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Fungal Culture From Yam Rot Using Different Dextrose Agars.

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Abstract

The effectiveness of cotton seed dextrose agar, groundnut seed dextrose agar and kernel dextrose agar in culturing fungi was studied by culturing fungi from yam rot (*Dioscorea rotundata)*. The research demonstrated the choice of the isolated fungi to various media, and revealed that some of the isolated fungi showed the same occurrence in different media. *Aspergillus flavus* showed preference for kernel dextrose and groundnut seed dextrose agar than Sabouraud dextrose agar and cotton seed dextrose agar. *Fusarium oxysporum* showed preference to kernel and cotton seed dextrose agar. The occurrence of *Rhizopus spp* was only higher in Sabouraud and groundnut seed dextrose agar. In comparison of cultures, the cotton seed dextrose agar culture showed the highest growth of the fungi isolates (66.8±2.35%) while groundnut dextrose agar culture showed the least growth of the fungi isolates (49.8±2.35%). No significant difference in the growth of the fungi isolates was found between cultures ($p>0.05$). This research demonstrated that groundnut seed and cotton seed dextrose agar can be used to culture specific fungi of interest. Hence, they would provide suitable alternative media for culturing fungi of interest and reduce reliance on potatoes dextrose agar. The use of these media may provide promising interest in research where interest may be to identify, enumerate and characterize fungi.

Keywords: Fungi, Yam rot, Sabouraud, Cotton seed, Groundnut seed, Kernel dextrose agars.

INTRODUCTION

Fungi are nucleated, spores bearing, achlorophylous organisms with absorptive nutrition which generally reproduce sexually or asexually and whose filamentous branch somatic structure are typically surrounded by cell walls containing cellulose or chitin or both (Mohotra and Ashok, 2003). Fungi are simple aerobic organisms such as mildew, molds, mushrooms, smuts, toadstools and yeast which can grow in low pH environment, their genetic materials are bound in a membrane they do not produce their own food but obtain nourishment from dead organic matter (Starr, 2014). They are among the most widely distributed organisms on earth, many of them are freely living in the soil or water while others form parasitic or symbiotic relationships with plants or animals.

Fungi may be beneficial or detrimental to plant, animal and human welfare. Many fungi are used as a direct source of food, such as mushrooms and truffles and in fermentation of various food products, such as: wine, beer, and soy sauce. More recently, fungi are being used as sources of antibiotics in medicine and various enzymes, such as cellulases, pectinases, and proteases, important for industrial use or as active ingredients of detergents (Ikechi–Nwogu and Elenwo, 2012). Fungi play an important whole in plant nutrition with the formation of mycorrhizae and in symbiotic relationship with algae (Dutta, 2002). Varieties of molds are used for drugs, cheeses, etc. Other edible mushrooms are used in oriental cooking delicacies and various strains of yeast such as *Saccharomyces cerevesiae* are used for beer making, wine and bread making. Humans have taken advantage of the metabolism in a tiny fungus called yeast to create beer and wine from grains and fruits (Alba-Lois and Segal-Kischinevzky, 2010). Fungi are important decomposers within the biosphere and are essential in recycling of elements, some of the materials attacked and decomposed by fungi may be building materials clothe leather, waxes, jet

photographic film, lenses of cameras and food products (Saxon et al. 2003).

The aim of the research is to culture fungi from yam rot using cotton seed, groundnut seed and kernel dextrose agars in different fungal growth media, and then to determine relatively cheap and more available dextrose agar because commercial Sabouraud dextrose agar is expensive. That is, to determine which other dextrose agar aside Sabouraud dextrose agar that supports fungi growth by comparing groundnut seed, cotton seed and kernel dextrose agars to know which is more suitable for fungi growth, and also better in isolating and identifying fungi associated with yam rot.

