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Recovery Rates of Moulds in Stored Products Cultivated on Three Mycological Media.

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Abstract

The choice of a culture media is important for a mycological analysis of fresh and stored food products like rice, maize, wheat, groundnut and beans. This will guarantee the reliability of the analysis. The medium should allow an excellent recovery of mould species present in the stored food and also avoid bacterial development. The efficacy of three media for moulds isolation and identification (sabouraud dextrose agar, potato dextrose agar and malt extract agar) were compared for analysis of fresh and stored food products. 2 genera were isolated and identified and they were *Aspergillus* and *Penicillium.* . The genus *Aspergillus* presented the highest frequency in Percentage occurrence among the media. Potato dextrose agar was the medium that presented the best results considering both the quantity and variety of isolated moulds. Comparing the results obtained in different media, it was observed that the mould recovered can vary according to the selected culture medium.

Keywords: Stored food products, Fresh food products, culture media, *Aspergillus* spp., moulds.

Introduction

In Africa, cereals, Rice (*Oryza sativa*), Maize *(Zea mays*), Wheat (*Triticum aestivum*) and legumes Groundnut (*Arachis hypogaea*), Cowpea (*Vigna sinensis*) are important food crops for the teeming population. Cereals and legumes provide cheap sources of energy and protein [1]. The abundant nutrient and low water activity make the seeds of these crops susceptible to moulds attack and deterioration [2], resulting in huge economic loss, poverty and hunger.

The cultivation of moulds on different mycological media has helped in their rapid characterization and identification. Several culture mycological media for the isolation and characterization of moulds had been documented [3]. Solid culture media are usually more useful, as they allow the mould to sporulate more easily [4]. These media may differ in their water activity, pH, nutrient content or composition. Moulds differ in their growth requirements. Therefore, no single medium is suitable for each and every mould. It is therefore important to select mould sampling agar media wisely. In general, culture media for mould

evaluation need to be highly selective, suppressing the fast growing bacterial contamination [5]. Traditionally, potato dextrose agar has been used for a general quantification of fungi. However, this medium does not present an adequate nutritive source and can inhibit the recovering of damaged cells due to its low pH (3.5) [6].The objectives are:

- To determine the colony forming units of fungal isolates on different media of fresh products and stored products.
- To determine the percentage occurrence of moulds in the fresh and stored food products.
- To determine the efficacy of three culture media for the recovery of moulds from fresh and stored food products.

Materials and Methods

Sample collection.

Whole grains/fine powder of rice, maize, wheat, groundnut and beans were stored in four different materials, such as, sack, polyethene (cellophane), plastic and metal containers. The storage lasted for two to four months. Two hundred and ten (210) samples were randomly collected following the method of [7]. Thirty grams (30g) of sample was weighed and packaged in the different materials, labelled and kept at room temperature prior to mycological assay.

Recovery Media.

Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MAE) were used for mould enumeration and identification [8]. An antibacterial agent chloramphenicol 50mg/l was used to inhibit the growth of bacteria. 0.1ml of lactic acid was added to prevent the growth of yeast [9].

Isolation of fungi.

Standard dilution and streaking technique methods were used. The samples were serially diluted up to 10^{-3} and 10^{-5}.One-tenth (0.1ml)of the dilutions were inoculated onto freshly prepared surface dried medium (PDA, SDA and MEA), streaked to obtain uniform and countable colonies. The plates were incubated at ambient temperature (28 ± 2°C) for 7 days and examined daily for fungal growth. Colonies were counted with a colony counter and expressed in colony forming unit per gram (CFU/g).Fungal colonies grown on the media were subculture on various media [10].

Enumeration of mould species.

The percentage occurrence of each isolate was done using this formula [11].

$$\% \text{ occurrence of species} = \frac{\text{Number of colonies of species}}{\text{Total number of colonies}} \times 100$$

Morphological and Microscopic identification.

The fungal isolates were transferred to sterilized plates for purification and identification. The isolated moulds were identified colonially by features such as spores, hyphae production and pigmentation. Arrangement of spores/conidia and septate was used to characterize the moulds microscopically. The grown fungi were mounted on a slide, stained with lacto phenol-cotton blue, covered with a cover slip and examined at low magnification to detect fungal structures and spore characteristics [8]. The identities of the isolates were confirmed with reference to standard manual [8].

Results and Discussion

Evaluating the isolates.

