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REGULAR ARTICLE

BASAL MEDIA FORMULATION USING *CANAVALIA ENSIFORMIS* AS CARBON AND NITROGEN SOURCE FOR THE GROWTH OF SOME FUNGI SPECIES

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ABSTRACT

The possibility of developing alternative media to commercial potato dextrose agar was assessed using, *Canavalia ensiformis* (Linn) (jack beans) as carbon and nitrogen source. Six leguminous meal media were used as substitute for either carbon or nitrogen or both, while potato dextrose broth (PDB) was used as a positive control and basal medium as a negative control. Six species of fungi *Aspergillus flavus, A. niger, Meria coniospora, Mucor sp, Neurospora crassa* and *Rhizopus oryzae* were aseptically inoculated into the formulated media and allowed to grow. Their mycelia dry weights were taken after 24, 48, 72, 96 and 120 hours. Growth of all fungal species was observed to be slightly lower, about the same or better in the formulated media relative to the control. *Aspergillus flavus* had its highest biomass of 1.70g in the media formulated with *Canavalia ensiformis* as the carbon source relative to 1.42g as the standard at the 120 hour. *A. niger* had a growth of 0.62g relative to 0.61g at 120 hours of the control. *Meria coniospora* had a growth of 0.27g relative to 0.38g at 120 hours. *Mucor sp* had a growth of 0.54g relative to 0.44g at 120 hours. *Neurospora crassa* had a growth of 1.05g relative to 0.24g at 120 hours. *Rhizopus oryzae* had a growth of 0.14g relative to 0.25g at 120 hours. The study revealed that *Canavalia ensiformis* contains minerals and nutrients that is able to provide the nutritional requirements of these fungi. Thus, it can be used as an alternative material in the preparation of culture media for *in vitro* cultivation of these fungi for teaching and research purposes.

Keywords: fungi, nutrients, minerals, *Canavalia ensiformis,* Basal media.

INTRODUCTION

Jack bean (*Canavalia ensiformis*, L) is an annual underutilized leguminous semi-erect, drought resistant plant originated from tropical South American bearing long pods containing white seeds **(Stephens, 1994)**. Jack beans have been reported to be rich in protein and could serve as potential protein source, and also has a high potential as protein replacer **(Seena *et al.*, 2006)**. Raw unprocessed seed of Jack bean contains about 300g crude protein and 600g carbohydrates kg-1 dry matter **(Udedibie, 1990)**. Although it is low in methionine, it is relatively high in lysine **(Stephens, 1994)**. The knowledge of the source of nutrients that encourage the growth of microorganisms is a useful determinant factor in considering the availability of the enzyme present in the microorganisms which can be industrially useful.

Fungi are a group of eukaryotic spore bearing, achlorophyllous organisms that generally reproduce asexually and sexually. Some are agent of diseases in plants and animals (parasitic), while some are saprophytic. Fungi are regarded as General Manager in Nutrients recycling department of nature **(Khalid *et al.*, 2006)**. Fungi due to their competitive saprophytic ability expressed by fast mycelia growth, spores production, presence of efficient and extensive systems of powerful enzymes are able to utilize complex polysaccharides and protein as their carbon and nitrogen sources **(Wubah, 1999)**.

Pelczar *et al.*, (1993) defined a culture medium simply as any material in which microorganisms find nourishment and can reproduce themselves. Therefore, for a microbiological medium to fulfill its specific purpose, it must contain all the substances and compounds necessary for the growth and reproduction of the organism. Various substances have been combined into nutritive concoctions media and have successfully been used to isolate important microorganisms from materials such as water, soil, food, clinical specimen and dairy products. Investigations into the composition of culture media, has established that the important ingredients such as nitrogen, carbon, as source of energy, vitamins and growth factors mainly essential mineral-salts. If an essential element is lacking from the medium or substratum, the fungus in question will not survive no matter how abundant other elements are. Hence, an optimal nutrient medium should provide not simply adequate growth, but the best possible growth in order to allow moulds grow without restriction and express all phenotypes **(Meletiadis *et al.*, 2001)**.

MATERIAL AND METHODS

Preparation of Raw Carbon and Nitrogen Source

The jack bean (*Canavalia ensiformis*) seeds used were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The clean healthy seeds were soaked with distilled water for two days to remove the seed coat and rinsed in changes of distilled water. The dehulled seeds were dried at 50^{o}C for 48 hours in the drying cabinet and pulverized into fine powder with the Marlex portable stainless grinder. The materials were stored in sterile transparent polythene bags, tightened and kept at -20^{o}C for further usage.

Biochemical Analyses

The proximate parameters were determined according to **AOAC (2005)** while minerals was analysed using Atomic Absorption spectrophotometer (AAS). Data were analyzed according to the analysis of variance (ANOVA) procedures and means separated using Duncan new Multiple Range Test.

Preparation of Growth Media

Eight different growth media were used, six of the broth were compounded by replacing either the carbon or nitrogen source in the basal medium formulated as modified by **Olutiola *et al* (2000)**, while the other two were Potato Dextrose Broth (PDB) (positive control) and basal medium (negative control).

Medium A – Standard/Positive Control was prepared according to manufacturer's specification.

Medium B – Basal medium is an enrichment culture medium used to test the ability of the microorganisms to degrade and utilize the carbon and nitrogen source (*Canavalia ensiformis,* L) as substrates for production of biomass. It contained 0.25grams - peptone, 0.26grams – KH_2PO_4, 1gram – $NaNO_3$, 0.025grams – $MgSO_4.7H_2O$, 0.5 grams – Na_2HPO_4, 5.0grams – sucrose; into 250millilitres distilled water.

