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Investigating the Use of Crude Atili Oil for Maintenance of Stock Fungal Cultures

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Abstract

Atili oil is obtained from the fruits of the Atili plant (*Canarium schweinfurtii*), a perennial tree commonly found in several parts of the country, especially the Northern and South-eastern Nigeria. In Pankshin local Government area of Plateau State, the oil extraction is done by several households both for commercial and domestic purposes. Atili oil obtained from Pankshin was incorporated in graded concentrations into Sabouraud's dextrose agar to obtain 10%, 20%, 30%, 50% and 100% oil in SDA medium. A portion (4mm plug) of an actively growing fungus from a pure culture of each of the isolates of *Microsporum ferrugineum, Trichosporon beigelii, Candida albicans, Trichophyton soudanense* and *Geotrichum candidum* was placed on the SDA-oil medium and incubated at room temperature. The cultures were observed weekly, for several months, for growth and evaluated for viability by sub-culturing onto SDA devoid of oil. Controls were set up for each of the organisms and these served as the basis for comparison. The results of this work indicate a good prospect for maintenance of stock fungal cultures with Atili oil. The benefits of this finding to the laboratory mycologist in Nigeria are discussed.

Key words: *Canarium schweinfurtii*, Atili plant, Pankshin, Sabouraud's dextrose agar

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Introduction

Fungi are found in various habitats, ranging from the soil to human and animal bodies. It is estimated that about 1.5 million species of fungi exist in the world (Hawksworth, 1991) out of which only about 90,000 have been described, many of which are available only as dead herbarium materials which have not been isolated into pure cultures. Their distribution all over the world is influenced by factors such as geographical location, climate, substrate type, fauna and flora distribution. Few countries have been explored for novel fungi discovery (Mycosphere, 2005). Maintenance of culture collections of different fungi is a way of preserving the fungal biodiversity. Culture preservation is a key procedure to having a mycology collection; and this in turn is a unique source of organisms for both fundamental and biotechnological researches. Maintenance of fungal cultures in media for a long time requires a lot of care because, with prolonged storage, the medium is used up and may dry up. As a result frequent sub-culturing may be required and this is time and material demanding, and may expose the culture to contamination, loss of sporulation and morphological properties (Silva et al, 1994; Borba and Rodrigues, 2000).

Different methods of culture preservation, among which are, oil overlay, freezing and liquid nitrogen cryogenic techniques, have been practiced in various laboratories, the one adapted depending, to a large extent on the equipment and facilities available. It is necessary to determine the right method of preservation for particular fungi species. Storage in mineral oil has been in use for a very long time and is generally considered a simple method with high rate of

cell viability after long period of storage (Diogo et al, 2005; Claudia et al, 2002; Santos et al,2005) and minimal physiological and morphological changes (Santos et al, 2005). Prolonged storage in, and the quality of the mineral oil have, however, been also reported to affect viability, sporulation and development of some fungi (Silva et al, 1994). Because of the wide diversity of fungal species, it is necessary for every mycology laboratory to have a culture collection. Investigations into novel techniques of preservation are necessary to maintain viable fungi for long periods of time (Diogo et al, 2005). Trials of new products for preservation are also important, to obtain suitable and readily available ones for different species of fungi.

Canarium schweinfurthii (Burseraceae), locally known in Nigeria as 'atili' (Hausa), ubeosa (Ibo) or origbo (Yoruba), is one of the members of the 75 species that make up the genus, Canarium. These are large evergreen trees of about 40 to 50 meters tall with alternate pinnate leaves, found in many tropical and sub-tropical countries of the world. The fruit is a drupe containing a single triangular shaped seed surrounded by a delicious purplish green pulp, which contains about 30 to 50 percent oil (Wikipedia, 2008; Agu et al, 2008). Atili oil is greenish yellow in colour and rich in fat, proteins and carbohydrates. It also contains water, ash and natural antioxidants, and of low acidity, less than 0.8% (Agu et al, 2008), similar to the olive oil (The Olive oil Source, 2008).

Preservation of cultures on agar medium at 4 – 5ºC is expensive and almost impracticable in this country, with the unstable electric power supply. Some laboratories may not have a refrigerator devoted to storage of fungi cultures, as a single refrigerator may serve for different materials. Other modern culture preservation methods are not available in many mycology laboratories in the country. The main approach to identification of filamentous fungi is still the macroscopic and microscopic examination of cultures, by observing the colonial appearance, colour, texture, sporulation, among others. To maintain viable fungi species for teaching and research it is necessary to investigate various resources at our disposal that may serve useful purposes of preservation.

Material and methods

Preparation of oil medium: Freshly prepared atili oil, made on request for this work, was obtained from Pankshin town, several kilometers from Jos, the state capital. Graded concentrations of the oil in Sabouraud's dextrose agar were prepared as follows: each of a measured quantity of the oil comprising 10ml, 20ml, 30ml, 50ml, and 100ml was added into a separate flask containing 100ml of Sabouraud's dextrose agar to constitute 10%, 20%, 30%, 50%, and 100% oil-SDA medium respectively. This was sterilized in the autoclave at 121ºC for 15 minutes. Each set was dispensed in 20-ml aliquots in sterile Petri dishes and allowed to set.