Table 1, shows the cultural and microscopic characteristics of identified isolates. A total of nine isolates were identified using their macroscopic and microscopic characteristics. They are as follows: *Aspergillus* sp. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium chrysogenum*, *Fusarium* sp, *Rhizopus stolonifer*, *Rhizopus nigricans*, *Mucor* sp.The colony forming units of isolates are shown in tables 2 to table 3 for fresh and stored samples.

The colony forming units of isolates are shown in tables (2,3,4,5,6,7,9,8,9,10 and 11) for fresh and stored samples. The number of colony forming units were more in Potato dextrose agar, sabouround dextrose agar and least in malt extract agar.

Results obtained in Table 12 showed that Fresh samples of Rice sample had *Aspergillus* sp as the highest occurring isolate followed by *Penicillium chrysogenum* and *Mucor* sp. Maize had *Aspergillus* sp followed by *Penicillium chrysogenum*,

Table 1: cultural and microscopic characteristics of identified isolates.

Cultural	Microscopic	Isolate
Colonies are usually fast growing, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores	Hyphae are septate , an unbranched conidiophore arises from a specialized foot cell. Vesicles are completely or partially covered with flask-shaped phialides.	*Aspergillus* sp
Colonies are typically plain green in colour or yellow-green becoming green with age.	Hyphae are septate and hyaline. Conidal head are short columneredbliseriate.	*Aspergillus flavus*
Blackish brown often with yellow mycelium moderately rapid growth rate.	Septated hyphae, long smoth and colourless conidiophores biseriatephalides, globose conidial head and presence of dark spores from the conidia head.	*Aspergillus niger*
Colonies are fast growing form pinkish to purple, irregular,pebblelikesclerotia.conidiophores appear as a powdery mass	smooth or finely roughened phialides are arranged on the conidial heads in a biseriate fashion.	*Aspergillusochraceus*
Blue green with a yellowish pigment, colonies fast growing in shades of green.	Septate hyphae branched conidiophores with phialides.	*Penicillium chrysogenum*
colonies are usually fast growing, pale or bright-coloured (depending on the species) with or without a cottony aerial mycelium	filaments are hyaline, septate, and 3–8 μm in diameter. They typically branch at acute and at right angles. They produce of both macro- and microconidia from slender phialides.	*Fusarium* sp
Colonies have very rapid growth, texture deeplycottony, white becoming gray- brown on surface pale white reverse	Hyphae broad, not or scarcely septate; rhizoids and stolons present; sporangiophores brown.	*Rhizopus stolonifer*
Filamentous, branching hyphae that generally lack cross-walls	branching mycelia composed of three types of hyphae: stolons, rhizoids, and usually unbranching sporangiophores. The black sporangia at the tips of the sporangiophores are rounded and produce numerous nonmotile multinucleate spores.	*Rhizopu snigricans*
Hyphae of Mucor is filamentous, aseptate or coenocytic.he colony of Mucor shows rapid growth. The colour of the colony is usually white to grey and turns to brown when the culture becomes old.	Hypha: Coenocytic and branched Spores: Generally black in colour but can vary with different species. The spores can be motile or non-motile and can exist in variable shapes.	*Mucor* sp

Table 2: Showing colony forming units of fungal isolates on different media of fresh products (Rice).

Sample code	SDA	PDA	MEA
RSG	$2.0x10^4$	NG	$3.0x10^3$
RCG	$3.0x\ 10^4$	NG	NG
RPG	$2.0x\ 10^4$	NG	NG
PMG	NG	NG	NG
RSP	NG	$2.0x10^3$	NG
RCP	NG	$2.0x10^3$	$2.0X10^3$
RPP	NG	$4.0x10^3$	NG
RMP	$2.0X\ 10^3$	$2.0x10^3$	$4.0X10^3$

Key :-
RSG - rice sac grain, RCG- rice cellophone grain, RPG - rice plastic grain, RMG- rice metal grain, RSP - rice sac powder, RCP - rice cellophone powder, RPP - rice plastic powder, RMP - rice metal powder, NG- no growth

Table 3: Showing colony forming units of fungal isolates on different media of fresh products (Maize).

