Screening of selected plant products as alternatives to agar in mycological studies

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Abstract

This study was carried out to examine the use of selected plant products as alternative to agar for mycological studies because agar is expensive and not readily available. Extracts from *Aloe vera* (aloe) leaves, *Terminalia ivorensis* stem bark (black afara), *Hevea brasiliensis* (rubber tree), *Abelmoschus esculentus* pod (lady finger), seeds of *Detarium macrocarpum* (sweet detar), *Brachystegia eurycoma* (naga), *Mucuna sloanei* (*hamburger bean*) and *Telfairia occidentalis* (fluted pumpkin), grains of *Triticum* sp. (wheat), *Zea mays* (maize) and *Oryza sativa* (rice), tubers of *Colocasia esculenta* (cocoyam), *Manihot esculenta* (cassava), *Dioscorea bulbifera* (aerial yam), *Ipomoea batatas* (sweet potatoes), *Dioscorea rotundata* (white yam) and *Dioscorea dumetorum* (three-leaved yam) were screened as alternative to agar for cost reduction. These plant extracts combined with potato and dextrose in varying concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 g), were tested for their gelling potentials. The different concentrations of all the plant products were tested at different periods (1 s to 1 h), under different temperature (20–90 ºC). All the media did not solidify except potato dextrose cassava starch (PDCS) which solidified at 50 g 100 mL in 30 min and at 29 ºC. Seven frequently occurring fungi (*Aspergillus niger, Aspergillus flavus, Botrydioplodia theobromae, Fusarium poae, Penicillium chrysogenum, Rhizopus oryzae* and *Trichoderma harzianum*) were cultured on PDCS and PDA (potato dextrose agar) for comparison to evaluate their abilities in supporting fungal growth. The radial growth and mycelial biomass of the test fungi were recorded and subjected to ANOVA. Mycelial biomass values (g) for each test organism for PDCS and PDA were: 3.77 ± 0.64 and 3.94 ± 0.53 for *A. flavus*; 4.09 ± 1.51 and 2.14 ± 0.41 for *A. niger;* 8.08 ± 0.19 and 4.97 ± 1.74 for *B. theobromae*; 1.04 ± 0.32 and 1.97 ± 0.25 for *F. poae*; 6.42 ± 0.14 and 5.40 ± 0.16 for *P. chrysogenum*; 7.10 ± 0.86 and 5.88 ± 0.14 for *R. oryzae* and 5.08 ± 1.02 and 4.30 ± 2.64 for *T. harzianum* respectively. The mycelia biomass of *A. flavus* and *T. harzianum* on PDCS, are not significantly different at $P \leq 0.05$ from those on PDA. While those of *A. niger, B. theobromae, F. poae, P. chrysogenum* and *R. oryzae* on PDCS were significantly higher than those on PDA. All the tested fungi exhibited normal growth and sporulation on PDCS when compared with PDA (control), but with better growth on PDCS at $P \leq 0.05$.

Keywords: Agar-agar, Cassava starch, Fungal growth, Media, Plant products.

Introduction

Agar is a sticky substance derived from red algae for example the *Gelidium* and *Gracilaria* genera. Commercially it is primarily derived from seaweed *Gelidium amansii* (Davidson and Tom, 2006). The majority of the world's agar is produced in Japan, which depends on their cold winters to do so. The reliance on winter weather for agar production led to irregular importation and an absence of agar in markets and laboratories. Agar is expensive because of the difficulty of growing red sea. As a result, there has been an increasing need to replace agar with natural plant products. Despite being costly and hard to come by, agar is frequently used in manufacturing numerous foods and commercial goods. Historically and in a modern context, agar is chiefly used as a solidifying agent (Smith, 2005; Ewen, 2015) to prepare culture medium for microbial growth (Agar, 2012) and also used for fungal culture preservation in the laboratory for identification, classification, future studies and extensive research work. It is also employed to thicken and emulsify fluids (Smith, 2005; Ewen, 2015). In addition, agar has long been used in Asia as a dessert.

The cost of agar will keep rising, and if nothing is done to replace it, studies on medicine, microbes, mycology, and plant pathology would be impacted negetively. A significant body of research has been developed as a result of the recent concern over the price of agar. The study of alternatives to agar has been ongoing for the past ten years using a variety of techniques and the finding that plant materials can work as solidifying agents has generated a lot of enthusiasm. Several studies have examined cheap alternatives to agar in plant tissue culture. Bhattacharya *et al.* (2007) and Naik and Sarkar (2001) used sago as a gelling agent, while Gerbre and Sathyanarayana (2001) used cassava starch and sago as gelling agents for *in vitro* culture of potatoes. Mohammed *et al.* (2010) reported corn starch and potatoes starch combination as efficient gelling agents for the *in vitro* propagation of potatoes. Daud *et al.* (2011) used a combination of alternative gelling agents with agar and reported that they regenerated shoots from the stem segment of *Celosia* sp. Maliro and Lameck (2004) used cassava flour as a gelling

agent while Pervez *et al.* (2014) used it in the production of bioethanol because of its availability and low market price.

The attempts to substitute agar has only addressed media for *in vitro* propagation and have not addressed media for fungal growth. However, the use of cassava starch without agar and other plant products, as solidifying media for the growth of fungi, have received a little attention (Ikechi- Nwogu and Elenwo, 2017). Many researchers have looked at alternative, less expensive options because using agar to prepare media is so expensive. The exorbitant price of agar paved the way for cheap and readily available plant products as growth media. A lot of researchers have acknowledged that a combination of cassava starch and little agar can supply alternative low-cost medium support but Dabai and Mohammad (2005) in their survey showed that cassava starch can stand alone as a solidifying agent. The objectives of the study were to obtain a substitute for agar that can be used as a solidifying agent in mycological studies, determine the gelling properties of indigenous plant products and determine the effect of the newly screened solidifying agent in supporting growth and development of selected field and storage fungi. To achieve this, cheap alternatives to agar were sought for, which led us to the screening of some selected plant produces.

Materials and Methods

Sources of materials and study area

The study was conducted in the Mycology and Plant Pathology Laboratory, Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba. The research was in three phases – extraction of solidifying agents from plants/the production of plant based solidifying agents, isolation of fungi from food materials and inoculation of isolated fungi on plant based solidifying agents.

To achieve screening of plant products as substitute to agar in mycological studies, the following were screened - plant exudates, starchy root vegetables, food thickeners, cereals and edible seeds such as:

Sap: *Aloe vera* was obtained from a garden at Rukpokwu in Obio/Akpor Local Government Area of Rivers State, Nigeria.

Resins: Resins were picked from black afara *(T. ivorensis)* located in Saviour's Chapel, University of Port Harcourt, Choba.

Latex: Latex was extracted from rubber plant (*H. brasiliensis*) from Delta Rubber Company Limited at Umuanyagu in Etche Local Government Area of Rivers State, Nigeria.

