. Incubate all the inoculated, uninoculated deep tube and plates at 98.6^0

F for four to seven days. After incubation, place the tubes into a refrigerator at 39.2^0 F for fifteen minutes. Pour on incubated agar plates with mercuric chloride solution and allow the plates to stand five to ten minutes.

Table 1: Isolated Fungi in Pulses

S. No/Particular	Red Gram	Green Gram	Black Gram	Massor
1. Jute Bag	10	5	6	7
2. Polythene Bag	3	4	3	2
3. Clay Jar	5	8	9	4

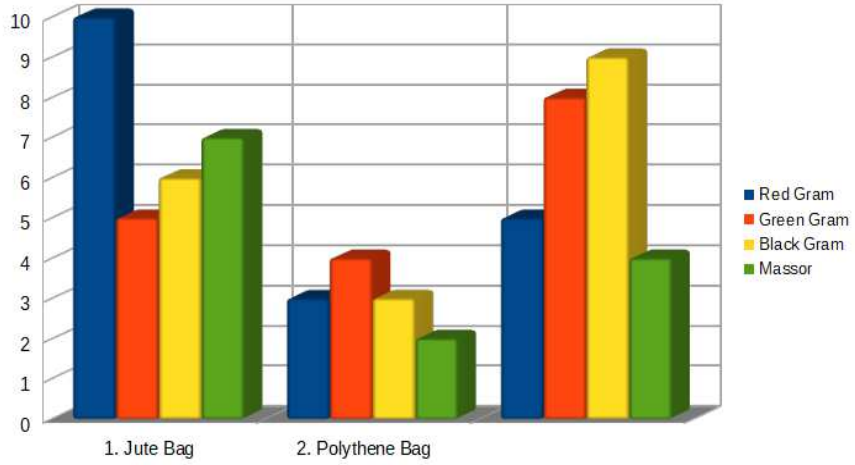

Chart 1: Types of Isolated Fungi in Pulses.

Data Analysis

- **Fungi Isolation from Pulses:** Fungi isolation from pulses Black gram (9), Green gram (8), Red gram (10), and Massor (6) has shown in table 1
- **Types of Fungi Isolated from Pulses:** Types of fungi are isolated in Black gram (9), Green gram (8), Red gram (10), and Massor (5)

Table 2: Types of Fungi Isolated in Pulses

S. No.	Pulses	Types of Fungi Isolated in Pulses
1.	Green Gram	10
2.	Black Gram	9
3.	Red Gram	9
4.	Massor	6

- **Total number of fungi isolated from pulses in different condition**: Majority of fungi are isolated in black gram and green gram. In different condition jute bag show a large number of fungi and in polythene show a lowest fungi infection. In black, green and red grams and massor ( ), ( ) ( ) and ( ). a very high considerable amount of fungi are isolated from jute bag.

Table 3: Total Number of Fungi Isolated from Pulses in Different Condition

S. No.	Pulses	No. of Isolated Fungi		
		Jute Bag	Polythene Bag	Clay Jar
1	Green Gram	11	4	10
2	Black Gram	10	3	9
3	Red Gram	10	4	10
4	Massor	11	3	8

Black Gram: Black Gram has stored in three conditions by two methods and fungi isolated in two medium (PDA and Sabourd) are shown in table 5.

Jute bag: Jute bag *Aspergillus niger, Aspergillus flavus (2), Fusarium sp. (1)* and *Penicillicum sp. a*re found.

Table 4

S. No.	Shake Method		Crush Method	
	PDA	SDA	PDA	SDA
1.	Aspergillus flavus	Aspergillus flavus	-	Aspergillus flavus
2	Fusarium sp.	-	-	Fusarium sp.
3.	Penicillium sp.	-	-	Penicillium sp.

Table 5: Jute Bag

S. No.	Shake Method		Crush Method	
	PDA	SDA	PDA	SDA
1.	Aspergillus flavus	Aspergillus flavus	-	Aspergillus flavus
2	Fusarium sp.	-	-	Fusarium sp.
3.	Penicillium sp.	-	-	Penicillium sp.

Polythene 6: *Aspergillus flavus, Aspergillus niger are found* in clay jar containing black gram and green gram.

Table 6: Polythene

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus flavus*		*Aspergillus flavus*	*Aspergillus flavus*

Clay Jar: *Aspergillus sp., Aspergillus flavus, Fusarium sp., Aspergillus niger, Pencillium sp.* and *Aspergillus ustus are found* in Clay containing black gram.

Table 7: Clay Jar

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
2	-	*Aspergillus niger*	*Aspergillus niger*	*Fusarium sp.*
3.	-	*Penicillium sp.*	*Fusarium sp.*	*Aspergillus ustus*
4.	-	-	-	*Aspergillus flavus*

Clay Jar: *Aspergillus sp., Aspergillus flavus, Fusarium sp., Aspergillus niger, Pencillium sp.* and *Aspergillus ustus are found* in Clay containing green gram.

Table 8: Clay Jar Green Gram

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
2	-	*Aspergillus niger*	*Aspergillus niger*	*Fusarium sp.*
3.	-	*Penicillium sp.*	*Fusarium sp.*	*Aspergillus ustus*
4.	-	-	-	*Aspergillus niger*

Red Gram: Red gram stored in three conditions by two methods and fungi isolated in two mediums of PDA and Sabourd.

