Mycopath (2015) 13(2): 71-80 **Research Article**

Growth assessment of three common soil fungi (*Trichoderma viride, Aspergillus niger* and *Penicillium* sp.) on formulated culture medium

*** M. Ubogu[1], E. Akponah[2] and F.E. Ejukonemu[3]**

[1]*Department of Biological Sciences, Federal University of Agriculture Makurdi, P.M.B. 2373, Makurdi, Benue State, Nigeria.*
[2]*Department of Microbiology, Delta State University, P.M. B 1, Abraka, Delta State.*
[3]*Department of Laboratory Sciences, Delta State Polytechnic, Oghara, Delta State, Nigeria.*
Corresponding author's email: ubomon@yahoo.co.uk

Abstract

The radial mycelial extension growth, mycelia extension growth rate and minimum sporulation time of three common soil fungi namely *Trichoderma viride*, *Aspergillus niger* and *Penicillium* sp. were investigated on Irish potato (*Solanum tuberosum* L.), cocoyam [*Xanthosomas sagittifolium* (L.) Schott], sweet potato [*Ipomoea batatas* (L.) Lam.], cassava (*Manihot esculenta* Crantz), yam (*Dioscorea rotundata* Poir.), shorgum [*Sorghum bicolor* (L.) Moench], rice (*Oryza sativa* L.), corn (*Zea mays* L.), millet (*Panicum miliaceum* L.), wheat (*Triticum aestivum* L.) based formulated culture medium in comparison to commercially produced packaged dehydrated potato dextrose agar (PDA) for a period of 96 h. All the formulated mediumsupported growth of the three fungi within the period of investigation. Sweet potato, Irish potato and corn based formulated culture medium consistently produced mycelial extension growth of *T. viride, A. niger* and *Penicillium* sp. that was remarkably higher than that of commercially produced PDA. The mycelial extension growth of *T. viride* and *Penicillium* sp. on tuber-based formulated medium were statistically the same with that of grain-based medium, while *A. niger* showed higher growth on grain-based medium. The minimum sporulation time for all the test fungi was 48 h in commercially produced PDA. While the minimum sporulation time for *A. niger* was 48 h, and that of *T. viride* and *Penicilium* sp. ranged from 48-96 h on the formulated medium.

Keywords: Commercially produced PDA, formulated medium, mycelial growth, sporulation time, test fungi.

Introduction

Studies in the field of microbiology for the most part, require the growth and maintenance of microorganisms in laboratory cultures which is highly dependent on the availability of appropriate culture media (Willey *et al*., 2011). Potato dextrose agar (PDA) has been regarded as one of the most important agar media in modern time (Amadi and Moneke, 2012), specifically suited for the growth and maintenance of fungi.

A number of registered companies are involved in the commercial production and marketing of packaged dehydrated PDA powder. Many microbiology laboratories in the world-over find it convenient to use commercially produced PDA because of quality assurance and the ease with which they can be reconstituted and used. In spite of these attractive features, the prohibitive cost of commercially produced PDA places additional financial burden on research students and their respective institutions especially in developing economy. In many developing countries such as Nigeria, there exist an array of starch-containing tubers and grains within the local communities which can serve as base ingredient for the formulation of culture media for growing fungi. It is on the bases of providing readily available and affordable alternatives comparable to commercially produced PDA that we examined and assessed the growth of three common soil fungi on formulated culture media from local tubers and grains.

Materials and Methods

Isolation and characterization of test fungal isolates

The three test fungi, *T. viride, A. niger* and *Penicillium* sp. used in this study were obtained from soil using the soil dilution pour plate method. Commercially produced potato dextrose agar

(PDA) was the medium for the isolation of the test fungal isolates. Plates were incubated at 30 ± 2 °C for five days for the growth of fungi. Test fungal isolates were characterized from pure plate culture based on cultural and morphological properties following the fungal identification schemes described by Rifai (1969), Barnett and Hunter (1972), Humber (2005) and Ellis *et al*. (2007).

Formulation of medium for the growth of fungal isolates

The three test fungal isolates were cultured in ten different formulated media in addition to commercially produced PDA (Oxoid, UK) (designated as S-PDA in this study) which served as control medium. Five of the media were formulated from the following tubers, Irish potato (*Solanum tuberosum*) (F-IPDA), cocoyam (*Xanthosomas sagittifolium*) (F-CYPDA), sweet potato (*Ipomoea batatas*) (F-SPDA), cassava (*Manihot esculenta*) (F-CSPDA), yam (*Dioscorea rotundata*) (F-YMPDA). While the other five media from the following grains, sorghum (*Sorghum bicolor*) (F-SHDA), rice (*Oryza sativa*) (F-RIDA), corn (*Zea mays*) (F-CNDA), millet (*Panicum miliaceum*) (F-MLDA), wheat (*Triticum aestivum*) (F-WHDA).