Mucilage: Okro (*A. esculentus*) obtained from a garden at Rukpokwu in Obio/Akpor Local Government Area of Rivers State, Nigeria.

Starchy root vegetables: Aerial yam *(D. bulbifera*), white yam (*D. rotundata*), three-leaved yam (*D. dumetorum*), taro corm (*C. esculenta),* were also collected from a farm at Rukpokwu in Obio/Akpor Local Government Area of Rivers State, Nigeria.

The research was run twice with purchased starch after it showed positive results. Cassava stems (*M. esculenta*), tropical *Manihot* species (TMS) 94/0026 variety were obtained from ADP Rumuodomaya in Obio/Akpor Local Government Area of Rivers State, Nigeria, planted, harvested and starch was extracted. Cereals such as wheat, barley, rye, maize and rice were bought from the market at Rumuodomaya in Obio/Akpor Local Government Area of Rivers State, Nigeria. Seeds such as ofor (*D. macrocarpum*), achi (*B. eurycoma*), hamburger seed okpor (*M. sloanei)* and fluted pumpkin (*T. occidentalis)* were bought from the market at Rumuodomaya in Obio/Akpor Local Government Area of Rivers State, Nigeria. Fungi were randomly collected from different food materials afterwards gelling agents were prepared and plant based solidifying agents were produced. This research consisted of three replicates, ten treatments and one control.

Isolation of fungi from food materials

Standard blotter method recommended by International Seed Health Testing Association (ISTA, 2016), was used to isolate fungi. The fungi were isolated from food materials such as red and white varieties of bitter kola, millet, maize, beans (white and brown), carrot, lemon, pepper, tomatoes, groundnut, guinea corn, plum, yam, Irish potatoes, soya beans and okro. Petri dishes (9 cm diameter) were lined with 3 layers of sterilized filter papers. Sterilized distilled water was used to wet them and excess water was poured out. The food materials used were sorted to remove diseased ones, then soaked in 70% ethanol for 2–3 minutes and rinsed twice in sterilized distilled water; after which they were placed in Petri dishes equidistantly and incubated at 25 ^{o}C in the laboratory for 3–7 days. The fungi found growing on the food materials were identified using CBS Laboratory Manual Series Food and Indoor Fungi, Samson *et al*. (2010) and Descriptions of Medical Fungi, David *et al*. (2007). The isolated fungi were sub-cultured on the various culture media prepared. The fungi isolated were *A. niger, A. flavus, B. theobromae, F. poae, P. chrysogenum, R. oryzae* and *T. harzianum.* These fungi belonged to different groups such as plant and human pathogens, post-harvest pathogens and biocontrol agents (Javaid *et al.*, 2008; Gardiner *et al.*, 2014; Khan and Javaid, 2021; Khan *et al.*, 2021).

Data collection

Data collection was based on the clarity of the test fungi seen with unaided eye on the media. The cultures were investigated from the plates and the clarity of the growth of fungi on PDCS was compared

with those on the PDA.

Production of plant based solidifying agents

Extraction of gel from *A. vera*: Various steps were involved in extracting gel from *A. vera* leaves. Tools and hands were washed to avoid contamination. The aloe plant was cut off from the mother plant near the base of the plant with a sharp knife. Then the leaves were placed in an upright position to allow the dark yellow resin coming from the cut base to drain out. After this, the outer layer of the mature *A. vera* plant leaf, were sliced off then a vegetable scrapper was used to carefully peel the green portion of the leaves making a canoe-shaped half filled with gel. The gel was scooped out with a spoon into a clean bowl. After this, it was stored in an air tight container and refrigerated since aloe vera gel is perishable, and different concentrations such as 5, 10, 15, …, 50 g of each were taken and added to 1 L of potatoes dextrose broth to verify solidification.

Extraction of gel from okro: The slimy liquid in okro was extracted by soaking the plant in water.

Tapping of exudates from black afara: The exudates were handpicked from the tree bark, dried and crushed into powder with the local hand blender then different concentrations (5, 10, 15, …, 50 g) were added to 1 L of PDB.

Tapping of exudates from rubber plant: A sharp tool (gouge) was used to make spiral incisions through the bark of the tree and a collecting vessel was positioned at the base of the tree for the latex to drop into it.

Starch extraction from maize

The traditional method of corn starch preparation and the method described by Nwokoro and Chukwu (2012) was used. Different quantities such as 5, 10, 15, …, 50 g were added to 1 L of PDB to verify if they could be used to substitute agar. Before adding to PDB, the starch was cooked in low heat to avoid clogging. After sterilizing, it was allowed to get cold and 3 drops of 25% lactic acid were added. After which dispensed into Petri-dishes and allowed to solidify.

For cereals like wheat and rice, they were wet milled but it was difficult to extract starch so they were ground and used without extracting the starch.

Starch extraction from cassava

The method of starch preparation adopted by Kwoseh *et al*. (2012) and the method described by some local farmers were used. This process of obtaining starch was divided into five main stages namely; peeling, washing, grating, purification (starch washing) and drying. Some stems of tropical *Manihot* species 94/0026 variety were obtained from ADP Rumuodomaya, planted, allowed to mature then harvested. After harvest, 100 kg of cassava tubers were weighed, peeled, washed and grated into a paste. The paste was then strained into a clean bowl using a cheese cloth and the solution obtained was topped up with 3 L of distilled water. The starch was sun-dried for 72 h at room temperature, crushed and stored for future use. After which, different quantities of the starch power (5, 10, 15, …, 50 g) were added to 1 L of PDB, sterilized, cooled, 3 drops of 25% lactic acid were added then dispensed into Petri-dishes and allowed to solidify.

For starchy root tubers like aerial yam, white yam, three-leaved yam and taro corm, the starch contents are very little so they were used whole without starch extraction. After this, different quantities (5, 10, 15, …, 50 g) of dried powder was added to 1 L of PDB after which they were sterilized, cooled and 3 drops of 25% lactic acid was added then dispensed into Petri-dishes and allowed to solidify.

Starch extraction from edible seeds

Naga, sweet detar, hamburger seed, and fluted pumpkin, were used whole without starch extraction because of their slimy and viscose nature. The process of preparation involves: boiling, peeling and washing, grating and drying then the addition of different quantities such as 5, 10, 15, 2, 25, 30, 35, 40, 45 and 50 g to 1 L water. After which, they were sterilized, cooled and 3 drops of 25% lactic acid was added, dispensed into Petri-dishes and allowed to solidify.