Jute Bag: Red gram containing in jute bag *Aspergillus sp., Aspergillus ustus, Fusarium sp. and Cladosporium sp.*

Table 9: Jute Bag

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus sp.*	*Aspergillus sp.*	*Aspergillus ustus*	*Aspergillus sp.*
2	*Cladosporium sp.*	*Aspergillus niger*	-	*Cladosporium sp.*
3.	*Fusarium sp.*	*Fusarium sp.*	-	*Fusarium sp.*
4.	-	*Aspergillus ustus*	-	*Aspergillus ustus*

Polythene: The polythene bag containing red gram has *Aspergillus sp., Fusarium sp.* and *Aspergillus niger are found.*

Table 10: Polythene

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus sp.*	*Fusarium sp.*	-	*Aspergillus niger*

Clay Jar: The red gram in clay jar containing *Aspergillus sp, Fusarium sp. Aspergillus niger* and *Cladosporium sp* are found.

Table 11: Clay Jar

S. No.	Shake Method		Crushe Method	
	PDA	SDA	PDA	SDA
1.	-	-	*Aspergillus sp.*	*Cladosporium sp.*
2	-	-	*Cladosporium sp*	*Fusarium sp.*
3.	-	-	*Fusarium sp.*	-
4.	-	-	*Aspergillus ustus*	-

Massor: Massor stored in three conditions by two methods and fungi isolated in two medium of PDA and Sabourd.

Jute Bag: Massor in jute bag contains *Aspergillus sp., Fusarium sp. Sarcinella sp.* and *Microsporium sp.* are found.

Table 12: Jute Bag

S. No.	Shake Method		Crush Method	
	PDA	SDA	PDA	SDA
1.	-	*Fusarium sp.*	*Fusarium sp.*	*Fusarium sp.*
2	-	*Aspergillus ustus*	-	*Aspergillus ustus*
3.	-	*Microsporium sp*	-	*Microsporium sp*
4.	-	*Sarcinella sp.*	-	*Sarcinella sp.*

Polythene: In Massor containing in polythene *Aspergillus ustus, Fusarium sp. are found.*

Table 13: Polythene Bag

S. No.	Shake Method		Crush Method	
	PDA	SDA	PDA	SDA
1.	-	*Fusarium sp.*	*Fusarium sp.*	*Fusarium sp.*

Clay Jar: Massor which is in clay jar contained *Aspergillus sp., Fusarium sp., Sarcinella sp.,* and *Microsporium sp.* are found.

Table 14: Clay Jar

S. No	Shake Method		Crush Method	
	PDA	SDA	PDA	SDA
1.	*Aspergillus sp*	-	*Fusarium sp*	*Sarcinella sp*
2.				*Microsporium sp*

ENZYMATIC ASSAY

Isolated Fungi Enzymatic Assay is carried out. All *Aspergillus sp.* produce the amylase, cellulase and gelatinase enzymes. Except *Fusarium sp.* and *Sarcinella sp.*, all show positive result with amylase production. Except *Fusarium sp.* and *Sarcinella sp.*, all show a positive result with amylase production. Except *Fusarium sp.* all other species show positive result for cellulase enzyme production. All species show negative results Other than *Asparigius sp.* with gelatinase production.

Table 15: Enzymatic Assay on Isolated Fungi

S. No	Isolate Fungi	Amylase	Enzyme Assay	
			Cellulose	Gelatinase
1.	*Aspergillus niger*	+	+	+
2.	*Aspergillus flavus*	+	+	+
3.	*Fusarium sp*	+	-	+
4.	*Penicillum sp*	+	+	+
5.	*Aspergillus ustus*	+	+	+
6.	*Cladosporium sp*	+	+	-
7.	*Microsporium sp*	+	+	-
8.	*Sarcinella sp*	-	+	-
9.	*Aspergillus sp*	+	+	+

Ten different types of Fungi are found in stored pulses like the species of *Aspergillus, Fusarium, Penicillium, Cladosporium, Microsporium* and *Sarcinella*. Most of the fungi from Black, Green and Red Grams are isolated in different stored conditions. It is mostly eliminated in polythene and its rate more in jute bag when we compare each other.

Most of the farmers throughout India are using bags to store the pulses with using any chemical compounds for the storage (Diope, 1996). The study has carried on four pulses which are stored in different conditions. It is found in the observation of the study that the Jute bag and Clay jar has impact on the storage quality of the pulses. In most of the cases it is found post harvest there is a threat to the storing crop in the form of fungi, termites, rats, etc. here these techniques helped to know how to protect pulses from taxinonenic fungi. The case has revealed better to clay jars and jute bags polythene bags are most efficient in isolation of fungi in pulses. Spoilage is the common incident in pulses by fungi but with available techniques we can protect it maximum.

Before harvest fungi threat is very least, but in majority of the storage food it has disastrous effect on not only seed but also on food and health of living beings which consume it. We know very well how 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).

Aspergillus niger is commonly associated with all the pulses with a frequency of 3-48 % since its distribution of the world wide it is a commonly found one all over the world in the pulses. *Alternaria* toxins *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 spp. also play a key role in spoilage of pulses that show a clear impact on their quality or nutrient level of pulses. In India pulses are eat as a main dietary food so that this study show that storage of pulses 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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