Medium C (as nitrogen source) – Contained all that is in medium B except that the nitrogen source ($NaNO_3$) was replaced with Jack beans powder (1.0gram).

Medium D (as carbon source) – Has all the reagents compounded for the basal medium but the carbon source (sucrose) was replaced with (5.0grams) Jack beans powder.
Medium E - All the reagents remains has in the basal medium except the replacement of both the nitrogen source with Jack beans and carbon source with glucose.
Medium F – Formulation was done according to medium E with change in the carbon source as maltose.
Medium G – Formulation was done according to medium E with change in the carbon source as lactose.
Medium H – Formulation was done according to medium E with change in the carbon source as galactose.

Test Organisms

The test organisms, isolated from the fermenting jack beans using Malt extract agar, were identified using standard microbiological methods **(Onions *et al*., 1995)**. The fungi isolates were *Aspergillus flavus, Aspergillus niger, Neurospora crassa, Meria coniospora, Mucor mucedo* and *Rhizopus oryzae.*

Inocula were drawn from pure cultures of the isolates before 72th hour of growth aseptically to ensure that growth was still high at logarithmic phase when it would have uniform physiological characteristics **(Nwachukwu and Akpata, 2003)**.

Determination of Fungal Biomass

The biomass produced in each broth medium was determined by dry weight measurement applying the method of **Nwanze *et al* (2005)**. The weight of biomass of the culture were determined by drying and weighing using a pre-weighed filter paper under aseptic condition with the Mettler balance (PM400).

Physicochemical Analysis

The pH of the fermenting substrate was taken twice daily (12 hourly intervals) using a potable pH meter. The temperature of the medium was also taken 12 hourly bases **(AOAC, 2005)**.

RESULTS AND DISCUSSION

Observation of the result of proximate and mineral analysis carried out on *Canavalia ensiformis* shows it contains appreciable amounts of nutrients required for the good growth of fungi. This can be seen in biomass yield of six fungi in the formulated media.

Table 1 Proximate Composition of Raw dehulled Jackbeans (*Canavalia ensiformis* L.)

Moisture content (mg/100g)	8.53 ± 0.43
Ash content (mg/100g)	2.72 ± 0.04
Crude Fat (mg/100g)	2.67 ± 0.13
Crude Protein (mg/100g)	27.22 ± 2.24
Crude Fibre (mg/100g)	10.3 ± 1.43
Carbohydrate (mg/100g)	58.56 ± 4.42
Sodium (mg/kg)	369.75 ± 8.45
Calcium (mg/kg)	131.75 ± 5.65
Magnesium (mg/kg)	338.32 ± 9.46
Potassium (mg/kg)	235.37 ± 7.35

Table 1 shows the moisture content of the sample (*Canavalia ensiformis*), as well as the ash content, percentage fat, percentage protein, crude fibre content and carbohydrate which were given as 8.53, 2.72, 2.67, 10.3, 27.22 and 58.56g/100g respectively. Although moisture (water) is required by all organisms for their life processes and fungi in particular require water for extracellular digestion of nutrients. The moisture content of the sample has negligible or no effect on the growth of the fungi tested because they were grown in solution (broth). However, water is important for the cultivation of fungi on solid substrate **(Olutiola *et al*., 2000; and Silva *et al*., 2005)**.

The ash content of the sample (jack beans) was recorded as 2.72g/100g; this shows that jack beans have a low percentage ash. The crude fibre is a representation of the organic matter in the sample. It gives an estimate of the proteins, lipids (fats), carbohydrate and the nucleic acid component of the sample. From table 1, the percentage crude fibre composition and fat composition are (10.3 and 2.67g/100g) respectively. Although there is a relationship between lipids content of media and fungal growth according to **Nwanze *et al*., (2005)** which

reported that lipids had significant effects on the mycelia wet and dry weight of *Lentinus squarrosulus* (Mont) singer and *Psathyrella atroumbonata* Pegler in submerged liquid culture. The carbon to nitrogen ratio (C/N) has been said to influence rapidly with a high degree of efficiency the rate of assimilation of nitrogen into microbial biomass **(Zibilske, 1999; Narasimha *et al.*, 2006)**. Also represented on table 1, is the percentage nitrogen content of Jack beans (*Canavalia ensiformis*) which was given as an index of the percentage protein content which is 27.22g/100g. This may be referred as a contributory factor to the massive growth of organisms cultured in the media with jack beans as the raw carbon source. Another observation from table 1 is the concentration of minerals (both macro and micro elements) in the raw carbon/nitrogen substrate which must have contributed to the growth of some of the fungi in the media. The raw carbon/nitrogen source contained the tested minerals in concentrations as shown below sodium: 369.75mg/Kg, calcium 131.75 mg/Kg, magnesium 338.32 mg/Kg, and potassium 235.37 mg/Kg.