Sample code	SDA	PDA	MEA
MSG	$3.0x10^4$	$7.0\ X\ 10^3$	$3.0x10^3$
MCG	$2.0x\ 10^4$	$3.0X10^3$	$3.0x10^3$
MPG	NG	NG	NG
MMG	$3.0\ x\ 10^3$	$4.0X10^3$	$4.0\ \ 10^4$
MSP	$2.0\ x\ 10^3$	NG	$1.0X10^4$
MCP	$2.0\ x\ 10^3$	NG	NG
MPP	NG	NG	NG
MMP	NG	$4.0x10^3$	NG

Key :-
MSG - maize sac grain, MCG- rice cellophone grain, MPG - maize plastic grain, MMG- maize metal grain
MSP - maize sac powder, MCP - maize cellophone powder, MPP - maize plastic powder, MMP - maize metal powder. NG- no growth

Table 4: Showing of colony forming units of fungal isolates on different media of fresh products (Wheat).

Sample code	SDA	PDA	MEA
WSG	$3.0x10^3$	$3.0\ X\ 10^3$	$3.0x10^3$
WCG	NG	$2.0X10^3$	$1.0x10^3$
WPG	NG	NG	$1.0\ X10^3$
WMG	$3.0\ x\ 10^3$	$3.0\ X10^5$	$1.0X10^3$
WSP	NG	NG	NG
WCP	NG	NG	NG
WPP	NG	NG	NG
WMP	$2.0X\ 10^3$	$1.0x10^3$	$2.0\ X10^3$

Key :-
WSG - wheat sac grain, WCG- wheat cellophone grain, WPG - wheat plastic grain, WMG- wheat metal grain
WSP - wheat sac powder, WCP - wheat cellophone powder, WPP - wheat plastic powder, WMP - wheat metal powder, NG- no growth

Table 5: Showing of colony forming units of fungal isolates on different media of fresh products (Groundnut).

Sample code	SDA	PDA	MEA
GSG	NG	6.0 X 10^3	NG
GCG	4.0x 10^3	2.0X10^3	NG
GPG	4.0x10^3	2.0X10^3	1.0 X10^3
GMG	NG	1.0 X10^5	4.0X10^3
GSP	2.0X 10^3	NG	NG
GCP	2.0X 10^3	7.0 X10^3	NG
GPP	1.0X 10^3	NG	4.0X 10^3
GMP	1.0X 10^3	2.0x10^3	3.0 X10^3

Key :-
GSG - groundnut sac grain, GCG- groundnut cellophone grain, GPG - groundnut plastic grain, GMG- groundnut metal grain, GSP - groundnut sac powder, GCP - groundnut cellophone powder, GPP - groundnut plastic powder, GMP - groundnut metal powder, NG- no growth

Table 6: Showing of colony forming units of fungal isolates on different media of fresh products (Beans).

Sample code	SDA	PDA	MEA
BSG	5.0x 10^3	1.0 X 10^3	NG
BCG	NG	4.0X10^3	5.0 X10^3
BPG	NG	NG	NG
BMG	NG	NG	NG
BSP	NG	1.0 X10^3	NG
BCP	5.0 x103	3.0 X10^3	5.0X 10^3
BPP	NG	3.0 X10^3	NG
BMP	NG	3.0x10^3	NG

Key :-
BSG - Beans sac grain, BCG- Beans cellophone grain, BPG - Beans plastic grain, BMG Beans metal grain BSP - Beans sac powder, BCP - Beans cellophone powder, BPP - Beans plastic powder, BMP - Beans metal powder, NG- no growth

Table 7: Showing colony forming units of fungal isolates on different media of stored products (Rice).

Sample code	SDA	PDA	MEA
RSG	4.0x10^5	1.0 X10^5	4.0x10^3
RCG	NG	2.0 X10^5	3.0 X10^5
RPG	3.0x 10^5	3.0 X 10^5	NG
PMG	NG	NG	2.0 X10^5
RSP	NG	4.0x10^5	NG
RCP	NG	1.0x10^5	1.0X10^3
RPP	2.0 X 10^6	3.0x10^5	NG
RMP	3.0X 10^5	1.0x10^5	NG

Key :-
RSG - rice sac grain, RCG- ricecellophone grain, RPG - rice plastic grain, RMG- rice metal grain, RSP - rice sac powder, RCP - rice cellophone powder, RPP - rice plastic powder, RMP - rice metal powder,NG- no growth

Table 8: Showing colony forming units of fungal isolates on different media of stored products (Maize).