The commercially produced PDA was reconstituted according to the manufacturer's (Oxoid) instruction. Thirty nine (39) grams of the powdered medium was dissolved in 1 L of sterile distilled water. On the other hand, the tubers used for the formulated media were separately peeled and sliced into pieces. 200 g of the sliced pieces for each of the tubers were then weighed and added to 1 L of distilled water respectively for media preparation. Mixture of tuber and water was allowed to boil for 30 min and the extract collected using cheesecloth. Twenty (20 g) of dextrose and 20 g of agar powder were added to tuber extract and distilled water was used to make up the mixture to 1 L mark in a conical flask. These same procedures were applied to the grains which were first grinded to powdery form. All the respective formulated media including the reconstituted commercially produced medium were then autoclaved for 15 min at 121 °C. These were allowed to cool to about 45 °C and tetracycline was added (to prevent the growth of bacteria and permit the selective isolation of fungi), mixed thoroughly under sterile condition.

Inoculation of commercially produced PDA and formulated media

All the formulated media including the commercially produced medium were inoculated with the respective test fungal isolates in a 12 cm Petri dish. This was done by placing in an upside down position a 5 mm agar disc inoculum obtained (using a cork borer and inoculating needle) from the edge of a young actively growing pure culture of test fungi. Each test fungal isolate was prepared in triplicate plates for the respective formulated media including the control. All plates were then incubated at room temperature (30 ± 2 °C) for 96 h close to laboratory windows to permit sufficient 12 h day lighting alternating 12 h darkness.

Measurement of radial mycelial extension growth of fungi

Radial mycelial extension growth of all the test fungi in their respective growth media was determined by measuring the radial growth of fungi in millimeters (mm) at 0, 48, 72 and 96 h using a graduated meter rule.

Determination of mycelial extension growth rate of fungi

The mycelial extension growth rate (mm h^{-1}) of each of the test fungi on the respective growth media was determined at the end of 96 h. study period using the formula:

$$\text{Mycelial extension growth rate} = \frac{\text{Total radial growth (mm)}}{\text{Total time (h)}}$$

Determination of minimum sporulation time of fungi on growth media

The minimum sporulation time for each of the test fungi on the respective growth media was determined by observing the commercially produced and formulated fungal growth culture media at interval of 24 h for the presence and appearance of hyphal-bearing spores under a low-powered objectives (10 X).

Statistical Analysis

Data obtained from replicate samples were analyzed using measure of central tendency (mean), dispersion (standard deviation), Student's t-test and Analysis of Variance (ANOVA) ($P \leq 0.05$).

Results

Radial mycelial extension growth of fungi

The results of the radial mycelial extension growth showed that all the formulated media (tuber and grain-based) including the commercially produced medium (control) supported the growth of *T. viride*, *A. niger*, *Penicillium* sp. throughout the period of study

(Fig. 1, 2,5,6,9 and 10). However, measurement of the radial mycelia extension growth of all the test fungi taken at 96 h revealed that the extent of fungal growth on formulated media was dependent on the tuber or grain species used in media formulation except for *T. viride* where grain species did not significantly affect fungal growth. Furthermore, comparative studies on the radial mycelial extension growth on tuber-based formulated media showed that there was no significant difference in the growth of *T. viride* on S-PDA (control), F-IPDA, F-SPDA and F-CSDA. However, *A. niger* and *Penicillium* sp., showed a higher radial mycelia extension growth on both F-IPDA and F-SPDA than S-PDA. In addition, *A. niger* also showed higher growth on F-CSDA than that of S-PDA, however, the growth recorded in these two latter media were statistically the same for *Penicillium* sp. Growths of the three test fungi were lower on F-YMDA and F-CYDA than the control and other tuber-based formulated media with F-YMDA consistently higher than F-CYDA.

Studies on the radial mycelial extension growth on the grain-based formulated media showed that there was no significant difference on the extent of growth of *T. viride* on all the grain-based formulated media as compared to S-PDA. However, for *A. niger*, while there was no significant difference on the extent of growth of the fungi on F-SHDA, F-CNDA, F-MLDA and F-WHDA; all formulated media including F-RIDA yielded higher radial mycelial extension growth than S-PDA. The radial mycelia extension growth of *Penicillium* sp. on F-CNPDA and S-PDA were the same but higher than that of F-SHDA, F-RIDA, F-MLDA and F-WHDA, with F-RIDA producing the least growth. Moreover, comparative study on the overall radial mycelia extension growth between grain and tuber-based formulated media showed there was no significant difference on growth performance of the test isolates except for *A. niger* where grain-based formulated media produced higher growth than that of tuber-based formulated media.

Mycelial extension growth rate of fungi

The mycelial extension growth rate of *T. viride*, *A. niger* and *Penicillium* sp. on S-PDA were 0.41, 0.29 and 0.19 mm h^{-1}, respectively. While that of tuber and grain-based formulated media ranged from 0.37-0.42, 0.13-0.34, 0.12-0.26 and 0.42-0.44, 0.36-0.43, 0.12-0.20 mm h^{-1} for *T. viride*, *A. niger* and *Penicillium* sp., respectively (Table 1-6).