Preparation of growth medium

The following media were prepared and used for this study: potato dextrose broth, potato dextrose agar, potato dextrose aloe vera gel, potato dextrose black afara exudates, potato dextrose okro exudates, potato dextrose rubber latex, potato dextrose white corn , potato dextrose yellow corn, potato dextrose cassava starch, potato dextrose aerial yam, potato dextrose white yam, potato dextrose three-leaved yam, potatoes dextrose taro corm , potatoes dextrose of seed , potato dextrose *Brachystegia eurycoma* seed, potato dextrose hamburger seed, potato dextrose fluted pumpkin seed, cassava starch and cassava starch dextrose. Potatoes (200 g), for each plant products; 5, 10, 15, …, 50 g plus 20 g of dextrose were added to 1 L of distilled water.

PDA preparation method as modified by Ikechi-Nwogu *et al.* (2019). was used with the different plant products above as agar substitute.

Direct microscopic mounts

Sterile technique was used to mount the slides for microscopic view to obtain the micrograph of the organisms below. A little portion of colony was picked using an inoculation needle and mounted on a clean microscope slide and lactophenol cotton blue was dropped. Then the colony was teased with a sterile needle, to spread out the filaments. A cover slip was placed over the slide, tapped the bottom of an inoculation needle to remove excess water and air bubbles. Protocols described by Ikechi-Nwogu *et al.*

(2021) were used to culture the test fungi.

Fungal growth studies

Fungal growth was studied by examining colonies for radial growth, number of spores and biomass. This study was carried out in 9-cm sterilized Petri dishes. Three-millimeters discs of each test fungus were placed in the middle of the Petri dishes containing different media. Three Petri dishes were used for each fungus for all the media. Two perpendicular straight lines were drawn on the bottom of each Petri dish. The crossing point coincided with the center of the 3 mm initial fungi disc. Radial growth measurements were recorded daily from the edge of the initial inoculum until the extreme area of fungi mycelia development, following the four segments formed by the two perpendicular lines. The dishes were incubated in the dark at 25±1 ºC for seven days. Daily fungal growth rate was calculated for each fungus and expressed in cm.

Evaluation of fungal biomass

Fungal biomass was the total weight of the fungal culture in each Petri dish. A sterile 3-mm diameter cork borer was used to cut fungal plugs from the edge of the fungal cultures and transferred into plates containing the new growth medium, using a sterile inoculating loop or needle or wire loop. The plates were sealed with masking tape to prevent contamination and to stop rodents from destroying and eating them. After this, they were incubated at 25±1 ºC for seven days. After the seventh day, the mycelial mass was harvested by placing the Petri dishes containing the solid medium on the wire mesh over a bunsen burner to melt. After melting, the fungi were harvested and washed in sterile distilled water thrice using forceps. Then Whatman's No 1, 9-cm filter papers were weighed and oven dried at 70 ºC for 24 h, the fungi were placed on a pre-weighed filter paper, oven dried and reweighed. The mycelial weights were calculated by subtracting the final from the initial weight (Nair, 1995).

Experimental design and data analysis

One-way analysis of variance (ANOVA) was conducted on each of the variables at significant level $P \leq 0.05$ was performed using SPSS 16.0 software for windows to compare the difference between means. Results were expressed as the mean± standard deviation of triplicate determination. Mean separation was done using Duncan Multiple Range Test. All analysis was done at 5% level of significance.

Results and Discussion

Proximate analysis

Proximate analyses were carried out using the methods of AOAC (2012) to ascertain the nutritional content of the plant products used. The results are presented in Table 1 below.

The presence of protein and lipid in cassava helped in the pasting properties and lipids are known to improve textural properties of starch leading to the viscosity hence improving the starch quality (Tukomane *et al*., 2007).

Visual observations of different plant products used as solidifying agents

Plant products screened as solidifying agents were visually observed. The results obtained from the observation are shown in Table 2. *A. barbadensis* did not solidify. In addition to this, it did not support fungal growth because of its anti-microbial properties that encouraged the inhibition of fungal growth. This corresponds with the results of Prakash *et al*. (2012) and Jeyasakthy *et al*. (2017). Similarly, Oana *et al*. (2007) also reported the effects of aloe vera gel on fungal organisms. These anti-bacterial properties may be cinnamic acid (Kouassi and Shelef, 1998), *p*-coumaric acid, ascorbic acid (Fite *et al*., 2003) and phenolic compounds (Cowan, 1999). The phenolic compounds such as pyrocatechol (hydroxylated phenol, known to be toxic to micro-organisms) act by denaturing protein and as a disinfectant. Cinnamic acid, *p*-coumaric acid and ascorbic acid, inhibit enzymatic activities of the microorganisms.

The PDRL medium formulated with rubber latex did not solidify because it was not exposed to air for sterility purpose. To explain this, Felips *et al*. (2014) reported that rubber latex is a substance that coagulates when it comes in contact with air. The bacteria in the air get into the latex and produces lactic acid that cause coagulation of the latex (Kuala, 2011). Another reason PDRL did not solidify is because potatoes added ammonia solution (OH- ion) which it possesses to the medium. This ammonization also prevents coagulation and possibly made the rubber particles to remain negatively charged.

B. eurycoma, D. macrocarpum and *M. sloanei* are used as soup thickeners. In addition to their thickening properties, they also have gelation properties and gummy texture. Food proteins respond to heat by unraveling, then bonding to one another before coagulating into solid mass. These thickening seeds are very rich in protein yet did not coagulate. This is as a result of the presence of sugar and fats since sugar and fats disrupt and slow down the rate of gelatinization (Janice, 2015).

Media formulated with *B. eurycoma, D. macrocarpum* and *M. sloanei* seed had challenges with fungal growth and this was confirmed by Binita and Khetarpaul (1997). Their poor performance is as a result of some antinutritional factors such as oxalate, phytate, saponin and tannin that prevented nutrient availability for fungi utilization. Saponins generally protect plant against microorganisms (Umaru *et al.*, 2006). This explains why fungi did not grow well on the PDOS, PDBES and PDHS. Tannin is a harsh, bitter plant polyphenolic compound that either binds

and precipitates or shrinks protein and other various organic compounds including amino acids and alkaloids. The compounds are widely distributed in many species of plants and they play a role in protection from predation and perhaps also in growth regulation. Tannins have the ability to precipitate certain proteins (Umaru *et al.*, 2006). They combine with digestive enzymes thereby making them unavailable for digestion. These chemicals found in these edible seeds contain ant-nutritional factors that affected the availability of nutrients required by the body of the fungi. As a result, their growth rates were inhibited (Umaru *et al.*, 2006). The growth observed, may be as a result of the storage or field fungi that invaded them.

Further investigations were carried out using starchy root tubers and from the result obtained, it was observed that there were some difficulties in extracting starch from aerial yam, white yam, three-leaved yam, taro corm and sweet potatoes. These tubers produced little quantity of starch so the tubers were used whole without starch extraction. Comparing their gelling behaviors and fungal growth; from the observation made, the media prepared with Aerial yam and white yam, did not solidify however they supported the growth of some fungi.