Sample code	SDA	PDA	MEA
MSG	$1.0x10^5$	$4.0 X 10^5$	$3.0x10^5$
MCG	$1.0x 10^5$	$2.0X10^6$	$3.0x10^5$
MPG	$1.0 X 10^5$	$1.0 X10^5$	$3.0 X10^5$
MMG	$4.0 x 10^5$	$4.0X10^5$	$2.0 X 10^5$
MSP	$1.0 x 10^5$	$4.0 X10^5$	NG
MCP	$2.0 x 10^5$	$4.0 X10^5$	$1.0 X10^5$
MPP	$5.0 X 10^5$	$3.0 X10^5$	$2.0 X10^5$
MMP	$4.0 X10^5$	$3.0x10^5$	$2.0 X10^5$

Key :-
MSG - maize sac grain, MCG- ricecellophone grain, MPG - maize plastic grain, MMG- maize metal grain MSP - maize sac powder, MCP - maize cellophone powder, MPP - maize plastic powder, MMP - maize metal powder,NG- no growth

Table 9:Showing of colony forming units of fungal isolates on different media of stored products (Wheat).

Sample code	SDA	PDA	MEA
WSG	$3.0x10^5$	NG	NG
WCG	$1.0 x10^5$	$1.0X10^5$	$3.0x10^5$
WPG	$1.0 x10^5$	$3.0 X10^5$	$2.0x10^5$
WMG	$1.0 x 10^3$	$3.0 X10^5$	$3.0 X10^5$
WSP	NG	$3.0 X10^5$	NG
WCP	NG	NG	NG
WPP	NG	$1.0 X10^5$	NG
WMP	$4.0X 10^5$	$2.0x10^5$	$2.0 X10^5$

Key :-
WSG - wheat sac grain, WCG- wheat cellophone grain, WPG - wheat plastic grain, WMG- wheat metal grain WSP - wheat sac powder, WCP - wheat cellophone powder, WPP - wheat plastic powder, WMP - wheat metal powder, NG- no growth

Table 10: Showing of colony forming units of fungal isolates on different media of stored products (Groundnut).

Sample code	SDA	PDA	MEA
GSG	2.0 x105	$3.0 X 10^5$	$1.0 X10^5$
GCG	$3.0x 10^5$	$2.0X10^5$	$3.0 X10^5$
GPG	$3.0x 10^5$	$3.0X10^5$	$3.0 X10^5$
GMG	3.0X 105	$1.0 x10^5$	$3.0X10^5$
GSP	$2.0X 10^5$	$3.0 X10^5$	$2.0 X10^5$
GCP	$4.0X 10^5$	$7.0 X10^5$	$2.0 X10^5$
GPP	$3.0X 10^5$	$3.0 X10^5$	$3.0X 10^5$
GMP	$4.0X 10^5$	$6.0x10^5$	$3.0 X10^5$

Key :-
GSG - groundnut sac grain, GCG- groundnut cellophone grain, GPG - groundnut plastic grain, GMG- groundnut metal grain, GSP - groundnut sac powder, GCP - groundnut cellophone powder, GPP - groundnut plastic powder, GMP - groundnut metal powder, NG- no growth

Table 11: Showing of colony forming units of fungal isolates on different media of stored products (Beans).

Sample code	SDA	PDA	MEA
BSG	NG	1.0 X 10^3	NG
BCG	NG	4.0X10^3	4.0 X10^3
BPG	4.0 X 105	NG	NG
BMG	3.0 X105	NG	NG
BSP	NG	1.0 X10^3	NG
BCP	4.0 x10	3.0 X10^3	4.0X 10^3
BPP	NG	3.0 X10^3	NG
BMP	NG	3.0x10^3	NG

Key :-
BSG - Beans sac grain, BCG- Beans cellophone grain, BPG - Beans plastic grain, BMG Beans metal grain BSP - Beans sac powder, BCP - Beans cellophone powder, BPP - Beans plastic powder, BMP - Beans metal powder, NG- no growth

Aspergillus niger, *Mucor* sp, *Aspergillus flavus*, *Aspergillus ochraceus* and *Rhizopus stolonifer*. Wheat had *Aspergillus* sp, *Rhizopus stolonifer and Aspergillus flavus*

Groundnut had *Rhizopus stolonifer*, *Mucor*sp, *Aspergillus flavus*, *Aspergillus sp.* and *Penicillium chrysogenum*. This was also reported by [12]. Beans had *Aspergillus* sp. followed by *Penicillium chrysogenum*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Mucor* sp (Table 12) .