Among the tuber-based formulated media, *T. viride* recorded the highest mycelial extension growth rate of 0.42 mm h^{-1} on F-SPDA; however this was not statistically different from that of S-PDA. On the other hand, the highest mycelial extension growth rate for *A. niger* and *Penicillium* sp. were recorded on F-IPDA (0.32 and 0.26 mm h^{-1}), respectively, which differ significantly from that of S-PDA. In a similar way, F-CNDA recorded the highest mycelial extension growth rate among the grain-based formulated media for *T. viride* (0.44 mm h^{-1}), *A. niger* (0.43 mm h^{-1}) and *Penicillium* sp. (0.20 mm h^{-1}) which differ significantly from that of S-PDA except for *T. viride*.

Minimum sporulation time of fungi on growth medium

The minimum sporulation time for *A. niger* on all formulated media (tuber and grain-based) including medium was 48 h. *T. viride* and *Penicillium* sp. also recorded minimum sporulation time of 48 h on commercially produced medium and all tuber-based formulated media except for F-CYDA and F-YMDA were minimum sporulation time was 72 hrs. (Fig. 3, 4, 7, 8, 11 and 12). In the grain-based formulated media, sporulation time for *Penicillium* sp. on F-RIDA and FWHDA was 48 h. While that of *T. viride* and *Penicillium* sp. on F-CNDA, F-MLDA and F-WHDA was 72 h. *T. viride* recorded the longest minimum sporulation time on F-SHDA and F-RIDA at 96 h.

Discussion

The three common soil fungi *T. viride*, *A. niger* and *Penicillium* sp used as index of growth performance on the formulated media in this study showed that all the local tubers: Irish potato, cocoyam, sweet potato, cassava, yam, and grains: sorghum, rice, corn, millet, wheat can be employed as base ingredient in media formulation for the general growth and maintenance of fungi. These fungi do not only produce vigorous growth within a relative short period of time in the formulated media comparative to commercially produced PDA but also sporulated considerably. Previous studies also lend credence to findings here reported. In a study carried out to investigated the growth of *Fusarium moniliforme, Fusarium oxysporium, Aspergillus niger, Aspergillus flavus, Penicillium notatum, Rhizopus stolonifer, Mucor mucedo, and Aspergillus fumigatus* on cocoyam, yam, sweet potato, Irish potato and cassava formulated media. Omodara (2003) reported growth in all tubers with some producing better performance than commercially produced PDA. Similarly, Amadi and Moneke

(2012) reported a remarkable mycelial growth and sporulation of *Aspergillus niger and Aspergillus carbonarius* on media formulated from purple sweet potato, whitish sweet potato, edible cocoyam, non-edible cocoyam and yam. Favourable growths of the following fungi, *Aspergillus niger, Fusarium moniliforme, Penicillium* sp., *Cerocospora* sp., *Curvulara palescens, Botryodiplopodia* sp., *Rhizospus* sp., and *Rodotolura* sp. on corn, sorghum and millet based formulated media has also been reported (Adesemoye and Adedire, 2005).

Among the tubers and grains used as base ingredient in the media formulation in this study, sweet potato, Irish potato and corn consistently produced mycelia extension growth of *T. viride, A. niger* and *Penicillium* sp. that were remarkably higher than that of commercially produced PDA. This implies that F-IPDA, F-SPDA and F-CNDA are an ideal media for the growth of the test fungi and perhaps many other soil fungi.

The overall mycelial extension growth on tuber-based formulated media were not statistically different from that of grain-based media for *T. viride, Penicillium* sp., save for *A. niger,* were grain-based media favoured higher growth than tuber-based media. This implies that grain-based formulated media may actually be more suited for certain species of fungi than tuber-based formulated media.

The minimum sporulation time of 48 h for *A. niger* in all the media implies that the sporulation of the fungus was independent of the media used for growth, unlike *T. viride* and *Penicillium* sp. where sporulation was delayed up to 72-96 h in some of the media. Though adequate daylight has been shown to stimulate sporulation in *T. viride* (Ellison *et al.*, 1981), considerable evidence of interaction between nutritional factors and sporulation does exist in *T. viride* (Brian and Hemming, 1950). The differential minimum sporulation time witnessed among fungi in the various media in this study may therefore be inherent in the nutritional demand and utilization capacity of the fungi vis- a-vis nutritional composition of the base ingredient employed in the media formulation as the test fungi and their respective culture media were subjected to the same amount of day lighting. Thus, the Kleb's principle that sexual and asexual sporulation in fungi is generally favoured by exhaustion of nutrients (Basu and Bhattacharyya, 1962) may have come into play.

From this study, it is incontrovertibly evident that all the local tubers and grains employed in the formulation of fungi growth media could serve as valuable alternatives to the commercially produced packaged dehydrated PDA powder.