MATERIALS AND METHOD

Study Design

Fungi are cultured to enumerate the quantity of fungi species associated with the culture and to identify and characterize the species present in the culture. Fungi need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce (Annan, 2010). Microbial culture media can be of different type, depending on the nutritional growth requirements of the microorganism (Basu et al. 2015). To prepare culture media, the nutrients necessary for the growth of specific organisms to be cultured must be added. Yam was therefore selected because it can be infected by fungi and it contains necessary nutrients which must be added in fungal culture so that any fungus (if present) would grow in the yam culture media. Culture media used in the laboratory for the cultivation of microorganisms supply the nutrients required for growth and maintenance (Tharmila et al. 2010). Different media like Sabouraud dextrose agar and potatoes dextrose agar can be used for growing fungi. The fungal decay of water yam (*Dioscorea alata*) has been investigated for the fungi responsible for postharvest rot of tubers in storage (Anwadike, 2018). The feasibility of using palm kernel agar (PKA) as an alternative culture medium to desiccated coconut agar (DCA) has been studied (Atanda et al. 2006). Khosravi et al. (2015) studied the efficacy of *Cuminum cyminum* essential oil using fungal strains of *Fusarium verticillioides* cultured from potato dextrose agar (PDA).

This study was therefore designed to culture fungi from yam rot using cotton seed, groundnut and kernel dextrose agars alongside the usually expensive Sabouraud dextrose agar.

Plant and other materials

Rotted Yam (*Dioscorea rotundata)* was selected from a yam farmer at Ifite Awka in Awka South LGA of Anambra State using the method of Anwadike 2018. Cotton seed was gotten from a farmer in Kaduna, Kaduna state. The groundnut seed was gotten from a farmer in Awka, Anambra state of Nigeria. Palm kernel was obtained from a bush at Ifite Awka, it was dried before use. Every other material was obtained from the Botany laboratory, Department of Botany of Nnamdi Azikiwe University, Awka Anambra State of Nigeria.

Other materials used in the study include 1. Cotton seed. 2. Groundnut seed. 3. De oiled nut (palm kernel). 4. Yam rot (*Dioscorea rotundata)*. 5. Nutrient Agar. 6. Glucose. 7. Ethanol. 8. Auto-Clave. 9. Cotton wool. 10. Aluminium foil. 11. Conical flask. 12. Petri-dish. 13. Spatula. 14. Weighing balance. 15. Distilled water. 16. Bunsen burner. 17. Antibiotics. 18. Masking tape.

Reagent Preparation (Yam culture media)

A slightly modified method of Anwadike (2018) was applied to conduct the experiment. The laboratory environment was swept and cleaned before the experiment commenced. Commercial Sabouraud dextrose agar was used to prepare the medium. The medium was prepared according to the instructions of the manufacturer: 35g of Sabouraud dextrose agar was dissolved in 500ml of distilled water. The medium was then sterilized in Auto clave at 121^{o}C and pressure for 15minutes. Antibiotics were added to the medium and mixed properly. The medium was handled carefully to avoid contamination.

Media Preparation and Isolation of Organisms

The methods of Muneera Al-Kahtani (2014) and Mailafia et al. (2017) were followed to isolate the organisms. The environment (table) was sterilized with ethanol, and then under the flame of light the liquid was dispensed into Petri-

dish. Cotton seed, groundnut seed and palm kernel were grounded. 20g each of the grounded materials was measured and put into conical flask containing 100g of distilled water and then boiled on a stirrer. Afterwards, the water was strained. 1g of Nutrient Agar, 0.17g of Glucose was added to each of them. Water was added in 500ml conical flask and then put inside the Autoclave for sterilization, after which antibiotics were added.

Yam tubers were cut open with surface sterilized kitchen knife to reveal the boundary area between healthy tubers and rotted side. The cut ends of the yam tubers were soaked with cotton wool to reduce bacterial interface. Sterile scalped blade was used to scrape the rotted part of the yam into the plate containing cotton seed dextrose agar, groundnut seed dextrose agar, kernel dextrose agar and Sabouraud dextrose agar. All the agars were externally wrapped round with masking tape, kept in the Laboratory bench and observed for seven days.

Inoculation

In order to inoculate the media, the table for inoculation was cleaned with ethanol to sterilize the table. A Bunsen burner was brought to sterilize the area of inoculation was taking place. An airtight glass jar containing cotton seed dextrose agar, groundnut seed dextrose agar, kernel dextrose agar and Sabouraud dextrose agar under the flame of light were poured into the petri-dishes to make it gel.

Statistical Analysis

The data obtained from the study were analyzed using simple descriptive statistics (frequency and mean) and analysis of variance ($p<0.05$).

RESULTS AND DISCUSSIONS

The results obtained from the experiments are presented in tables. Table 1 shows the occurrence of fungi isolates in Sabourand, kernel, cotton seed and groundnut seed dextrose agars.