Stored samples analysis, Table 13,showed that Rice had *Aspergillus* sp as the highest occurring isolate followed by *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Aspergillus flavus and Rhizopus nigricans*.. Maize had *Rhizopus stolonifer*as the highest occurring isolate followed by *Penicillium chrysogenum Aspergillus* sp, *Aspergillus flavus*, *Mucor* sp. and *Fusarium* sp. Wheat had *Penicillium chrysogenum*as the highest occurring isolate followed by*Rhizopus stolonifer Aspergillus* sp, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus* and *Rhizopus nigricans*. Groundnut had *Penicillium chrysogenum*, as the highest occurring isolate followed by *Aspergillus* sp, *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus ochraceus* and *Rhizopus nigricans* Beans had *Aspergillus* sp as the highest occurring isolate followed by *Penicillium chrysogenum Aspergillus flavus*, *Aspergillus niger*and *Rhizopus nigricans*. Stored samples recovered more isolates (73%) than fresh samples (27%), from Table (14).

The overall best organism was from the genus *Aspergillus* followed by *Penicillium chrysogenum* this was also reported by [12].All the moulds reported in this research have already been isolated in foods as reported by [13].

The genus *Aspergillus* was the most prevalent. The majority of *Aspergillus* species in this study are potential producer of toxic metabolites. However the minimum water activity (Aw) required to synthesize toxins is superior to the minimum required to fungal multiplication this was reported by [14]

Evaluation of mycological media.

Potato dextrose agar recovered the highest number of isolates from both fresh and stored samples followed by sabouraud dextrose agar then malt extract agar (tables15 and 16) This agreed with the work of [15] and [16] that said that potato dextrose agar has a very high recovery rate of fungi compared to other mycological media.

Evaluation of sample.

Groundnut had the highest number of isolates followed by maize, beans, rice and wheat was the least. (Tables 12 and 13). This result agreed with the work of [17] saying that the control of fungal development during the storage of food is mainly reach through the low Aw in the final product.

Table 12: Percentage occurrence of isolates from fresh products.

Isolates	Rice	Maize	Wheat	Groundnut	Beans	Total	Percentage(%)
Aspergillus sp	6	5	4	2	4	21	29
Aspergillus flavus	-	1	2	3	3	9	13
Aspergillus niger	-	2	1	-	-	3	4
Aspergillus ochraceus	-	1	-	-	-	1	1
Penicillium chrysogenum	4	5	-	1	4	14	19
Rhizopus stolonifer	-	1	3	7	2	13	18
Rhizopus nigricans	-	-	-	1	-	2	3
*Mucor*sp	1	2	-	5	1	9	13
Total	12	17	10	19	14	72	100