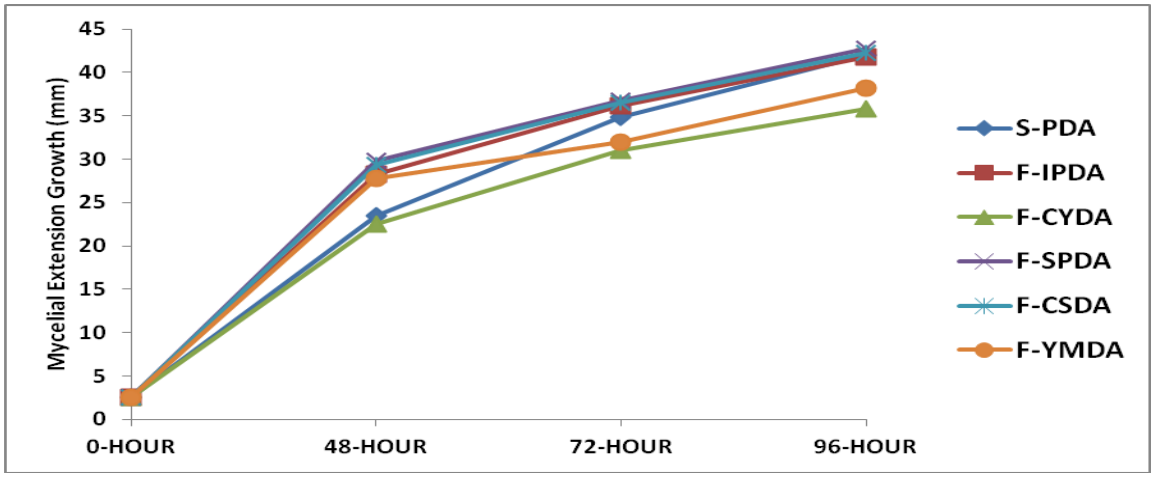

Fig. 1: Radial mycelial extension growth trajectory of *Trichoderma viride* on tuber-based formulated medium

*S-PDA (Control) = Synthetic Potato Dextrose Agar (a)
F-IPDA = Formulated Irish Potato Dextrose Agar (a)
F-CYDA = Formulated Cocoyam Dextrose Agar (b)
F-SPDA = Formulated Sweet Potato Dextrose Agar (a)
F-CSDA = Formulated Cassava Dextrose Agar (a)
F-YMDA = Formulated Yam Dextrose Agar (c)

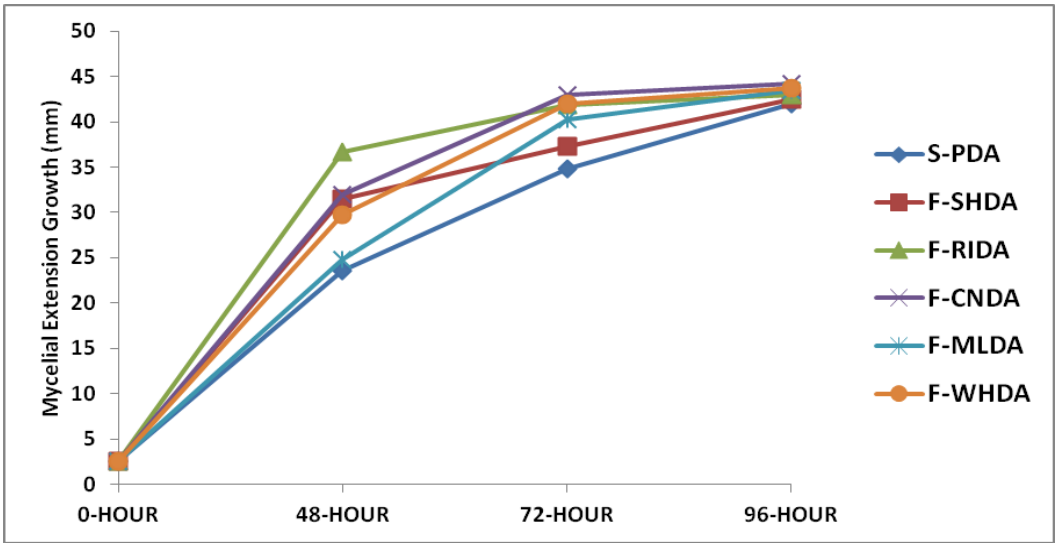

Fig. 2: Radial mycelial extension growth trajectory of *Trichoderma viride* on grain-based formulated media.

Key: *S-PDA (Control) = Synthetic Potato Dextrose Agar
F-SHDA = Formulated Sorghum Dextrose Agar
F-RIDA = Formulated Rice Dextrose Agar
F-CNDA = Formulated Corn Dextrose Agar
F-MLDA = Formulated Millet Dextrose Agar
F-WHDA = Formulated Wheat Dextrose Agar

Table 1: Mycelial extension growth rate (mm h^{-1}) of *Trichoderma viride* on tuber-based formulated medium.

Media	Mycelial extension growth rate (mm h^{-1})
S-PDA (Control)	0.41 ± 0.01^{a}
F-IPDA	0.41 ± 0.01^{a}
F-CYDA	0.35 ± 0.01^{b}
F-SPDA	0.42 ± 0.01^{a}
F-CSDA	0.41 ± 0.01^{a}
F-YMDA	0.37 ± 0.03^{c}

Values with the same alphabet in a column show did not differ significantly (P≤0.05).

Table 2: Mycelial extension growth rate (mm h^{-1}) of *Trichoderma viride* on grain-based formulated medium.

Media	Mycelial Extension Growth Rate (mm h^{-1})
S-PDA (Control)	0.41 ± 0.01^{a}
F-SHDA	0.42 ± 0.01^{a}
F-RIDA	0.42 ± 0.01^{a}
F-CNDA	0.44 ± 0.01^{a}
F-MLDA	0.43 ± 0.01^{a}
F –WHDA	0.43 ± 0.02^{a}

Values with the same alphabet in a column show did not differ significantly (P≤0.05).