Table 1. Occurrence of Fungi Isolates in Sabouraud Dextrose Agar, kernel Dextrose Agar, Cotton seed Dextrose Agar and Groundnut seed Dextrose Agar.

		Agar Type			
S/N	**Fungi Isolates**	**Sabouraud**	**Kernel**	**Cotton Seed**	**Groundnut**
1	*Aspergillus flavus*	-	+	+	+
2	*Aspergillus fumigates*	-	+	-	-
3	*Aspergillus niger*	-	-	-	+
4	*Aspergillus spp*	-	-	+	-
5	*Aspergillus terreus*	+	-	+	-
6	*Botryodiplodia theobramae*	+	-	-	-
7	*Cercospora spp*	+	-	-	-
8	*Fusarium oxysporum*	+	+	+	-
9	*Fusarium spp*	-	-	-	+
10	*Rhizopus spp*	+	-	+	+
11	*White fungus*	-	+	-	-

Key: + Presence; absent

As shown in Table 1, ten (10) fungi isolates were gotten from Sabouraud, kernel, cotton seed and groundnut dextrose agars. *Aspergillus flavus* was found in agar culture of the cotton seed and groundnut. *Aspergillus fumigatus* was found in kernel agar culture. *Aspergillus niger* was found in groundnut agar culture. *Aspergillus terreus* was found in agar culture of Sabouraud and cotton seed. *Botryodiplodia theobramae* was found in Sabouraud agar culture. *Fusarium oxysporum* was found in agar culture of Sabouraud, kernel and cotton seed. *Aspergillus spp* and *Fusarium* spp were found in groundnut agar culture. *Rhizopus spp* was found in agar culture of cotton seed and groundnut while white fungus was found in kernel agar culture. The percentage occurrence of five fungi isolates (*Aspergillus*

terreus, *Botryodiplodia theobramae*, *Rhizopus spp*) in Sabouraud dextrose agar C*ercospora spp*, *Fusarium oxysporum* and culture is shown in Fig. 1.

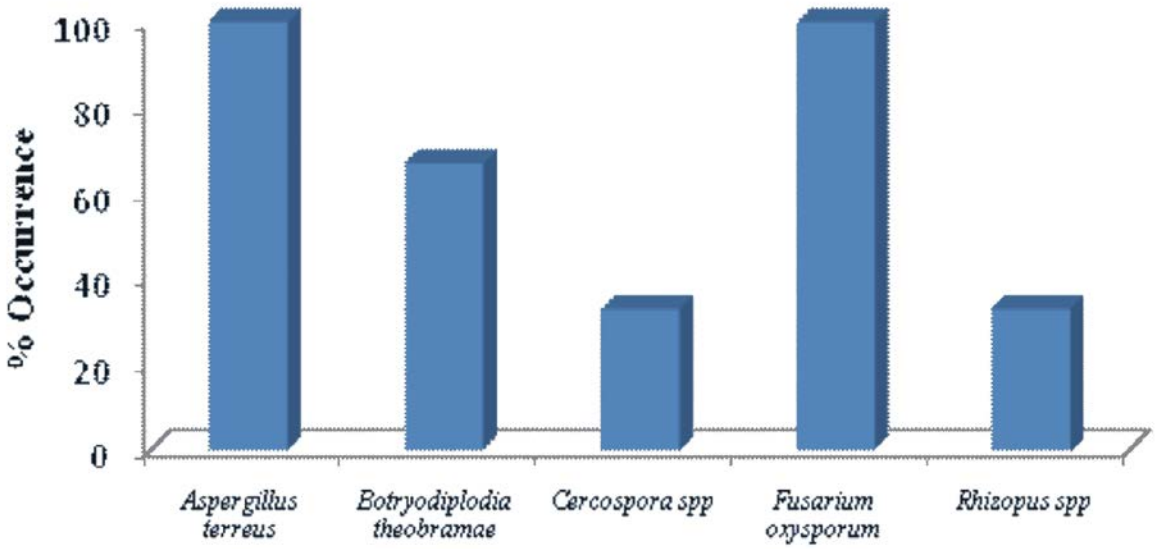

Fig. 1. Percentage occurrence of fungi isolates in Sabourand dextrose agar culture.