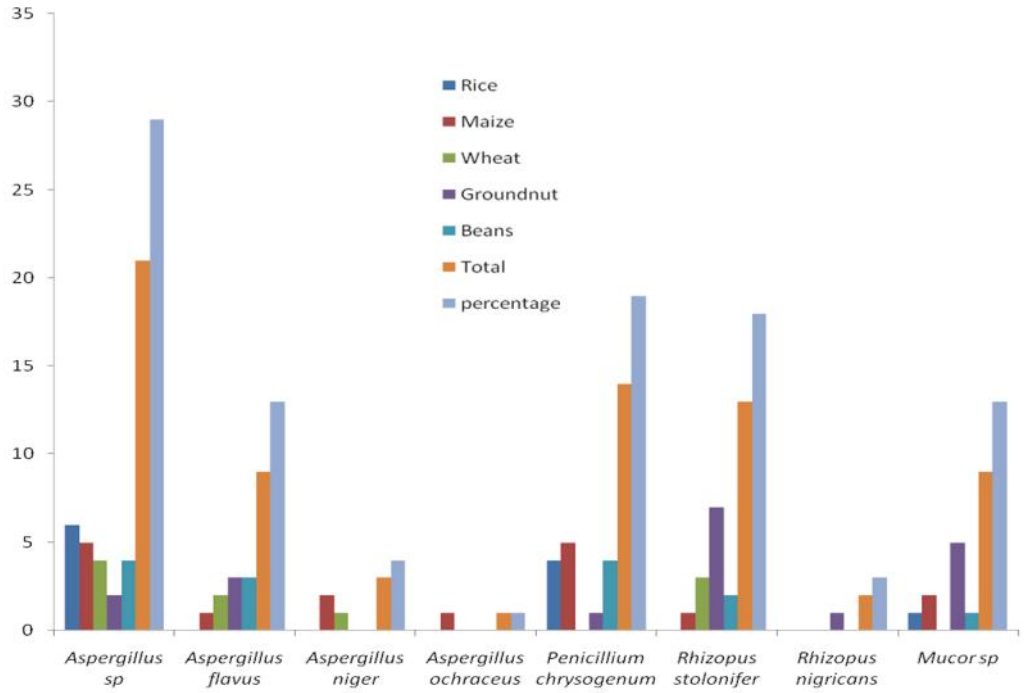

Figure 1: Percentage occurrence of isolates from fresh products.

Table 13: Percentage occurrence of isolates from stored products.

Isolates	Rice	Maize	Wheat	Groundnut	Beans	Total	Percentage (%)
*Aspergillus*sp	11	11	4	15	12	53	27
Aspergillusflavus	2	8	2	6	4	22	11
Aspergillusniger	-	-	4	-	4	8	4
Aspergillusochraceus	-	-	2	3	-	5	3
Penicilliumchrysogenum	9	12	8	17	10	56	28
*Fusarium*sp	-	1	-	-	-	1	1
Rhizopusstolonifer	7	16	6	14	-	43	22
Rhizopusnigricans	1	-	1	1	2	5	3
*Mucor*sp	-	1	-	-	-	1	1
Total	30	49	27	56	32	194	100

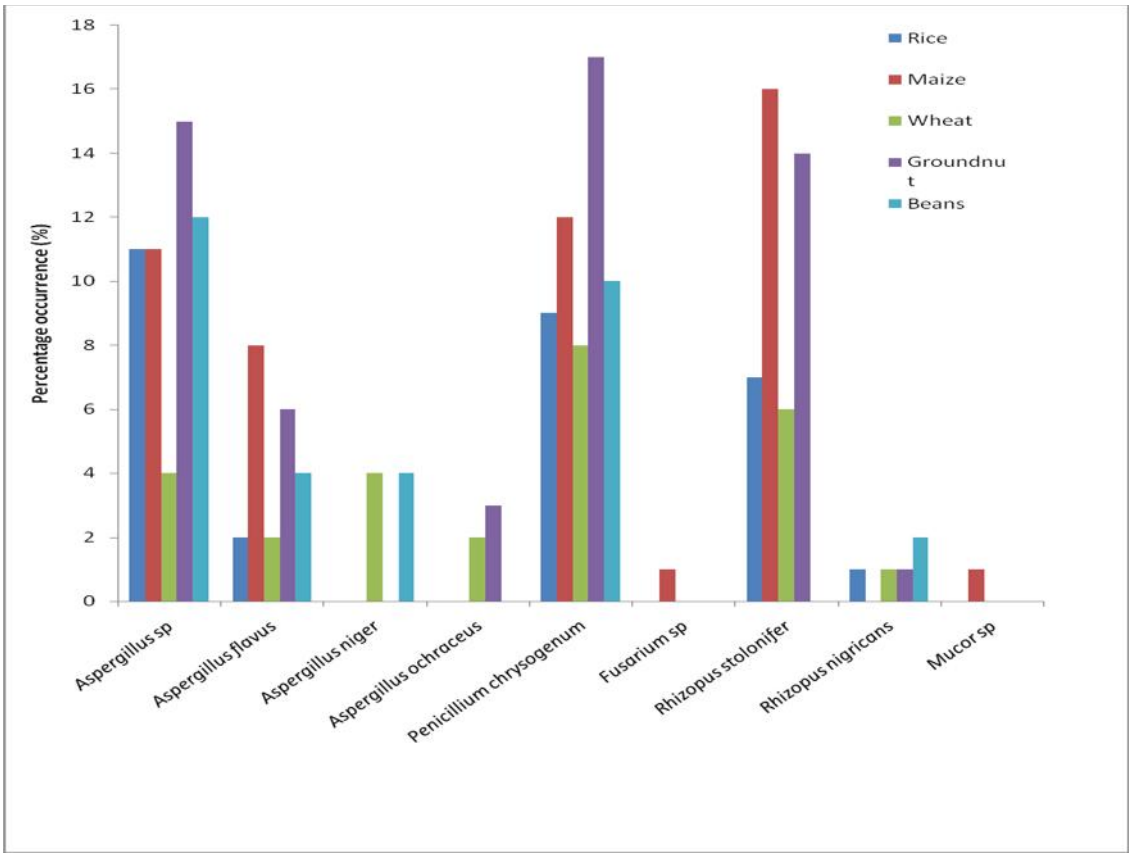

Figure 2: Percentage occurrence of isolates from stored products.