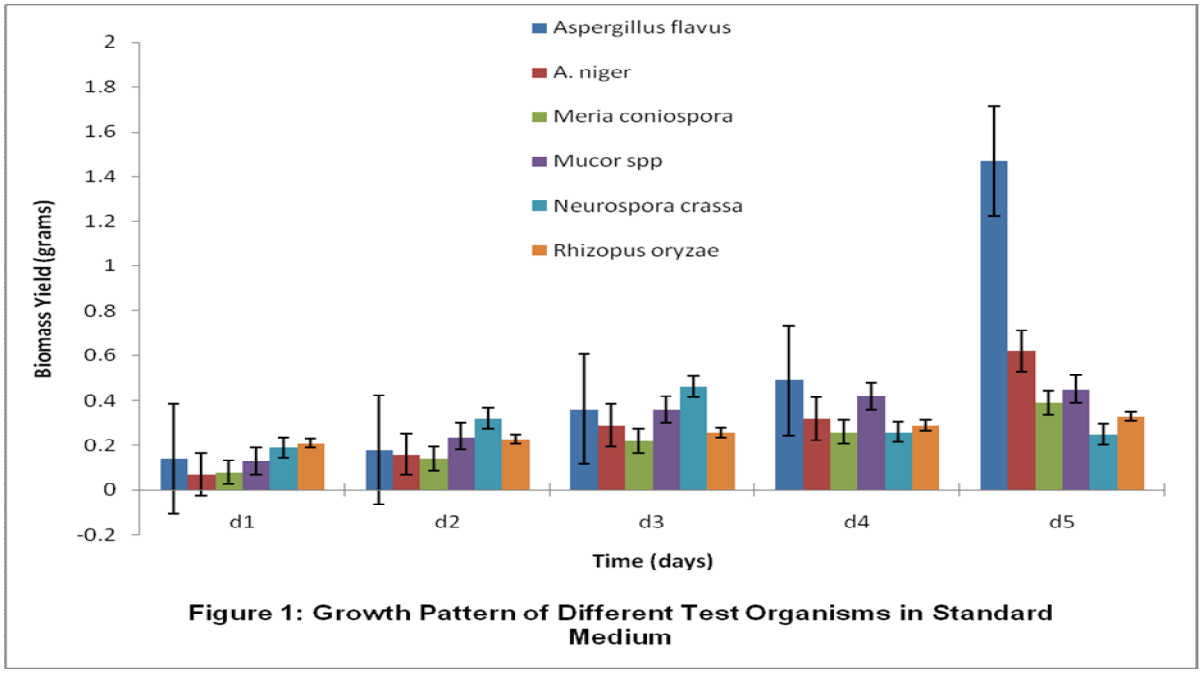

Figure 1: Growth Pattern of Different Test Organisms in Standard Medium

Legend: Potato Dextrose Broth (PDB). d1 – d5 represents day 1 to day 5.

Growth of the Selected Fungi on the Formulated Media and the Controls

Maximum growth of *Aspergillus flavus* (figure 4) was observed within 120 hours of inoculation of spores into sample formulated with jack beans as the carbon source and $NaNO_3$ as the nitrogen source (medium D) with a biomass yield of 1.696 grams. This was closely followed by potato dextrose broth which was the standard (figure 1), sample formulated with

Canavalia ensiformis as nitrogen source and sucrose as carbon source (medium C that is figure 3) and sample formulated with *Canavalia ensiformis* as nitrogen source and lactose as carbon source (medium G/ figure 7) with maximum biomass yields of 1.148, 1.137 and 1.01grams respectively. Minimum biomass yield was recorded in the sample with nitrogen source as *Canavalia ensiformis* and carbon source as galactose (medium H) as 0.653grams. Although, medium B (basal formula) and the standard were expected to have supported the growth of the fungus within few days because their carbon sources are easily digestible than that of the raw carbon source maximum growth was still recorded in medium 4 after five days. This is an indication that natural substances contain a lot of complex substances such as vitamins and minerals (table 1) that are essential for good growth. This is in line with the findings of **Akinyele and Adetuyi (2005)** and **Silva *et al* (2005)**, which shows that agricultural waste materials supports the good growth of fungi.