Fig. 1 shows the percentage occurrence of five fungi isolates obtained from Sabouraud dextrose agar culture. From the figure, percentage occurrence of *Aspergillus terreus* and *Fusarium oxysporum* in the Sabouraud dextrose agar culture are higher (100%), followed by *Botryodiplodia theobramae* (67%) and lastly *Cercospora spp* (33%) and *Rhizopus spp* (33%) respectively. Fig. 2 shows the percentage occurrence of four Fungi isolates obtained from kernel dextrose agar culture.

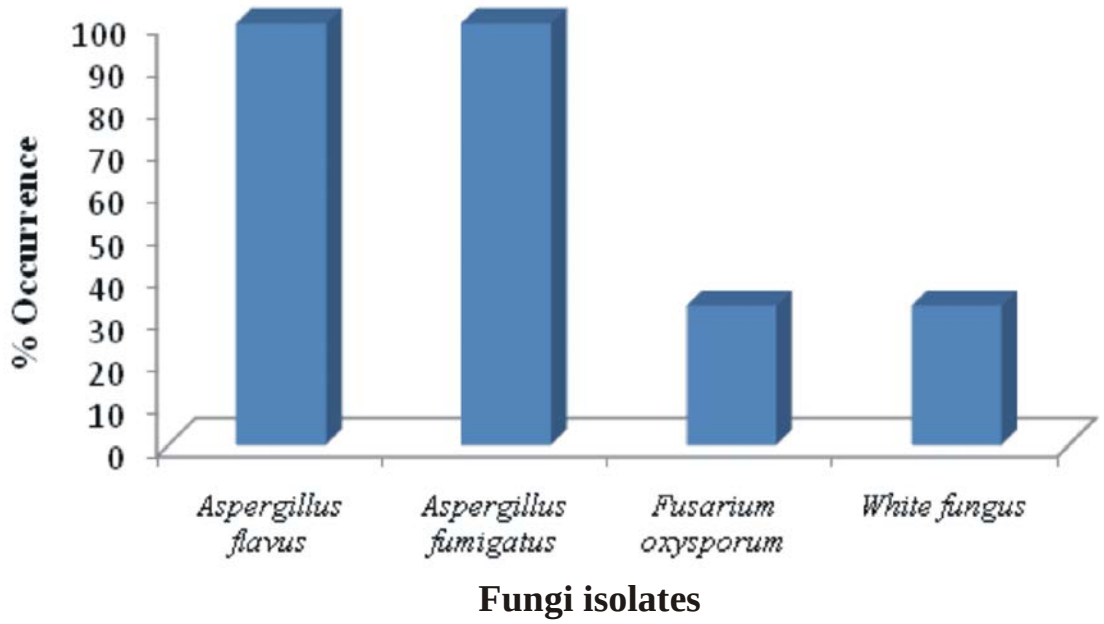

Fig. 2. Percentage occurrence of fungi isolates in Kernel Dextrose agar culture.

Fig. 2 indicates that percentage occurrence of *Aspergillus flavus* and *Aspergillus fumigatus* in the kernel dextrose agar culture are higher (100%) than those of *Fusarium oxysporum* (33%) and White fungus (33%). Fig. 3 shows the percentage occurrence of four fungi isolates (*Aspergillus terreus, Aspergillus spp, Aspergillus flavus and Fusarium oxysporum*) obtained from cotton seed dextrose agar culture.