Table 14 Percentage occurrence of isolates between fresh and stored products.

Samples	Frequency	Percentage (%)
Fresh	72	27
Stored	194	73
Total	266	100

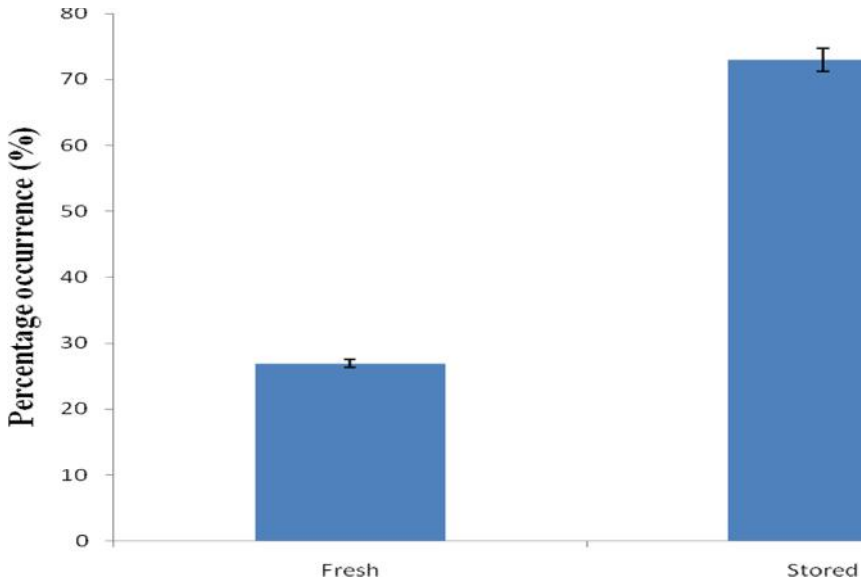

Figure 3: Percentage occurrence of isolates between fresh and stored products.

Table 15: Recovery rate of isolates using different media from fresh samples.

Samples	SDA	PDA	MEA	Total
Rice	4(33%)	5(42%)	3(25%)	12(17%)
Maize	6(35%)	7(41%)	4(24%)	17(24%)
Wheat	3(30%)	5(50%)	2(20%)	10(14%)
Groundnut	6(32%)	7(36%)	6(32%)	19(26%)
Beans	4(29%)	7(50%)	3(21%)	14(19%)
Total	23(32%)	31(43%)	18(25%)	72(100%)

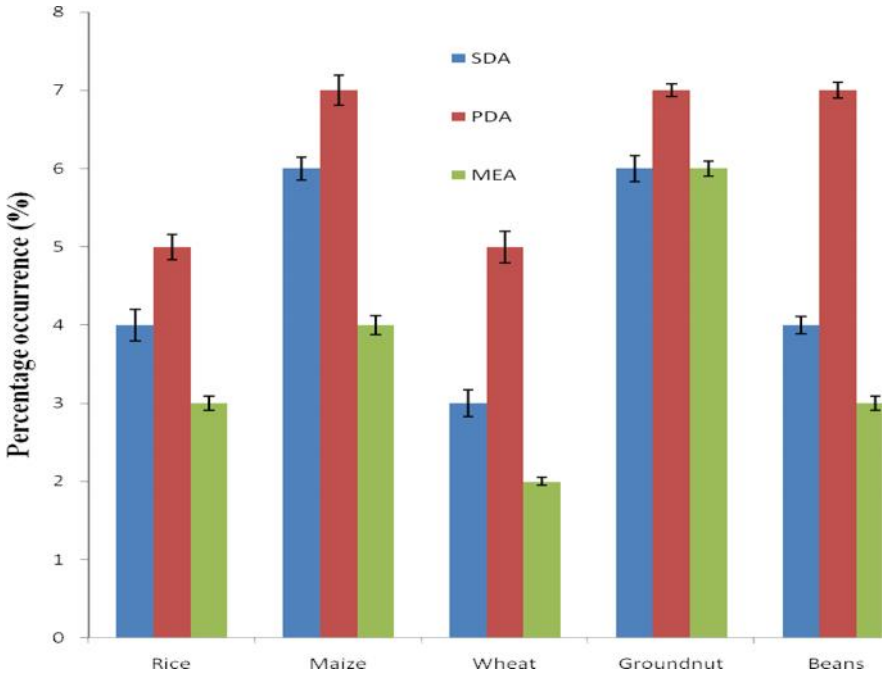

Figure 4: Recovery rate of isolates using different media from fresh samples.