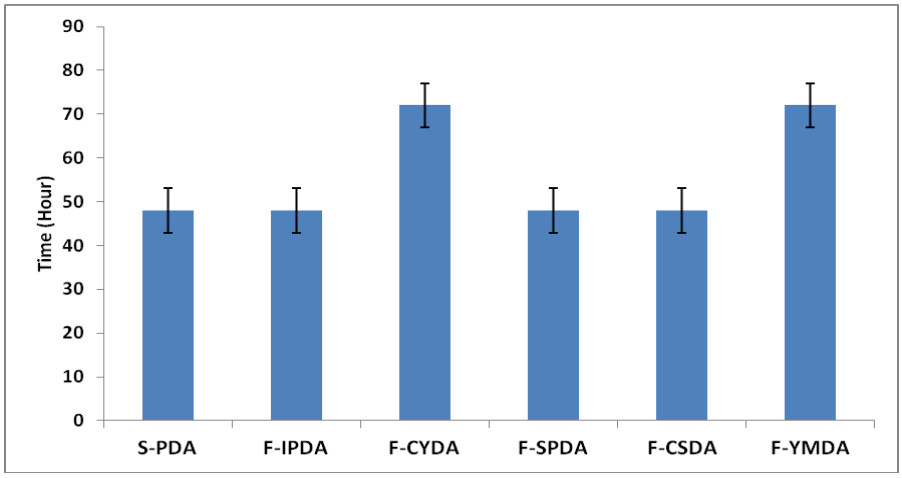

Fig. 3: *Trichoderma viride* minimum sporulation time on tuber-based formulated medium.

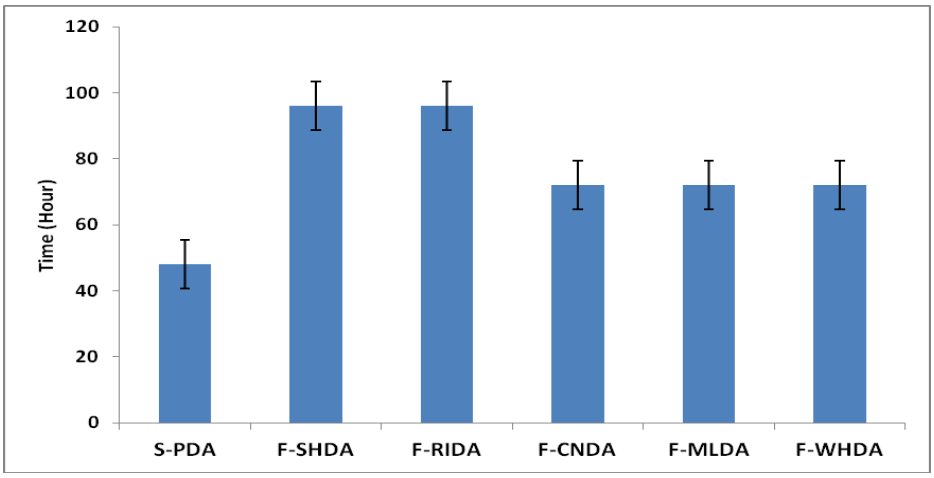

Fig. 4: *Trichoderma viride* minimum sporulation time on grain-based formulated medium.

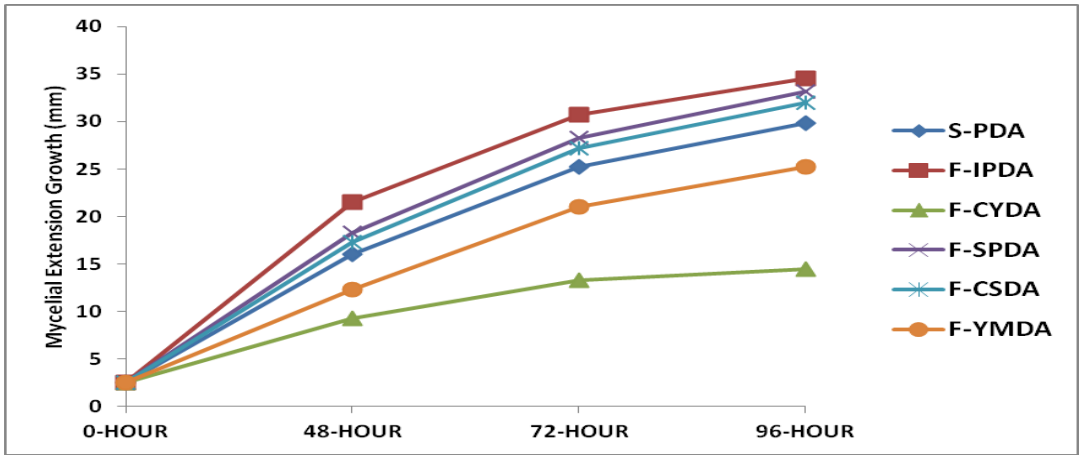

Fig. 5: Radial mycelial extension growth trajectory of *Aspergillus niger* on tuber-based formulated medium.