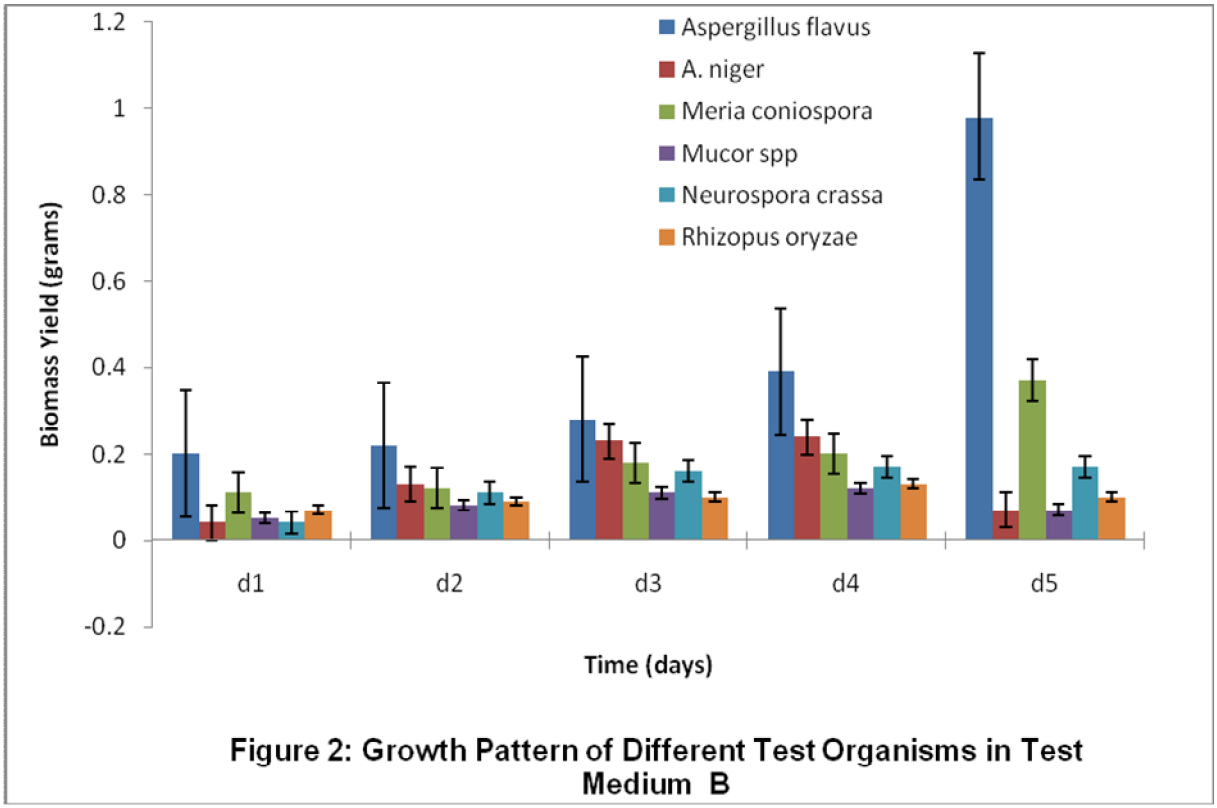

Figure 2: Growth Pattern of Different Test Organisms in Test Medium B

Legend: peptone (0.25g), KH_2PO_4 (0.26g), $NaNO_3$ (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), sucrose; (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

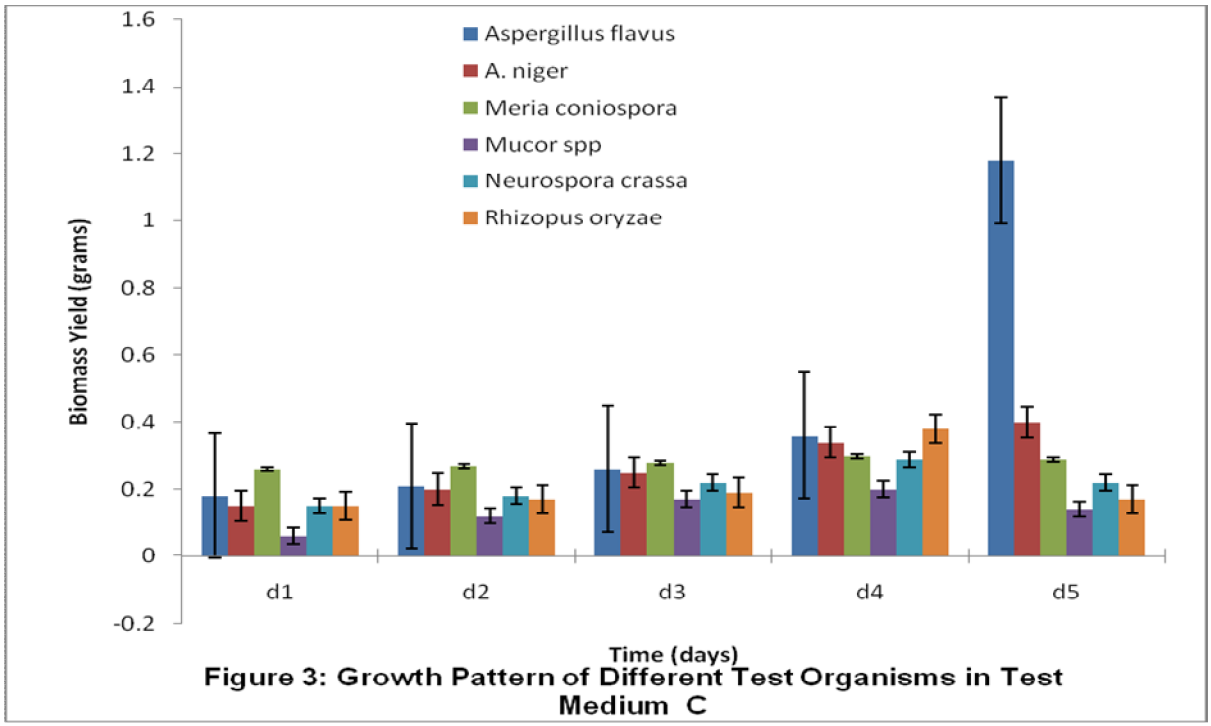

Figure 3: Growth Pattern of Different Test Organisms in Test Medium C

Legend: peptone (0.25g), KH_2PO_4 (0.26g), Jack beans (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), sucrose; (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

Figure four gives the best growth support to *Aspergillus niger* which has 0.610g as its maximum growth on day five, this was closely followed by 0.607g in the standard (figure 1). The media with nitrogen source as *Canavalia ensiformis* had their biomass as 0.586 and 0.501grams in medium F and H respectively while medium B which is the basal medium had a growth of 0.235g which was the minimum growth recorded for the fungus on day five.