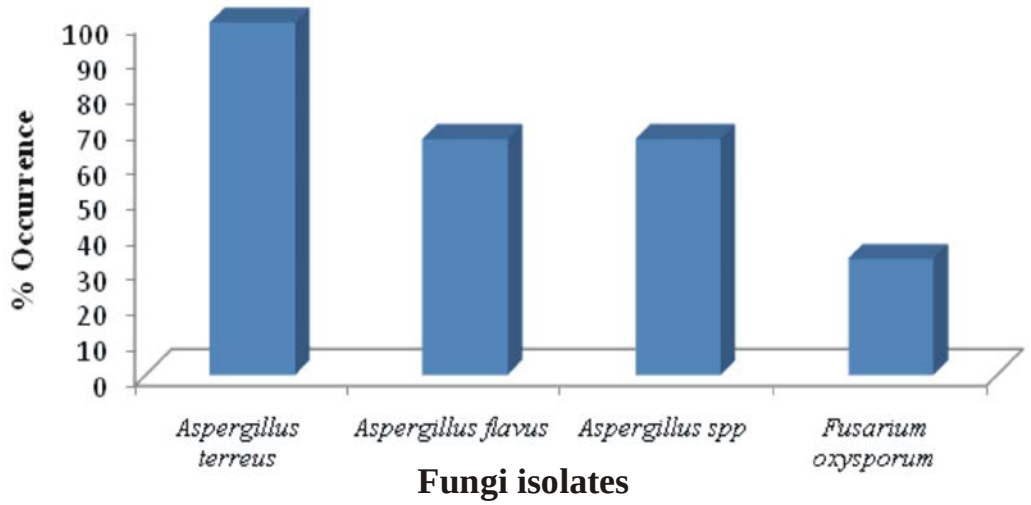

Fig. 3. Percentage occurrence of fungi isolates in cotton seed dextrose agar culture.

As shown in Fig. 3, the percentage occurrence of *Aspergillus terreus* in the cotton seed dextrose agar culture is higher (100%), followed by those of *Aspergillus flavus* (67%) and *Aspergillus spp* (67%) and lastly *Fusarium oxysporum* (33%). Fig. 4 shows the percentage occurrence of four fungi isolates (*Aspergillus niger*, *Rhizopus spp*, *Aspergillus flavus and Fusarium spp*) obtained from groundnut dextrose agar culture.

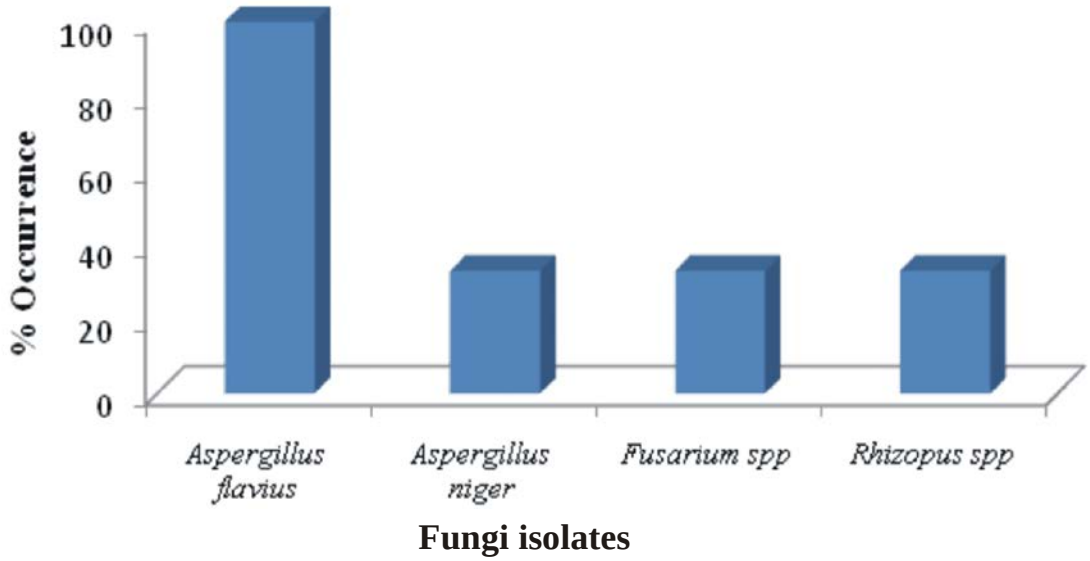

Fig. 4. Percentage occurrence of fungi isolates in groundnut dextrose agar culture.

As shown in Fig. 4, the percentage occurrence of *Aspergillus flavus* in the groundnut dextrose agar culture is higher (100%) than *Rhizopus spp* (33%), *Aspergillus niger* (33%) and *Fusarium spp* (33%). Table 2 shows the percentage occurrence of fungi isolates in dextrose agar culture of Sabouraud, kernel, cotton Seed and groundnut.

Table 2. Percentage Occurrence of Fungi Isolates in Dextrose Agar Culture of Sabouraud, Kernel, Cotton Seed and Groundnut.