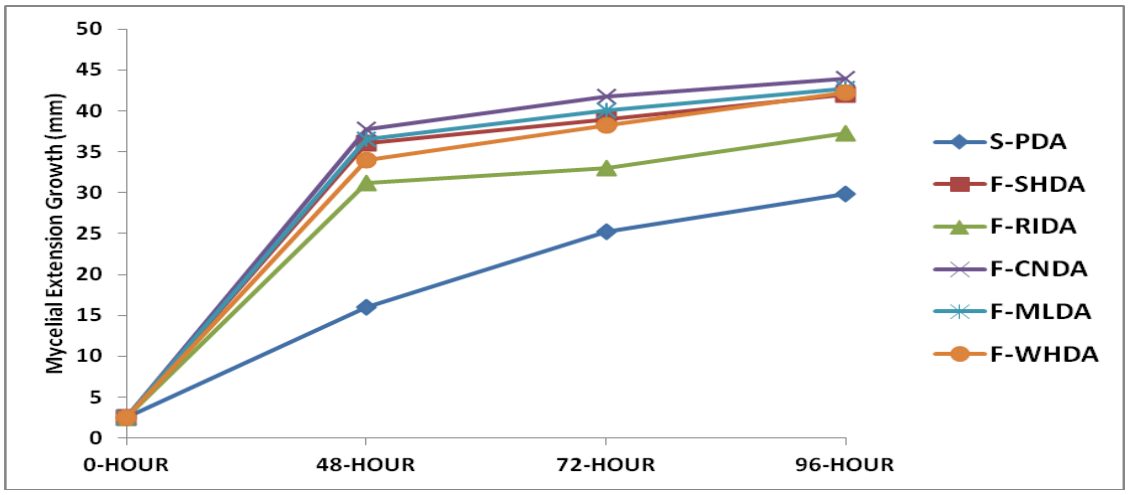

Fig. 6: Radial mycelial extension growth trajectory of *Aspergillus niger* on grain-based formulated medium.

Table 3: Mycelial extension growth rate (mm h^{-1}) of *Aspergillus niger* on tuber-based formulated medim.

Media	Mycelial Extension Growth Rate (mm h^{-1})
S-PDA (Control)	0.29 ± 0.01^{a}
F-IPDA	0.34 ± 0.04^{b}
F-CYDA	0.13 ± 0.03^{c}
F-SPDA	0.32 ± 0.02^{b}
F-CSDA	0.31 ± 0.01^{b}
F-YMDA	0.24 ± 0.02^{d}

Values with the same alphabet in a column show did not differ significantly (P≤0.05).

Table 4: Mycelial extension growth rate (mm h^{-1}) of *Aspergillus niger* on grain-based formulated media

Media	Mycelial Extension Growth Rate (mm h^{-1})
S-PDA (Control)	0.29 ± 0.01^{a}
F-SHDA	0.41 ± 0.03^{b}
F-RIDA	0.36 ± 0.02^{c}
F-CNDA	0.43 ± 0.01^{b}
F-MLDA	0.42 ± 0.01^{b}
F –WHDA	0.41 ± 0.01^{b}

Values with the same alphabet in the same row did not differ significantly (P≤0.05).

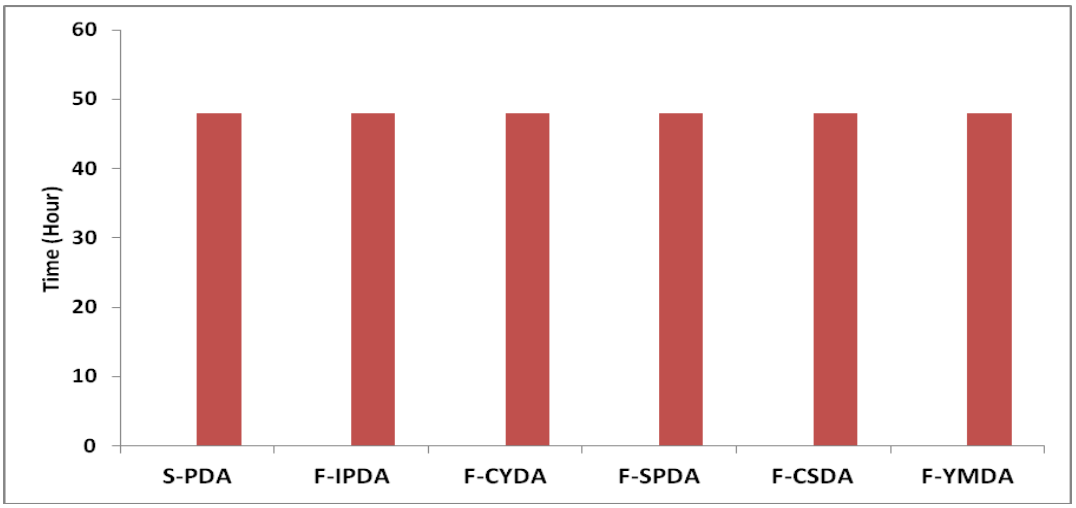

Fig. 7: *Aspergillus niger* minimum sporulation time on tuber-based formulated medium.