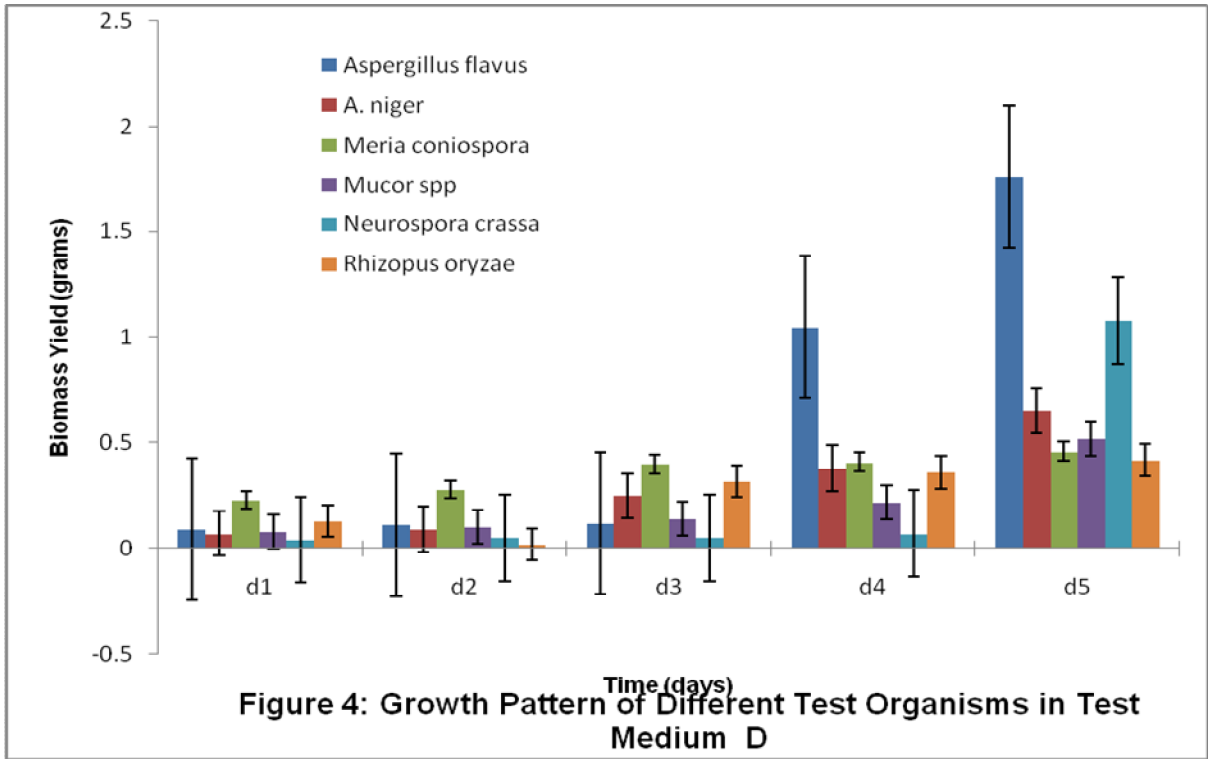

Figure 4: Growth Pattern of Different Test Organisms in Test Medium D

Legend: peptone (0.25g), KH_2PO_4 (0.26g), $NaNO_3$ (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), Jack beans (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

The maximum growth of *Meria coniospora* was recorded as 0.455grams in figure 4, relative to 0.07 g in the standard (figure 1) on the third day of both medium. The change on the fifth day as a growth 0.382g was recorded as the maximum in the standard (figure 1) and the growth in figure 4 declined to 0.268grams. This may lead to the inference that *Meria coniospora* easily digests raw carbon sources than refined and that raw carbon sources contains the essential vitamins and minerals (table 1) is capable of supporting the rapid growth of *Meria coniospora* within 72hours. The media in which jack beans was used as nitrogen sources had their maximum growth of the fungus recorded as 0.334g in figure 5 on the day five. This could be as a result of the fungus being able to digest glucose (a monosaccharide) faster than maltose, lactose, sucrose etc (which are disaccharides). In addition, the presence of glucose in a medium represses the synthesis of enzymes needed for the utilization of other carbohydrate substrates in the medium **(Ruijter and Visser, 1997; Suto and Tomita, 2001)**.

As observed, the maximum biomass growth of 0.536grams was recorded for the *Mucor sp* with the medium D, relative to 0.442g in the standard, 0.079g in basal medium and 0.149g as the highest recorded in the media with nitrogen sources as sample *Canavalia ensiformis* on day five. This can be attached with the findings of **Valhidi *et al.*, (2004)**, that

some fungi grow profusely in organic substances than inorganic. The medium D has the same growth pattern as the standard medium, that is, a gradual increase in biomass, while media C and F shows an initial increase followed by a decrease to day 5.

The maximum growth of *Neurospora crassa* was recorded as 1.052 grams on the fifth day while the standard had a growth of 0.238grams on the same days. Rather, the maximum growth in the standard was recorded on the third day as 0.481grams and later declined. This may be caused by maximum utilization of the nutrients by the fungi which led to the shortage of nutrient after days three in the standard. Optimal nutrient medium should provide not simply adequate growth but the best possible growth in order to allow molds grow without restriction and express all phenophytes **(Meletiadis *et al*., 2001)**.

The highest biomass of Rhizopus oryzae was recorded in medium D with a mass of 0.486gm and not only thus the medium has the highest growth for the fungus but shows the best support for the fungus which is evident in its growth from day 3 to 5 when compared with that of the standard medium. Medium E shows good support for the growth of fungus up to day 4 with a increase biomass which start decreasing from day 5.