The culture medium containing corn (PDC), thickened however lost its thickening ability due to the fact that it lacks protein gluten (Norsildmel, 2015; Palik and Suresh, 2003). Gluten is a mixture of protein that has viscoelastic properties and gives elasticity to liquids. Meaning it contributes to viscosity. The viscosity of the medium was affected since maize lacks some essential amino acids. PDC supported different fungal growth such as: *A. niger, A. flavus, P. chrysogenum, F. oxysporium and T. harzianum.* Although PDC lost its thickening power when autoclaved at a high temperature, it can be used for the formulation of alternative culture medium for fungi. This result is similar to the observations reported by Adesemoye and Adedire (2005). Further investigations carried out on starchy root tubers indicated solidification was not obtained. These root crops also have some amount of antinutritional factors (FAO, 1990) that are responsible for lack of coagulation. In addition, these tuberous crops are rich in sugar and sugar reduces gelatinization and decreases viscosity. There were difficulties in starch extraction from yam. This observation agrees with Moorthy (2002) who reported that extraction of starch from yams was difficult due to presence of mucilage in the tubers. The study on the gelling behavior of yam showed that yam supported fungal growth though it did not solidify (Weststeijn and Okafor, 1971).

Potato dextrose yam encouraged the growth of *A. niger* and *B. theobromae* and coincidentally these are storage fungi of yam. Also, potato dextrose sweet potato (PDSW), growth medium became very slimy at 50 g at a temperature of 32 ^{o}C but after about two hours, it turned into a liquid medium and it encouraged the growth of *A. niger* and *F. oxysporium.* Among agar substitutes tested, potato dextrose cassava starch (PDCS) at 50 g was the only gelling agent that gave a positive end result when compared to agar. At 40 g, the cassava medium became semi-solid but it was observed that PDCS lost its viscosity after two weeks. However, at 50 g, the medium thickened though not as strong as agar and retained its viscosity until after four weeks.

After the 50 g concentration of the starch was obtained, standard blotter method as recommended by Seed Health Testing Methods of ISTA (2016) were used to isolate the field and storage fungi namely *A. niger, R. oryzae, A. flavus, T. harzianum, P. chrysogenum, B. theobromae* and *F. poae* and were identified using the methods of Samson *et al.* (2010) and Ellis *et al.* (2007). The isolated fungi were sub-cultured on the cassava and agar culture media as test fungi to compare the media. The test on cassava culture medium was to check its efficiency as agar substitutes to support fungal growth and sporulation. Five days into incubation on cassava and agar culture media, the micrographs of all the test fungi used were taken.

Fungal growth studies

The colors of the colonies grown on the media that were solidified with agar and starch were similar and the micromorphology descriptions of the colonies in both media were also the same. The test fungi were incubated for seven days to check the effect of the media on their growth. First day after inoculation, radial growth for *A. flavus, B. theobromae* and *T. harzianum* on PDCS gave values significantly higher ($P \leq 0.05$) than those recorded for PDA (Fig. 1A).

The same trend was also observed on the second day after inoculation. Radial growth values for *F. poae* for PDCS was significantly lower than what was recorded for PDA ($P \leq 0.05$). On the third day after inoculation, radial growth values (cm) of *A. flavus, A. niger, F. poae, P. chrysogenum* and *R. oryzae* on PDCS as gelling agent were not significantly different from those recorded for PDA ($P \leq 0.05$). However, the values for *B. theobromae* and *T. harzianum* on cassava starch were significantly higher than those of agar ($P \leq 0.05$) (Fig. 1B). On the fourth day after inoculation, the values of radial growth of *B. theobromae, R. oryzae* and *T. harzianum* on PDCS were significantly higher than those of PDA. For *F. poae*, PDA gave a significantly higher value of radial growth (Fig. 1C). On the fifth day after inoculation, radial growth of the values (cm) for *P. chrysogenum* and *R. oryzae* on cassava starch were significantly higher than those of agar ($P \leq 0.05$). For *F. poae*, agar gave a significantly higher value of radial growth. Radial growth values for *A. flavus, P. chrysogenum* and *R. oryzae* on cassava starch as gelling agent were significantly higher than what was recorded for agar on the sixth day after inoculation. For *F. poae*, agar gave a significantly higher value of radial

growth. Seven days after inoculation, the values of radial growth of *A. flavus*, *B. theobromae*, *P. chrysogenum*, *R. oryzae* and *T. harzianum* on cassava starch as gelling agent were significantly higher than those observed on agar. For *A. niger*, there was no significant difference on the radial growth values between cassava starch and agar as gelling agents. *F. poae* on agar gave a significantly higher value of radial growth ($P \leq 0.05$) (Fig. 1D).

Determination of mycelia density of test fungi

The mycelia density was determined using the method suggested by Kadiri (1998). Aerial mycelia growth of the test fungi were determined based on the appearance of the vegetative growth of the selected fungi and also by visual observation by a panel ranking of three persons (Table 3). From visual observation, PDCS showed that *R. oryzae* had a very dense growth. This organism grew profusely and extended out of the plate. For *A. niger* as shown above, the organism that grew on PDCS did better than that of the PDA. It was observed that the growth of *A. niger* on PDCS was better than those on PDA. The same result observed in *A. niger* was also observed in *A. flavus. F. poae* growth on PDA was moderate compare to PDCS that was scanty. Finally, the growth of *P. chrysogenum* in PDCS was scanty and it remained sterile, even after one week, but that of PDA grow very scanty and was contaminated after 2–3 days. Comparing the growth of organisms in PDCS with PDA (the control) visually, the cassava medium did better.

Evaluating the performance of the organisms on each medium

From the result obtained, it is evident that the *P. chrysogenum* performed better on PDCS than on PDA. Similarly, B. *theobromae* performed better on PDCS than on PDA. Then for *F. poae*, the trends changed. *F. poae* on PDA did better than the ones on PDCS. *A. niger* on PDCS, performed better than those on PDA. The same growth pattern was observed for *A. flavus*, *T. harzianum* and *R. oryzae*. It is such that the growth performance on PDCS was higher than those on PDA. The superior growth of some of the fungal species tested on PDCS over agar corresponds to the report of Moraes-Cerdeira *et al*. (1995). This is also affirmed by the report of Mateen *et al*. (2012). Babbar and Jain (2004) also recorded normal growth of fungi such as *A. flavus, A. nidulans, Sclerotium rolfsi, Trichoderma* sp., F. *moniliforme, F. semitactum, Cladosporium sphaerospermum* and *Circinella musco* on cassava starch. The essential elements required by fungi to grow elucidate why the organisms on PDCS performed better than those on the PDA.

Biomass determination

Fungal biomass was assessed 7 days after radial growths were measured. The biomass was taken to ascertain the effects of the media on the fungi and the result is represented below. Comparing the biomass of selected test fungi on the two-culture media (Fig. 2), *B. theobromae* had the highest growth followed by *R. oryzae, P. chrysogenum, T. harzianum* and *A. niger* in PDCS but *A. flavus* and *F. poae* on PDA have the highest growth. The value of *A. niger* and *T. harzianum* on PDCS and PDA were not significantly from different each other according to Duncan's multiple range test. *A. flavus* and *F. poae, B. theobromae, R. oryzae* and *P. chrysogenum*, were significantly from different each other.