Fig. 8: *Aspergillus niger* minimum sporulation time on grain-based formulated medium.

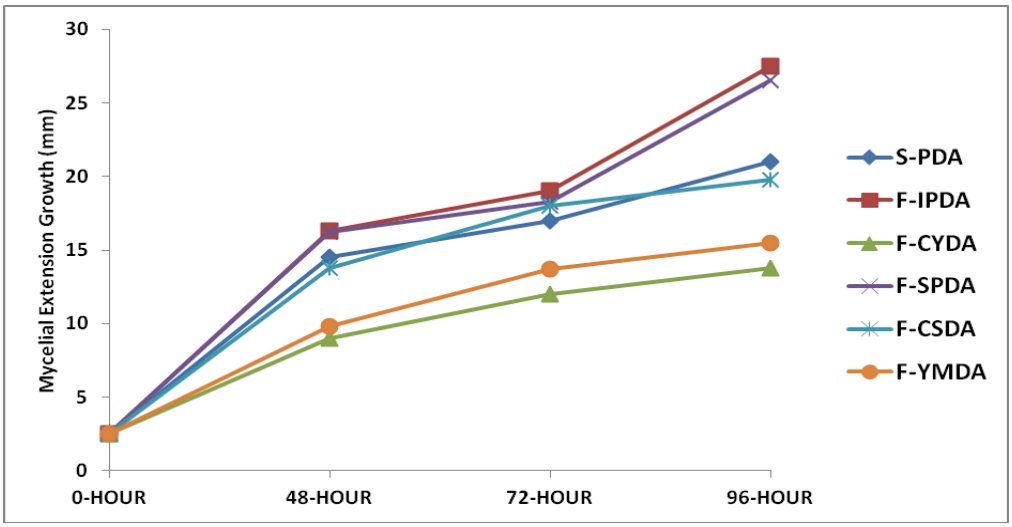

Fig. 9: Radial mycelial extension growth trajectory of *Penicillium* sp. on tuber-based formulated medium.

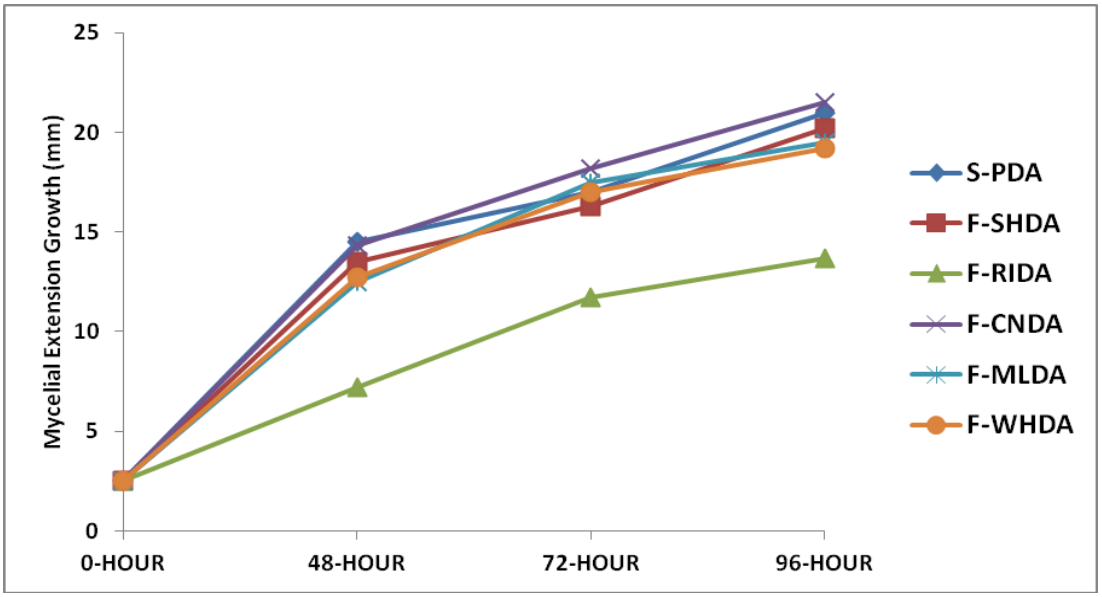

Fig. 10: Radial mycelial extension growth trajectory of *Penicillium* sp. on grain-based formulated medium.

Table 5: Mycelial extension growth rate (mm h^{-1}) of *Penicillium* sp on tuber-based formulated medium.

Medium	Mycelial Extension Growth Rate (mm h^{-1})
S-PDA (Control)	0.19 ± 0.01^{a}
F-IPDA	0.26 ± 0.05^{b}
F-CYDA	0.12 ± 0.02^{c}
F-SPDA	0.25 ± 0.02^{b}
F-CSDA	0.18 ± 0.03^{a}
F-YMDA	0.13 ± 0.01^{c}

Values with the same alphabet in the column did not differ significantly (P≤0.05).

Table 6: Mycelial extension growth rate (mm h^{-1}) of *Penicillium* sp. on grain-based formulated medium

Medium	Mycelial Extension Growth Rate (mm h^{-1})
S-PDA (Control)	0.19 ± 0.01^{a}
F-SHDA	0.18 ± 0.02^{a}
F-RIDA	0.12 ± 0.01^{b}
F-CNDA	0.20 ± 0.02^{a}
F-MLDA	0.18 ± 0.02^{a}
F –WHDA	0.17 ± 0.05^{a}

Values with the same alphabet in the column did not differ significantly (P≤0.05).