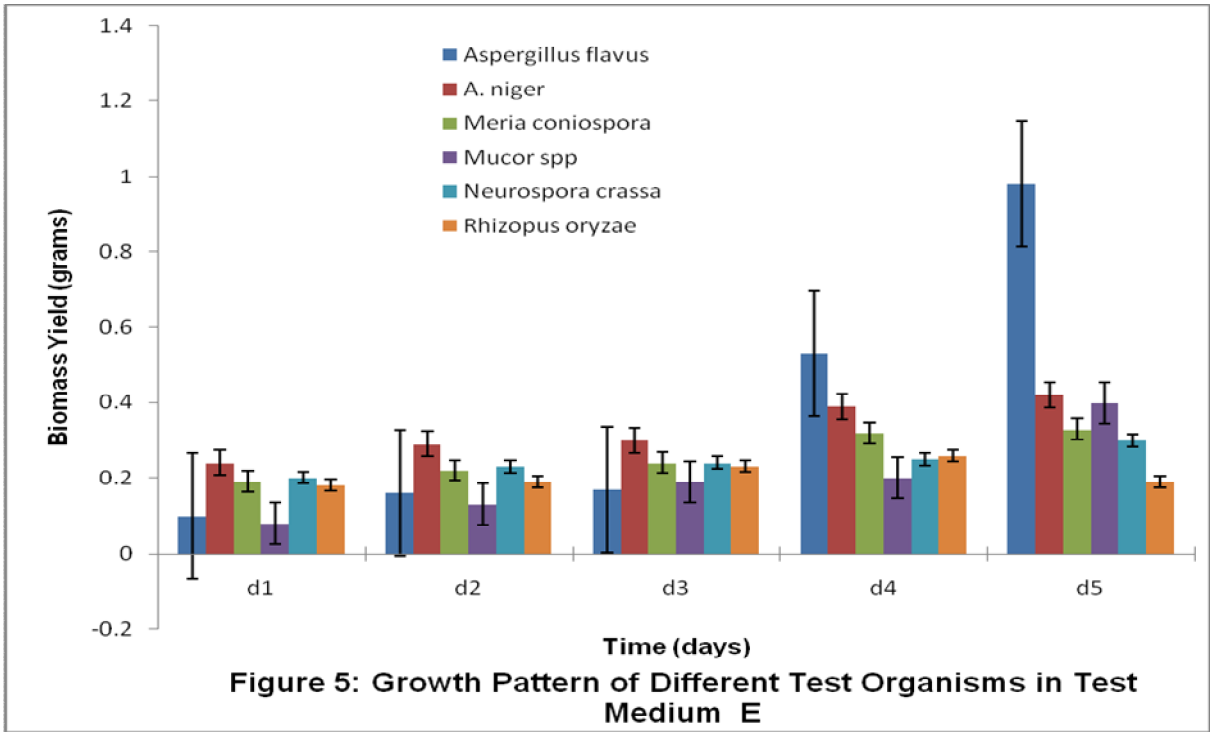

Figure 5: Growth Pattern of Different Test Organisms in Test Medium E

Legend: peptone (0.25g), KH_2PO_4 (0.26g), Jack beans (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), glucose (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

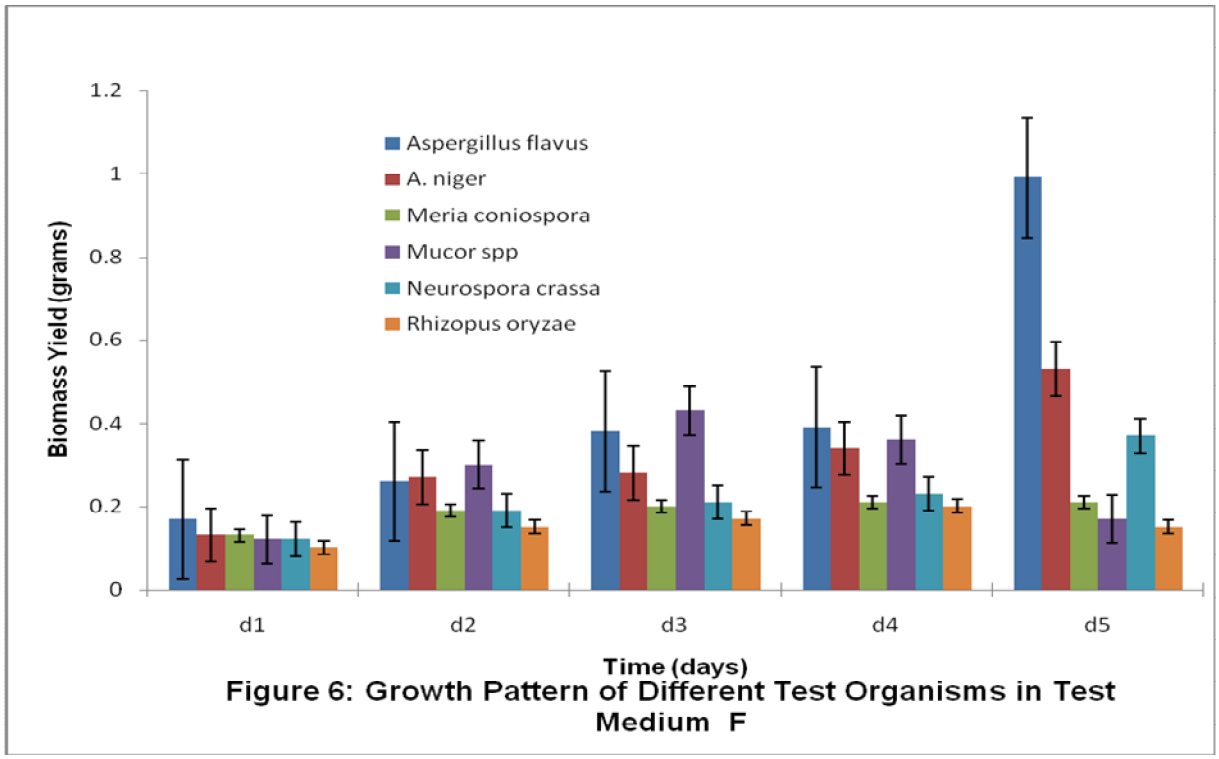

Figure 6: Growth Pattern of Different Test Organisms in Test Medium F

Legend: peptone (0.25g), KH_2PO_4 (0.26g), Jack beans (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), maltose; (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