Table 16: Recovery rate of isolates using different media from stored samples.

Samples	SDA	PDA	MEA	Total
Rice	10(33%)	12(40%)	8(27%)	30(15%)
Maize	16(32%)	18(37%)	15(31%)	49(25%)
Wheat	9(33%)	10(37%)	8(30%)	27(14%)
Groundnut	19(34%)	21(37%)	16(29%)	56(29%)
Beans	11(34%)	12(38%)	9(28%)	32(17%)
Total	65(33%)	73(38%)	56(29%)	194(100%)

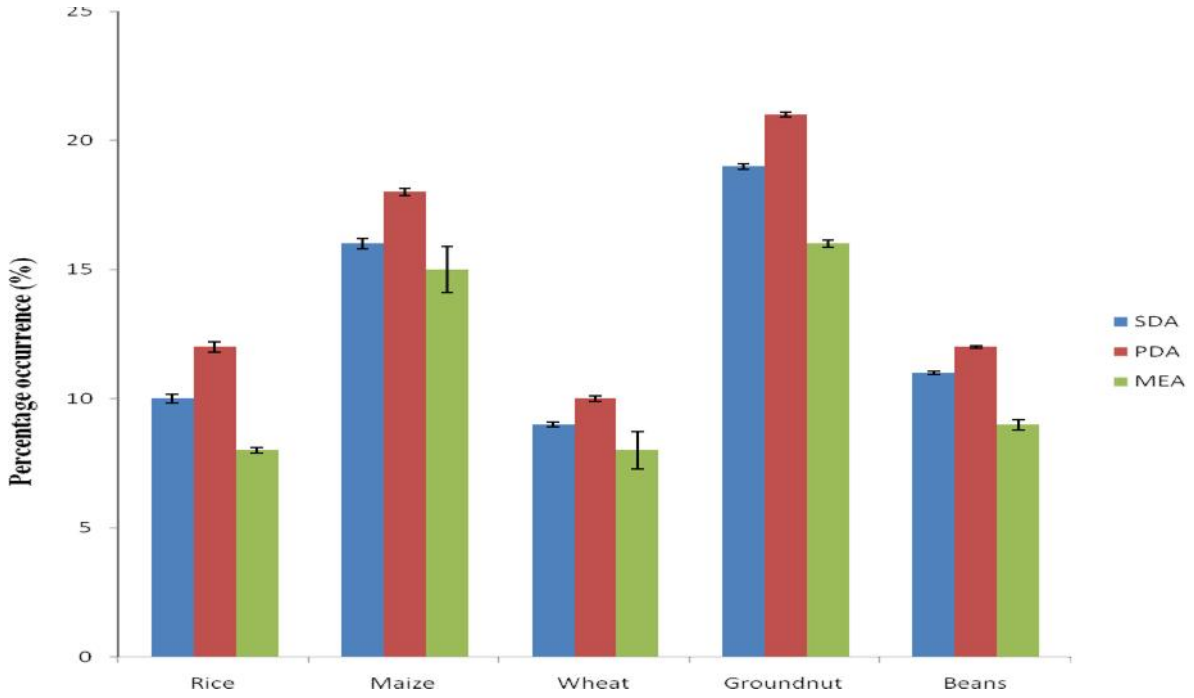

Figure 5: Recovery rate of isolates using different media from stored samples.

Comments:

- It seem the table and the figures are the same. Either use the table or the figure, or better still crop the tables side by side with the figures.
- Good attempt
- See comments as regards references
- See title recast and introduction edited
- Abstract not read yet
- Let's see to make the work better.
- Results and discussion needs improvement
- Which reference pattern did you adopt?

Conclusion

Evaluating the recovery rate of the various media, it was observed that potato dextrose agar had the highest rate of mould recovery, followed by sabouraud dextrose agar then malt extract agar. As a recommendation, potato dextrose agar should be used as a medium for fungal growth.

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