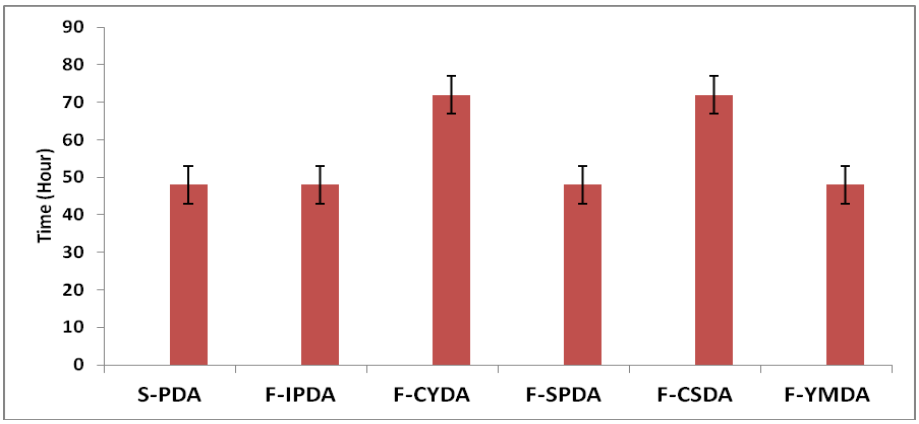

Fig. 11: *Penicillium* sp. minimum sporulation time on tuber-based formulated medium.

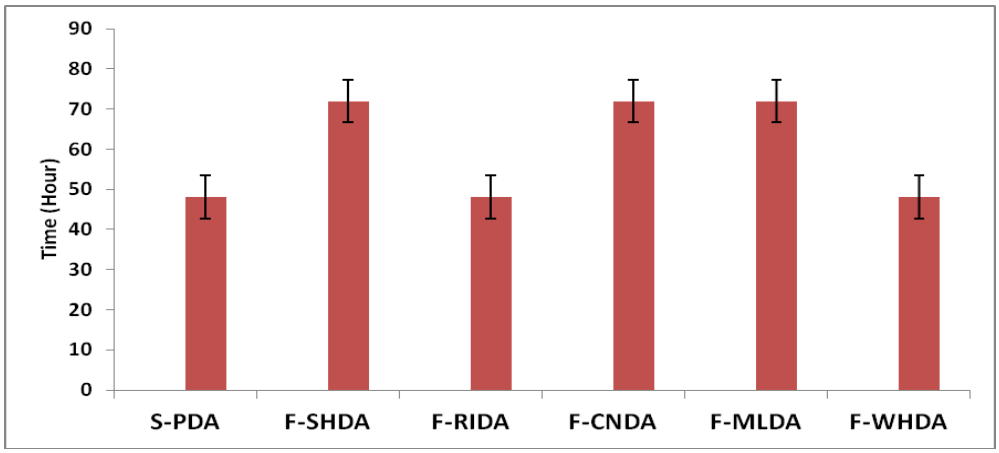

Fig. 12: *Penicillium* sp. minimum sporulation time on grain-based formulated medium.

References

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

Amadi OC, and Moneke AN, 2012. Use of starch containing tubers for the formulation of culture media for fungal cultivation. *Afr. J. Microbiol. Res.*, **6**: 4527-4532.

Barnett HL, Hunter BB, 1972. *Illustrated Genera of Imperfect Fungi*. Third edit. New York: Burgess, 225 p. ISBN 978-08-087-0266-5.

Basu SN, Bhattacharyya JP, 1962. Studies on the growth and sporulation of some species of *Penicillium. J. Gen. Microbiol*., **27**: 61-73.

Brian PM, Hemming HG, 1950. Some nutritional conditions affecting spore production by *Trichoderma viride. Tran. Brit. Mycol. Soc*., **33**: 132-141

Ellis D, Davis S, Alexiou H, Handke R, Bartley R, 2007. Descriptions of Medical Fungi, 2nd Edition. Mycology Unit Women's and Children's Hospital North Adelaide 5006 Australia.

Ellison, PJ, Harrower, KM, Owens, JD, 1981. Patterns of sporulation in *viride*. *Tran. Brit. Mycol. Soc*., **76**: 441-445.

Humber RA, 2005. *Entomopathogenic Fungal Identification*. USDA-ARS Plant Protection Research Unit US Plant, Soil & Nutrition Laboratory. Tower Road Ithaca, NY 14853-2901, USA.

Omodara OTR, 2003. Effect of formulated culture media from tubers on the growth of some fungal species. M.Sc. Desertation, Federal Uni. of Technology Akure, Nigeria.

Rifai, MA, 1969. A Revision of the Genus *Trichoderma*. Commonwealth Mycological Institute Key Survey, England.

Willey JM, Sherwood LM, Woolverton CJ, 2011. *Prescott's Microbiology*. Eight edit. McGraw-Hill, New York.