Comparison of Growth on the Formulated Media

Of all the medium formulated with Jack beans whether as a source of carbon or nitrogen, medium D where Jack beans act as the source of carbon gave the best support for the growth of all the fungi used as test organism when compared to the standard medium. Even though, the standard medium shows a great support for the growth of all the fungi but not as good as those of medium D. The basal medium shows a continuous growth support for *A. flavus* and *M. coniospora*, while its support for the other fungi diminished after the fourth day. The replacement of the nitrogen source with an organic source in medium C shows a better growth support for all the fungi except *Mucor sp, R. oryzae* and *N. crassa* which are *Mucoraceae* and *Sordariaceae* when compared with the basal medium (B). The substitution of the carbon source with glucose and the present of Jack beans as the nitrogen source gave a better growth support to all the fungi with the exception of *R. oryzae* (on the fifth day) throughout the experiment. The present of Jack beans as nitrogen source and maltose as the carbon source only shows good growth support for *A. flavus, A. niger* and *N. crassa* up to day five, while others shows regression after day four. Medium G with the presence of lactose as the carbon source and Jack beans as the nitrogen source demonstrate a progressive growth

support for all the fungi until day 4, while there was a growth regression for the others except the *Aspergilli*. Medium H that has galactose as its carbon source while Jack beans still remains the nitrogen source displays a progressive growth support for all the fungi with the exception of *Meria coniospora* on the fifth day. This change in carbon source results was in line with the finding of **Akinyosoye and Akinyanju (1989)**.

Although other sources of carbon (such as sucrose, maltose, lactose, galactose and glucose) were expected to have supported the growth of the tested organisms (fungi) better than the raw carbon sources (jack beans), because their carbon sources are easily digestible than that of the raw carbon source, minimum biomass yield was recorded more on these refined carbon sources than that of the raw carbon sources (figures 1-8). This is an indication that natural substances such as vitamins and mineral (table 1) are essential for good growth.

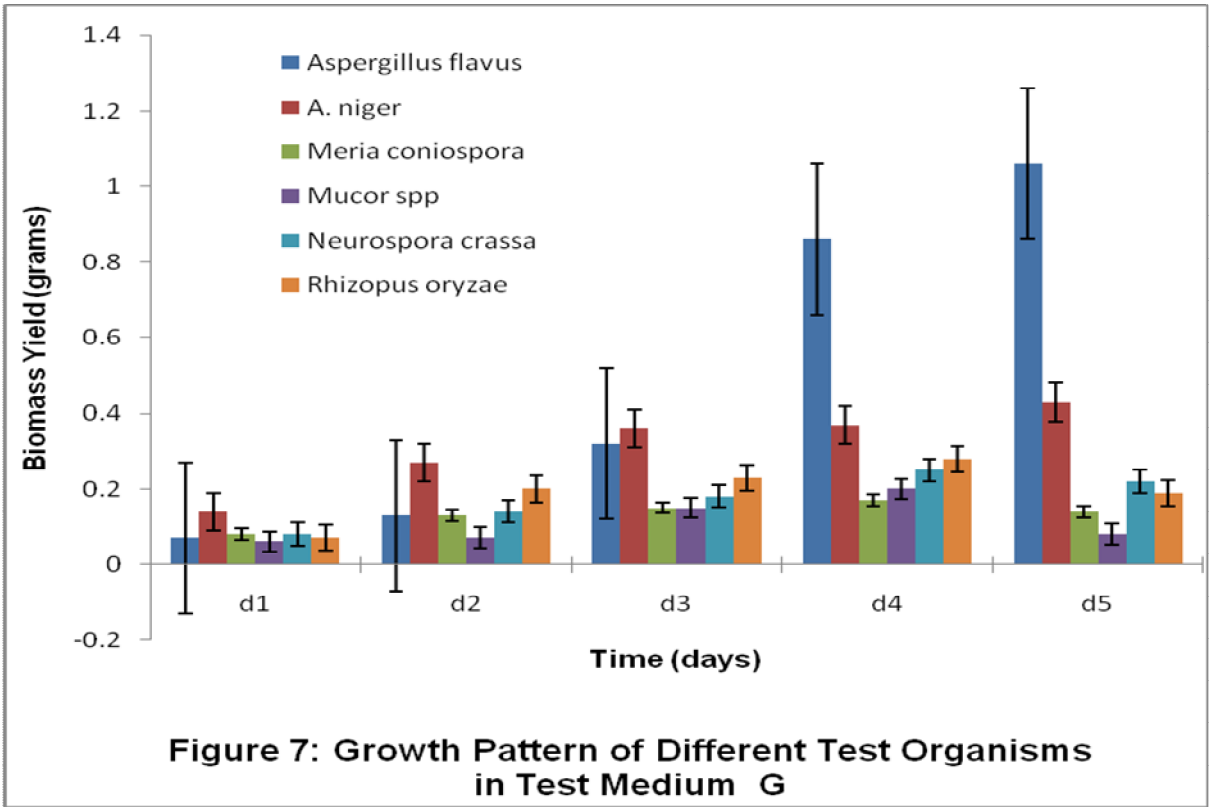

Figure 7: Growth Pattern of Different Test Organisms in Test Medium G

Legend: peptone (0.25g), KH_2PO_4 (0.26g), Jack beans (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), lactose; (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