S/N	Fungi Isolates	Percentage Occurrence				
		Sabouraud	Kernel	Cotton Seed	Groundnut	p-value
1	*Aspergillus flavus*	-	100[c]	67[b]	100[c]	0.15*
2	*Aspergillus fumigatus*	-	100[c]	-	-	NA
3	*Aspergillus niger*	-	-	-	33[a]	NA
4	*Aspergillus spp*	-	-	67[b]	-	NA
5	*Aspergillus terreus*	100[c]	-	100[c]	-	NA
6	*Botryodiplodia theobramae*	67[b]	-	-	-	NA
7	*Cercospora spp*	33[a]	-	-	-	NA
8	*Fusarium oxysporum*	33[a]	33[a]	33[a]	-	NA
9	*Fusarium spp*	-	-	-	33[a]	NA
10	*Rhizopus spp*	33[a]	-	-	33[a]	NA
11	*White fungus*	-	33[a]	-	-	NA
	Mean total (%)	53.2±3.02	66.5±3.68	66.8±2.35	49.8±2.35	0.819

*NA = Not applicable, *significant difference exist. Rows with same superscript are not significantly* different.

Table 2 summarizes the percentage occurrence of fungi isolates in dextrose agar culture of Sabouraud, kernel, cotton seed and groundnut. As can be seen from Table 2, the percentage occurrence of *Aspergillus flavus* is significantly higher in kernel dextrose agar culture (100%) and groundnut dextrose agar culture (100%) than cotton seed culture (67%). The percentage occurrence of *Aspergillus fumigatus* is 100% in kernel dextrose agar culture. The percentage occurrence of *Aspergillus niger* is 33% in groundnut dextrose agar culture. The percentage occurrence of *Aspergillus spp* is 67% in cotton seed dextrose agar culture. The percentage occurrence of *Aspergillus terreus* is 100% in both Sabouraud and cotton seed dextrose agar culture. The percentage occurrence of *Botryodiplodia theobramae* is 67% in Sabouraud dextrose agar culture. The percentage occurrence of *Cercospora spp* is 33% in Sabouraud dextrose agar culture.

The percentage occurrence of *Fusarium oxysporum* is 33% in Sabouraud, kernel and cotton seed dextrose agar culture. The percentage occurrence of *Cercospora spp* is 33% in Sabouraud dextrose agar culture. The percentage occurrence of *Fusarium spp* is 33% in Sabouraud, kernel and cotton seed dextrose agar culture. The percentage occurrence of *Rhizopus spp* is 33% in both Sabouraud and groundnut dextrose agar culture while the percentage occurrence of *White fungus* is 33% in kernel dextrose agar culture. In comparison between cultures, the cotton seed dextrose agar culture showed the highest growth of the fungi isolates (66.8±2.35) while groundnut dextrose agar culture showed the least growth of the fungi isolates (49.8±2.35). No significant difference in the growth of the fungi isolates was found between cultures ($p>0.05$) which is in tandem with the findings of Mailafia et al. (2017).

These findings are consistent with the study of Brus *et al.* (2005) who observed that fungi can grow on varieties of simple and complex food products. The findings also agreed with the results of Anwadike 2018 who used yam tubers (*Dioscorea alata*) to isolate and identify fungal species that cause rot and deterioration of tubers in storage. A total of seven fungi namely *Botryodiplodia theobromae*, *Aspergillus sp*, *Aspergillus niger*, *Fusarium sp*, *Fusarium sp*, *Penicillium sp* and *Trichoderma sp* were isolated from healthy or sound yam tubers. Results of the experiments also agreed with the findings of Nweke (2015) who isolated and identified three fungal pathogens (*Aspergillus niger* Van Tiegh, *Botryodiplodia theobromae* Pat and *Sclerotium rolfsii* Sacc.) of yam (*Dioscorea spp.*) in storage and the effects of their infection on the yam nutrient composition. The results are also in harmony with the findings of Muneera Al-