Evaluation of sporulation of test fungi

After biomass was taken, the test fungi were tested to determine the effect of PDCS and PDA on spore production. In general, the media used for the study supported growth and sporulation of the test fungi. Some fungi do not produce spores and many others can produce spores (sexual and asexual). For a medium to be considered good, it must encourage sporulation because sporulation is necessary for dispersal, preservation (the spores help them survive unfavorable conditions) and genetic variation. The result of the spore count is recorded as follows. The number of spores of *A. niger* per Petri dish is 32×10^4 on the PDA and 40×10^4 on PDCS. For *F. poae* on culture media, spores were not found. Spores of *A flavus* on PDA had higher sporulation rate at 40×10^4 than the PDCS at 25×10^4. Calculating the sporulation of *B. theobromae*, the number of spores recorded were fewer than those of *A. niger* at 20×10^4 for PDA and 23×10^4 for PDCS. For *T. harzianum*, the spore produced from organisms on PDA (35×10^4) is higher than those of PDCS (33×10^4). The following solidifying agents specifically, isubgol (Jain and Babbar, 1998), guar gum (Jain and Babbar, 2004), corn starch agarose, alginates carrageenan and Cassava starch (Jain *et al.*, 2005) in the past have been used as agar substitute and agar blend in plant tissue culture. The present study however, focuses on the role of cassava as agar substitute for mycological studies. Several scientists have used cassava as an alternative gelling gent and have reported the possibility of using cassava as a substitute for agar. Cassava powder alone or its combination with agar was found a suitable substitute as a gelling agent. Among agar substitutes tested, cassava starch gave a promising result when compared to agar (Gebre and Sathynarayana, 2001; Mbanaso *et al.*, 2001; Daud *et al.*, 2011; Umeh and Uguru, 2013). The other plant products were either runny or slimy after autoclaving. Under this experimental condition, remarkable radial growth and biomass was observed on PDCS compare to PDA. Babbar and Jain (1998), claim the starch yields sugar upon autoclaving at 121 ^{o}C for 15–20 min. When it yields sugar, the viscosity is affected. On the other hand, PDCS at 50 g was autoclaved for just 10 min at 121 ^{o}C still retained its viscosity until after four weeks. Agar is superior to cassava starch in gelling

ability. However, because of its exorbitant price, it can be substituted for cassava starch for routine experiments. The assessment of gelling behavior of cassava starch, has shown clearly that organisms can actually be grown on cassava starch. Gregory *et al*. (2003) reported that fungi are capable of growing on cassava starch at a temperature of 50 and 55 ºC. Cassava starch has the ability to form hydrogen bonds, which results in increased water holding capacity**.** It also forms a gelatinous matrix that can be autoclaved and preserved after it has been melted (Kasanadze, 2000; Nene and Sheila, 1996). Cassava starch has some advantages over agar such as high purity level, exceptional thickening quality, neutral taste, clear paste, high concentration of starch that is better than the starches of other staple foods (wheat, maize, sweet potatoes, rice et cetera) and simple extraction method. Maliro and Lameck (2004) in their statement said cassava starch is odorless, paste clear, sticky and low in impurities. Above all, starch can be produced on a large and small-scale basis even with little funds.

The growth of the different species of fungi tested in this study on culture media prepared with cassava starch as gelling agent, showed that PDCS is not toxic to fungi. Also, the increase in biomass of the organisms tested on PDCS as gelling agent is as a result of potato metabolism by the organisms. The fact that the PDCS containing some organisms did not melt after one month (4 weeks), proves that it is as good as agar. It was also discovered that after 1-2 months in Petri dishes, the organism on PDA started dying but the ones on PDCS were thriving. It was also observed that cassava starch gelled at a temperature of about 39 ºC and agar at 32 ºC. Among agar substitutes tested, 50grams weight of cassava starch increased the viscosity of the medium and yielded promising results when compared to agar and it was used for fungi growth studies. It has been observed in this study that the use of cassava starch as solidifying agent in mycological culture media preparation to grow fungi, is as good as using agar. Moreover, being of plant origin, it is biodegradable and it will not pose a threat to our environment when disposed after use. Although agar was considered biologically static and non-toxic, its adverse effects have been accounted for (Debergh, 1983). Cassava starch decomposes in cold water, and it is practically clear in gel form. All these and many more stated earlier are indicative of the potential of PDCS to become a universal gelling agent in culture media for fungal growth of *A. niger, P. chrysogenum, R. oryzae, B. theobromae, F. poae, T. harzianum* and *A. flavus.* From the results obtained, this study has shown the potential of cassava starch in providing a suitable substitute to agar since it solidified, supported growth and sporulation of the test fungi. Therefore, it is suggested that this inexpensive plant product and low-cost gelling agent be approved for routine mycological studies in a developing country like Nigeria where agar is imported. Cassava is ever-present, has a simple extraction method and an exceptional thickening quality.

Potato dextrose cassava starch medium containing *B. theobromae* and *P. chrysogenum* did not lose their gel strength after two weeks contrary to the report of Maliro and Lameck (2004). Rather the medium lost its gel strength after four weeks of incubation. The organisms on PDCS were still preserved even after the medium lost its gel strength. Therefore, it is recommended that the organisms growing on PDCS be sub-cultured every four weeks.

Comparing the nutritional facts of agar and cassava, the nutrients in cassava were higher than those of agar. Assessing the carbohydrate content of cassava and agar, the portion of carbohydrate in cassava is 38 g while agar was 6.8 g. Carbohydrates are the major source of nutrient and the preferred carbon source for fungi. They absorb and metabolize different soluble carbohydrates, such as glucose, xylose, sucrose, and fructose. Fungi have a unique ability to utilize insoluble carbohydrates such as starches and cellulose as a source of carbon and many of them can also use protein as a source of carbon and nitrogen. The protein content of cassava is also higher than that of agar. For fungi to make use of insoluble carbohydrates and proteins, they extracellularly absorb them. This is similar to the report of Onuweme (1982) that cassava has 90% starchy content. Also, Dabai and Muhammad (2005) in their study, reported that cassava starch is an acidic polysaccharide consisting of 77% carbohydrate, 21% lipid and 2% protein. Comparing the essential vitamins fungi require for growth, vitamin B1 (thiamin), in cassava is 6% and it is higher than that of agar which is 1%. Niacin in cassava is 4% and it is higher than that of agar which is 0% but Vitamin B5 (pantothenic acid), in agar is 3% and it is higher than that of cassava which is 1%. The vitamin B12 (cyano) content of both is 0%. To explain further why the organisms in the cassava did better, the mineral contents were assessed and from the assessment, it was noticed that for all the minerals such as copper, manganese, zinc, molybdenum, scandium, vanadium and gallium needed for fungi growth, those in cassava are higher than those in agar.