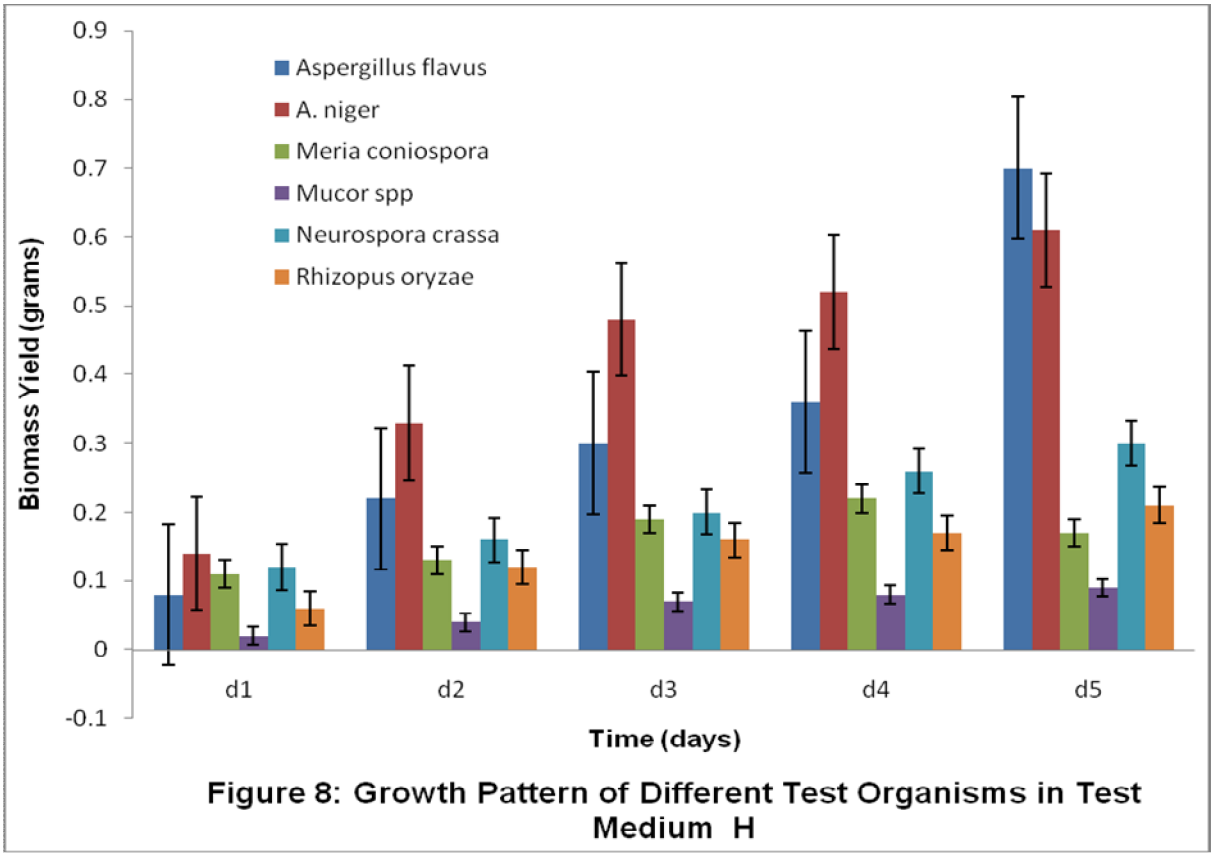

Figure 8: Growth Pattern of Different Test Organisms in Test Medium H

Legend: peptone (0.25g), KH_2PO_4 (0.26g), Jack beans (1.0g), $MgSO_4.7H_2O$ (0.025g), Na_2HPO_4 (0.5 g), galactose; (5.0g) & distilled water (250.0ml). d1 – d5 represents day 1 to day 5.

Generally, it can be drawn from this work that when organic substances are used as carbon or nitrogen sources for the cultivation of some species of fungi, the growth is found to be about the same or sometimes better in the formulated media relative to that of the standard as reported by **Adesemoye and Adedire (2005)**.

CONCLUSION

The study has revealed that *Aspergillus flavus, Neurospora crassa, Meria coniospora, Aspergillus niger, Mucor sp* isolated from fermenting jack beans and *Rhizopus oryzae* isolated from decomposing rice can utilize a variety of carbon source (raw and refined) for growth. The raw carbon source that was used for the growth studies was jack beans (*Canavalia ensiformis*) powder. The reason for this observation is not farfetched as fungi in general elaborate many hydrolytic enzymes in ailed that nature. Therefore, the ability of the above named fungi to grow on the raw and refined carbon sources is an oligomeric and polymeric unit of the carbon substrates.

In addition, the study has revealed that this raw carbon substrate contains the minerals and nutrients that can meet the nutritional requirements of the fungi, thus it can be utilized as an alternative material in the preparation of culture media for the *in vitro* cultivation of fungi for teaching and research purposes. Further research are still needed in the application of modern tools and methods to the study of fungal physiology as this will assist in the use of manipulation of waste materials into forms that can be useful.

Also, in solving the problem of shortage of culture media for laboratory practical, the results of this research will go a long way in ameliorating this problem and also assist in conserving the much foreign exchange for other developmental projects.

RECOMMENDATION

The scarcity of synthetic culture media in the local market due to its high price and the difficulties encountered in obtaining foreign exchange for the importation have led to the on-going research being abandoned by microbiologists an students of Microbiology **(Alonge,1992)**.

However, with the help of this work, the problem can be minimized by making use of locally sourced alternative food materials such as jack beans (*Canavalia ensiformis*).In this study, all the organisms showed good growth in the medium/sample formulated with jack beans as the carbon source. Therefore, it is evident that this alternative medium could be a better substrate for the culturing of fungi.

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