Kahtani (2014) who identified the presence of fungi and their mycotoxins in wheat and other grain samples. According to the findings, the most common genera were *Alternaria* (isolated from 68.96% of the tested samples), *Aspergillus* (24.14%) and in a lesser extent *Fusarium* (6.9%). In addition, the results obtained from the study agree with the findings of Mailafia et al. 2017 who isolated and identified fungi from spoilt fruits which include pawpaw (*Carica papaya*), orange (*Citrus sinensis*), tomato (*Lycopersicon esculentum*), pineapple (*Ananas comosus*), and watermelon (*Citrullus vulgaris*). Results indicated that *Aspergillus niger* had the highest occurrence in pineapple, watermelon, oranges, pawpaw, and tomatoes with a frequency of 38%. *Fusarium avenaceum* followed with the frequency of occurrence of 31% in fruits such as pineapple, watermelon, oranges, pawpaw, and tomatoes while *Penicillium digitatum* and *Rhizopus stolonifer* had the least frequency of 4% each in tomato; and orange and tomato, respectively. Other fungal species were identified as yeast (*Saccharomyces* species) (10%), *Fusarium solani* (8%), and *Aspergillus flavus* (5%). The highest prevalence rate was 70% of *A. niger* from orange followed by *F. avenaceum* of which 65% isolates were recovered from pawpaw. Other fungal organisms such as yeast (*Saccharomyces* species), *P. digitatum* and *R. stolonifer* were isolated with varying prevalence (40%, 20%, and 5%) from watermelon, tomato, and orange, respectively. Similarly, Hassan and Zanuddin (2018) examined and identified the presence of fungal organisms with three spoilt fruits which include banana (*Musa paradisiaca*), mango (*Mangifera indica*) and pineapple (*Ananas comosus*). The isolated fungal genera were *Apergillus sp*., *Fusarium sp*. and *Clasdoporium sp*. Results showed that mango demonstrated the highest frequency of fungus isolate which was seen in 2 of a total of 3 isolates (67%), followed by banana with one fungi isolate (33%). However, pineapple showed negative result with no occurrence of fungal isolated observed. Thiyam and Sharma (2013) also isolated and identified fungi associated with local fruits, namely: *Citrus limon, Mangifera indica, Musa paradisiaca, Psidium guajava, Elaeocarpus floribundus, Phyllanthus emblica, Artocarpus heterophyllus, and Carambola sp.* Samples were plated out on potato dextrose agar (PDA) medium and incubated at 28℃±2℃. Twenty three fungal pathogens were isolated which caused spoilage of fruits. Resulting growth was microscopically screened for fungal species. *Aspergillus* was found to be the commonest fungus found in all the fruits during storage of fruits. Other genera like; *Acremonium, Alternaria, Aspergillus, Chalaropsis, Cladosporium, Curvularia, Fusariumm, Mucor, Penicillium, Rhizopus,*and *Trichoderma* were common in the fruits stored in warm and humid condition.

The occurrence of the fungi was found to vary from culture media to another. The kernel dextrose agar culture was found to record higher occurrence of *Aspergillus fumigatus* and *White fungus*. This may suggest its potential as better source for culturing *Aspergillus fumigatus* and *White fungus* which agrees with the findings of previous studies. For example, Khosravi et al. (2007) showed that kernel dextrose agar culture provides suitable growth nutrients that make *Aspergillus fumigatus* and white fungus easy to thrive. Ikechi–Nwogu and Elenwo (2012) isolated and evaluated the response of fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus terreus*, *Aspergillus glaucus*, *Fusarium oxysporium* and *Rhizopus stolonifer* on some growth media - soybean, groundnut, ofor (*Detarium macrocarpum*), sawdust and potatoes dextrose broths - for the cultivation of fungal cultures. Soybean Dextrose Broth performed better than other broths probably because it contained more vitamins and minerals vital to fungal growth.

The choice of the isolated fungi to various media as observed in this study agrees with the work of Anjisha and Vrinda (2012) who proved that fungi would show variation in their growth and development when grown on various nutrient media, by evaluating seven fungi isolated from different infected host for their growth and development in define media. The fungi were: *Aspergillus niger, Fusarium sp., Antrodia sitchensis, Curvularia intermedia* and three representative isolates of *Macrophomina phaseolina* (a-Castor, b-Mango, c-Rose) isolated from different infected host. According to Khosravi et al. *(*2007), the reason for this can attributed to the fact that some culture media

share some nutrient in common which may make it possible to record the same fungi occurrence.

CONCLUSION

The research demonstrated that dextrose agar culture of palm kernel, groundnut seed, Sabouraud and cotton seed can be used to culture specific fungi of interest. Hence, their use may provide suitable alternative media for culturing fungi and reduce reliance on the use potato dextrose agar. Additionally, the uses of these culture media may provide promising interest in research where interest may be to enumerate, identify and characterize fungi.

RECOMMENDATIONS

Other crops such as carrots, beans and peas are recommended to be used to produce agars since they support the growth of fungi. Hence, their use may suitably provide alternative media for culturing fungi in order to reduce reliance on the use of potatoes.

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