Conclusion

Cassava starch being over 80 times cheaper than agar has provided evidence to be a cheap alternative. It is an easily cultivated plant, and therefore, increase in its demand could be easily met by increasing the area of cultivation and encouraging farmers and garri (flour made from cassava root) manufacturers not to waste the liquid that comes out of the production process. It is also not going to mount some pressure on our cassava because it is only in some parts of Nigeria that starch is consumed. This study has shown the potential of cassava starch in providing a suitable substitute to agar. It is an inexpensive plant product and is therefore recommended that this cheap

alternative gelling agent be adopted for routine mycological studies especially in a developing country like Nigeria where we have the constant need to import agar.

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Table 1: Nutrient content of plant products (per 100 g).

Nutrient content	Maize	Sweet potatoes	Cassava	Irish potatoes	Yam	Ofor	Achi
Moisture (%)	7.8	60.36	53.40	73.65	55.90	8.05	65.20
Energy (kJ)	1508	317	669	355	413	487	504
Protein (%)	9.48	3.07	2.18	5.59	3.07	13.09	6.12
Fat (g)	4.38	0.97	1.02	0.60	1.51	14	1.59
Carbohydrates (%)	76.16	32.41	34.97	18.42	36.24	55.6	24.3
Fibre (%)	0.73	2.08	7.04	0.79	2.03	6.13	1.22
Sugar (g)	0.59	0.74	1.4	4.14	0	0.2	11
Ash (%)	1.45	1.11	1.38	0.95	1.24	3.13	1.56
Calcium (mg)	0.925	9.55	19.55	2.65	7.925	120.30	233.30
Iron (mg)	201.325	37.075	37.30	39.15	30.20	956.63	248.375
Magnesium (mg)	281.45	71.60	217.55	76.925	131.10	917.00	422.83
Phosphorus (mg)	210	57	27	47	287	55	34
Potassium (mg)	2618.98	1662.25	3004.08	1567.43	2566.23	2931.85	780.33
Sodium (mg)	348.73	334.08	170.55	46.725	135.05	163.08	1,177.35
Zinc (mg)	24.35	3.00	8.70	3.60	18.175	23.65	29.00
Copper (mg)	2.30	0.875	0.50	0.325	1.125	3.175	4.775
Manganese (mg)	3.80	1.05	1.10	0.25	1.175	45.05	75.80
Selenium (µg)	14.9	0.1	0.3	0.2	0	0.4	1.1
Vitamin C (mg)	443.26	653.17	653.11	547.28	252.95	400.15	590.39
Thiamin (B1) (mg)	0.34	0.04	0.05	0.04	0.19	0.07	0.01
Riboflavin (B2) (mg)	0.15	0.02	0.03	0.03	0.10	0.01	0.02
Niacin (B3) (mg)	3.58	1.00	0.83	0.51	2.88	0.51	0.65
Pantothenic acid (B5) (mg)	0.37	0.25	0.06	0.77	0.1	0.27	0.22
Vitamin B6 (mg)	0.57	0.26	0.05	0.18	-	0.25	0.27
Folate Total (B9) (µg)	17	13	24	10	1	19	20
Vitamin A (IU)	210	0.9	10	13063	0	133	1120
Vitamin E alpha - tocopherol (mg)	0.45	0.01	0.14	0.21	0	0.33	0.09
Vitamin K1 (µg)	0.29	1.8	1.7	1.5	0	2.3	0.4
Beta-carotene (µg)	93	0.8	6	8417	0.1	76	445
Saturated fatty acids (g)	0.60	0.02	0.04	0.03	0.43	0.04	0.16

Table 2: Gelling behavior of plant screened indicating temperature and treatment conditions.

Plant products	Concentration (g L^{-1})	Temperature (ºC)	Observation	Fungal growth
A. barbadensis	5–50	10–90	Liquid	No growth
T. ivorensis	5–50	10–90	Liquid	*A. niger* and *A. flavus*
H, brasiliensis	5–50	10–90	Liquid	*A. niger* and *A. flavus*
A. esculentus	5–50	10–90	Liquid	*R. stolonifer*

D. macrocarpum	10–25	10–90	Liquid	Poor growth of *A. niger*, *F. oxysporium*, *A. flavus*, *R. stolonifer* and *P. chrysogenum*
B. eurycoma, and *M. sloanei*	10–25	10–90	Liquid	*A. niger*, *A. flavus* and *F. oxysporium*
	30–50	10–30	Slimy	
O.sativa	5–50	10–90	Liquid	*A. niger*, *A. flavus and R. stolonifer*
Z. mays	5 –35	10–90	Liquid	*A. niger*, *A. flavus*, *P. chrysogenum*, *F. oxysporium* and *T. harzianum*
	40–50	30	Solid	
T. occidentalis	5–50	10–90	Liquid	*A. niger*, *A. flavus*, *P. chrysogenum* and *F. poae*
Triticum spp.	5g -50	10–90	Liquid	*A. niger*, *A. flavus and R. stolonifer*
White yam (*D. rotundata*),	10–25	10–90	Semi -slimy	*A. niger* and *B. theobromae*
	30–50	10–30	Liquid to Slimy	
Aerial yam *(D. bulbifera*), three-leaved yam (*D. dumetorum*) and Taro corm (*C. esculenta*)	10–25	10–90	Liquid to Slimy	*A. niger* and *B. theobromae*, *A. niger* and *F. oxysporium*
	30–50	10–30		
Cassava starch	5–	28	Liquid	*A. niger*, *Rhizopus oryzae*, *A. flavus*, *T. harzianum*, *P. chrysogenum*, *B. theobromae* and *F. poae*
	30	29	Very slimy	
	40	28	Semi-solid	
	50	Room temp.	Solid	

Table 3: Mycelia density of test fungi.

Fungi	Aerial growth characteristics of test fungi			
	PDCS	**Score**	**PDA**	**Score**
A. flavus	Good growth (moderate)	++	Scanty	+
A. niger	Good growth (moderate)	++	Scanty	+
T. harzianum	Very good growth (abundant)	+++	No growth	-
B. theobromae	Very good growth (abundant)	+++	Very good growth (abundant)	+++
R. oryzae	Profuse growth (very abundant)	++++	Good growth (moderate)	+++
P. chrysogenum	Very good growth (abundant)	+++	Good growth (moderate)	++
F. poae	Scanty	+	Good growth (moderate)	++

Note: Profuse growth (very abundant) = ++++, Very good growth (abundant) = +++, Good growth (moderate) = ++, Scanty = + and No growth = -

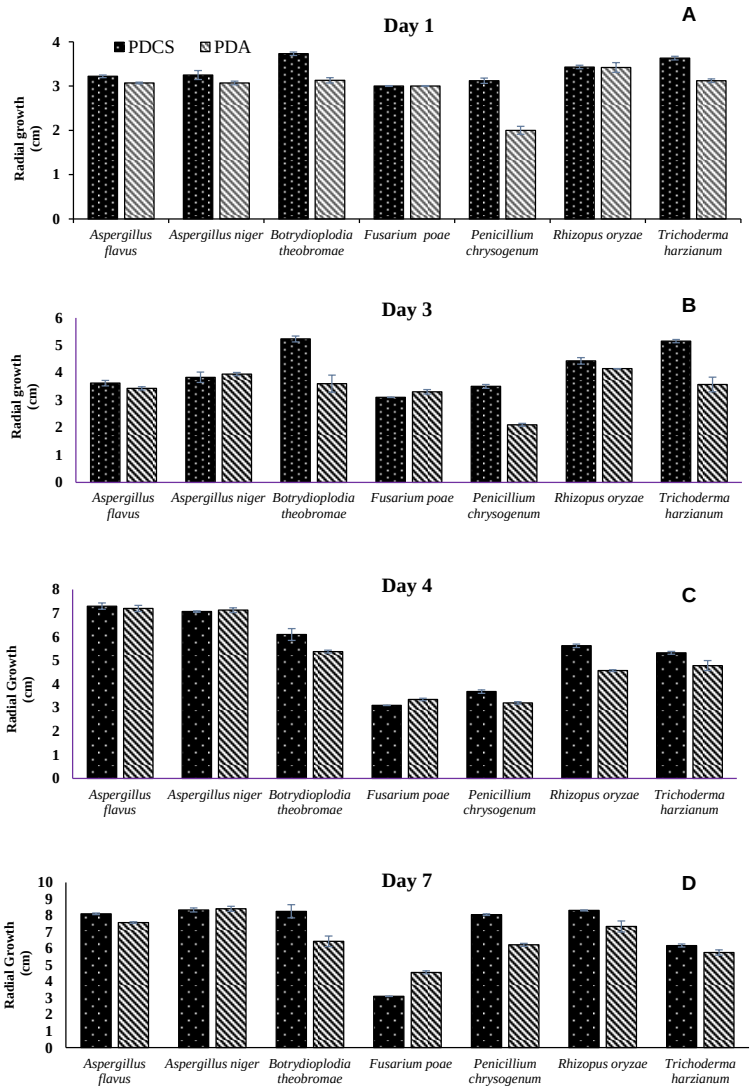

Fig. 1 (A-D): Mycelia extension of selected fungal species on PDCS and PDA at different time periods.

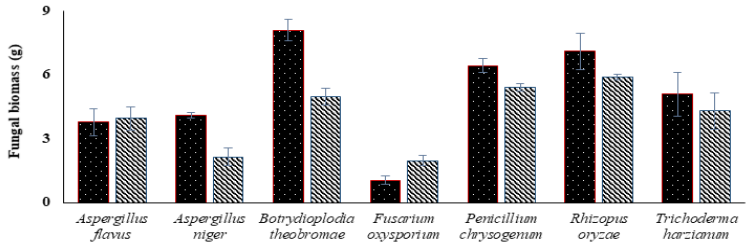

Fig. 2: Biomass of selected test fungi on culture media prepared with cassava starch and agar as gelling agent.

References

Adesemoye AO, Adedire CO, 2005. Use of cereals as bBasal medium for the formulation of alternative culture medium for fungi. *World J. Microbiol. Biotechnol.*, **21**: 329-336.

Agar. (2012). In Wikipedia. Available Online: *http.//en.wikipedia.org/wiki/ agar*. Retrieved on 13- 10-. 2011.

Alfred OU, Ahamefula II, 2014. Cassava Starch Exploring its potential as an alternative gelling agent for *in vitro* regeneration and

multiplication of sweet potatoes plantlets. *Am-Eur. J. Agric. Environ. Sci.*, **14**: 748-756.

Babbar SB, Jain N, 1998. Isubgol: As an alternative gelling agent for plant tissue culture media. *Plant Cell Rep.*, **17**: 318-322.

Babbar SB, Jain R, Walia N, 2004. Guar gum: As a gelling agent for plant tissue culture media. *In vitro Cell Dev. Biol. Plant,* **41**: 258-261.

Binita R, Khetarpaul N, 1997. Probiotic fermentation. effect on antinutrients and digestibility of starch and protein of indigenous developed food mixture. *J. Nutr. Health*, **1**: 139-147.

Cowan MM, 1999. Plant products as anti-microbial agent. *Clinical Microbiol. Rev.*, **12**: 564-582.

Dabai YU, Muhammad S, 2005, Cassava starch as an alternative to agar-agar in microbiological media. *Afr. J. Biotechnol.*, **4**: 5-4.

Daud N, Rosna MT, Nafizak MN, Hasimah A, 2001. Potential of alternative gelling agents in media for the *in-vitro* micro-propagation of *Celosia sp. Int. J. Bot.*, **7**: 183-188.

David E, Stephen D, Alexious H, Handke R, Bartley R, 2007. Descriptions of medical fungi. Second Edition. School of molecular and biomedical science. University of Adelaide, Adelaide, Australia.

Davidson A, Tom J, 2006. *The oxford companion to food*. Oxford University Press, USA.

Debergh PC, 1983. Effects of agar brand and concentration on the tissue culture medium. *Physiol. Plant*, **59**: 270-276.

Ellis D, Davis S, Alexiou H, Handke R, Bartley, R. 2007. Descriptions of medical fungi. Second edition. Mycology unit women's and children's hospital. School of molecular & biomedical science, University of Adelaide, Adelaide, Australia. pp. 1-204.

Felip AB, Lius FCB, Alberto T, Bruno DD, Larisa B, Paulo NLF, Perri EG, Graeff CF, Matheus C RM, Rondinelli DA, 2014. Natural rubber latex. *J. Int. Scholarly Res. Notices*, **1**: 5.

Fite A, Dykhuizen R, Litterick A, Golden M, 2003. Effect of ascorbic acid, glutathione, thiocyanate and iodine on antimicrobial activity of acidified nitrite. *Antimicrob. Agent Chemother.*, **48**: 655-658.

Food and Agricultural Organization of the United Nations, 1990. A manual for the processing of agar from *Gracilaria*. Available at. www.fao.org. Retrieved on 15-07-2016.

Gardiner BJ, Simpson I, Khuu MH, Kidd SE, Lo CH, Jenkin GA, 2014. An unusual ulcer: A case of cutaneous mucormycosis caused by *Rhizopus oryzae*. *Med. Mycol. Case Rep.*, **7**: 8-11.

Gebre E, Sathyanarayana BN, 2001. *Tapioca*. A new and cheaper alternative to agar for direct *in-vitro* regeneration and microtuber production from nodal cultures of potatoes. ethiopian agricultural research organization (EARO). *Afr. Crop Sci. J.*, **9**: 1-8.

Gregory KF, Reade AE, Santos-Nunez J, Alexander JC, Smith RE, Macleans SJ, 2003. Further thermotolerant fungi for the conversion of cassava starch to protein. *Anim. Feed Sci. Technol.*, **2**: 7-19.

Ikechi – Nwogu CG, Elenwo EN, 2017. Potentials of cassava starch extraction by-product as isolate for fungal species (unpublished manuscript). University of Port Harcourt, Nigeria. Faculty of Science, Department of Plant Science and Biotechnology.

Ikechi-Nwogu CG, Odogwu BA, Pikibo A, 2019. Morphological and molecular characterization of post-harvest fungal pathogen of ginger rhizomes. *Nig. J. Mycol.*, **11**: 196-203.

Ikechi–Nwogu GC, Okere SE, Elendu NR, 2021. The effectiveness of some plant leaves in the preservation habanero pepper (*Capsicum chinense*). *J. Appl. Sci. Environ. Manage.*, **26** (1): 47-50.

International Seed Testing Association (ISTA), 2016. International Rules for Seed Testing. Seed health testing changes and editorial corrections adopted at the ordinary general meeting 2016, Tallinn, Estonia. Chapter 7, pp. 1-12. Published by the International Seed Testing Association (ISTA) Zürichstr. 50, CH-8303 Bassersdorf, Switzerland.

Jain R, Anjaiah V, Babbar SB, 2005. Guar Gum. A cheap substitute of agar in microbial culture media. *Lett. Appl. Microbiol*., **41**: 345-349.

Janice L, 2015. How starched thicken sauces and fillings. Published by The Kitchen Magazine. www.thekitchn.com

Javaid A, Javaid A, Akhtar N, 2008. *In vitro* chemical control of *Botryodiplodia theobromae,* the cause of dying back disease of mango. *Pak. J. Phytopathol*., **20**: 195-199.

Jeyasakthy S, Rosdan S, Irfan M, Azian H, 2017. Antifungal effects of *Aloe vera* leaf extract on the selected fungal species. *J.Med.*, **32**: 41-46.

Kadiri M, 1998. Spawn and fruit body production of *Pleurotus sajor-caju* in Abeokuta, Nigeria. *Nig. J. Bot.*, **11**: 125-131.

Kasanadze AK, 2000. Replacement of agar by cassava flour in microbial media (B.Sc. Dissertation University of Malawi, Bunda College of Agriculture, Lilongwe).

Khan IH, Javaid A, 2021. *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina*. *J. Plant Pathol.*, **103**: 1195-1205.

Khan IH, Javaid A, Ahmed D, 2021. *Trichoderma viride* controls *Macrophomina phaseolina* through its DNA disintegration and production of antifungal compounds. *Int. J. Agric. Biol.*, **25**: 888-894.

Kouassi Y, Shelef LA, 1998. Inhibition of *Lysteria Monocytogenes* by Cinnamic Acid: Interaction of the acid with cysteinyl residue. *J. Food*

Safety, **18**: 231-242.

Kuala L, 2011. Coagulation of latex. Powered by blogger chemistry2u.blogspot.

Kwoseh CK, Asomani-Darko M, Adubofour K, 2012. Cassava starch-agar blend as alternative gelling agent for pathological culture media. *Botswana J. Agric. Appl. Sci.*, **8**: 8-15.

Maliro MFA, Lameck G, 2004. Potential of cassava flour as a gelling agent for plant tissue culture. Bunda College of Agriculture; Lilongwe. *Afr. J. Biotechnol.*, **3**: 244-274.

Mateen A, Hussain S, Rehman SU, Mahmood B, Khan MA, Rashid A, Sohail M, Farooq M, Shah SJA, 2012. Suitability of various plant derived gelling agents as agar substitute in microbiological growth media. *Afr. J. Biotechnol.*, 45: 10362-10367.

Mbanaso ENA, Crouch J, Onofeghara FA, Pillay M, 2001. Cassava starch as alternative to agar for gelling tissue culture media. A paper presented at a conference on cassava, an ancient crop for modern times held at Donald Danforth plant Science Centre, St. Louis, Missouri. pp. 4-9.

Moraes-Cerdeira, RM, Krans JV, McChesney JD, Pereira AMS, Franca SC, 1995. Cotton fibre as a substitute for agar support in tissue culture. *HortSci.*, **30**: 1082-1083.

Nair NN, 1995. Seed pathology of some important legume crops of Chhattisgarh. PhD. Thesis, Ravishankar University, Rarpur (M. P).

Nene LY, Shiela KV, Moss JP, 1996. A Manual on tapioca as a potential Substitute for Agar in microbiology and tissue culture media. ICRISAT, Andhra Pradesh India. *Indian J. Myco.* **17**: 511-512.

Norsildmel AS, 2015. Corn gluten. Retrieved from http://www.norsildmel.no.

Nwokoro OC, Chukwu BC, 2012. Studies on Akamu, a traditional fermented maize food. *Children Nutr.*, **39**: 4.

Oana RC, Marcel P, Laurian V, Mircea T, 2007. Antifungal activity of *Aloe vera* Leaves. Fitoterapia **78**: 219-222.

Onwueme IC, 1982. The tropical crops, yams, cassava, sweet potatoes and cocoyam; University of Ife; Ile-Ife, Nigeria, pp. 145.

Palik KK, Suresh CB, 2003. Food systems for improved human nutrition. Haworth Press. p.193.

Prakash PA, Borthakur BJ, Genesan SB, 2012. Evaluation of antimicrobial efficacy of *Aloe vera* and its's effectiveness in decontaminating. *J. Cons.*, **3**: 246-248.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B, 2010. Food and indoor fungi. *CBS Laboratory Manual Series*. Published by CBS-KNAW fungal biodiversity Centre Utrecht, The Netherlands.

Ssamula A, Mukasa SB, 2015. Use of cassava starch as gelling agent during *in-vitro* micropropagation of banana. *J. Exp. Biol. Agric. Sci.*, **3**: 34-38.

Umaru HA, Adamu R, Dahiru D, Nadro MS, 2006. Levels of antinutritional factors in some wild edible fruits of Northern Nigeria. *Afr. J. Biotechnol.*, **6**: 1935-1938.

Ume BU, Uguru MI, 2013. Comparative study on agar and cassava gelled media in *in vitro* propagation of ginger. *Afr. J. Agric.*, **8**: 2793-2798.

United Nations Food and Agriculture Organization. *Agriculture and Consumer Protection*. "Dimensions of need - staple foods. What do people eat?" Retrieved 15-10-2010.

Weststeijn G, Okafor N, 1971. Comparison of cassava, yam and potatoes dextrose agar as fungal culture media. *Netherlands J. Plant Pathol.*, **77**: 134-139.