Organisms: Pure cultures of *Microsporum ferrugineum*, *Trichophyton soudanense*, *Trichosporon beigelii*, *Geotrichum candidum*, and *Candida albicans* were obtained on slants of Sabouraud's dextrose agar. All the organisms were obtained from the Mycology laboratory of the Medical Microbiology Department, University of Jos, Nigeria.

Experiment: Each plate of SDA-oil medium was inoculated with about 4mm plug of fungus from a young pure culture of each organism and incubated at room temperature. This was to determine the effect of the oil on the fungi at different concentrations. The cultures were monitored weekly for growth, extension of the colony from the inoculum, measured in millimeters. This was indicated by a plus sign preceding the numbers 1 to 5, representing the minimum and maximum growth, respectively. Controls were set up for all the tests.

Viability Test: At intervals of one month, a portion of growth from each of the cultures of the organisms in 100% oil cultures (completely overlaid with oil) was taken to inoculate slants of SDA devoid of oil. This was incubated for one to two weeks at room temperature and examined macroscopically, and then microscopically mounted in lactophenol cotton blue. This procedure was repeated monthly for five months.

Results

Luxuriant growth was obtained at 10% to 20% concentrations of atili oil in SDA medium for most of the fungi, except *Trichophyton soudanense*. *Trichosporon beigelii* exhibited enhanced growth in the oil medium even at a concentration up to 20% (Table 1). From 20% to 100% concentrations, a varying reduction of growth was observed for the different fungi, growth appearing almost absent for all the fungi at 100% concentration (Table 1). Microscopic examination of the fungi from the cultures completely overlaid with oil (100%) at the end of five months revealed little or no sporulation in all the fungi.

Subcultures of the fungi made in oil free medium to test the viability of the fungi yielded appreciable growth within two weeks of incubation. Macroscopic and microscopic features of some of the fungi were different when compared with the controls (Table 2). Three of the fungi, *Trichosporon beigelii, Geotrichum candidum* and *Candida albicans*, maintained typical macroscopic and microscopic features of their individual species as described in literature (Larone, 1995).

Table 1: Effect of various concentrations of Atili oil on the fungi

Fungus species	Concentration of oil in SDA medium (%)				
	10	20	30	50	100
Microsporum ferrugineum	+5	+5	+1	+1	0
Trichophyton soudananse	+5	+5	+1	+1	0
Trichosporon beigelii	+5	+5	+4	+3	0
Candida albicans	+5	+5	+4	+4	0
Geotrichum candidum	+5	+5	+5	+4	0

+5 = Maximum growth; +1 = Minimum growth; 0 = No visible growth

Table 2: Macroscopic and microscopic characteristics of the fungi subculture in SDA medium at the end of five months.

Fungus isolate	Colour	Texture	Reverse	Microscopic Features
Microsporium ferrugineum	Light brown	Flat with white velvety surface	Colourless	Thin septate hyphae
Trichophyton soudanense	Cream	Heaped, waxy	Colourless	Thin hyphae, few chlamydospores
Trichosporon beigelii	Yellowish-gray	Yeast-like, heaped with fine wrinkles	Colourless	Hyphae, arthroconidia and blastoconidia
Geotrichum candidum	White	Yeast-like with a granular surface	Colourless	Hypae, rectangular arthro- conidia with rounded ends
Candida albicans	Cream	Moist and pasty smooth surface	Colourless	Budding cells

Discussion

Atili oil at tolerable concentrations may enhance morphological appearance of some fungi, as shown in this study for *Trichosporon beigelii* at 10 to 20% concentrations. There was also indication of antifungal activity of this oil against some fungi even at very low concentrations. *Trichophyton soudanense* did not grow beyond 10% concentration of the oil in SDA. This further stresses the need to determine the correct preservation method for individual fungi as recommended by other reports (Deshmukh, 2003; Diogo et al, 2005; ten Hoopen et al, 2004; Claudia et al, 2005).

The variation in growth rate observed for all the fungi with increase in length of incubation was remarkable. It is possible that the oil exerted a static effect on the fungi, probably due to reduced oxygen consumption which may have led to reduction in metabolic rate (Buell and Weston, 1947). This also corroborates suggestions for periodical monitoring of preserved cultures (ten Hoopen et al, 2004).

All the fungi were recovered by sub-culture in oil-free medium after five months of preservation in SDA medium overlaid with atili oil, an indication that they were still viable, corroborating the viability of fungi preserved by oil overlay technique as highlighted in several reports (Bezerra et al, 2006; Mycosphere,2005; Claudia et al, 2002; Santos et al, 2005). *Candida albicans, Geotrichum candidum* and *Trichsporon beigelii* maintained morphological characteristics typical of their species, suggesting that the oil had no detrimental effect on their development. Yeast-like fungi of the genera Candida, Geotrichum, and many others produce lipolytic enzymes and have been shown to adapt to substrates rich in fats (Paokevieius, 2001). Two of the fungi, *Trichophyton soudanense* and *Microsporum ferrugineum* showed varying macroscopic morphology and microscopic features, an observation similar to what other workers had found with prolonged storage of some fungi in mineral oil (Silva et al, 1994; Borba and Rodrigues, 2000). A different response was anticipated from this work considering that atili oil is a vegetable oil.

From the findings in this investigation it is obvious that atili oil may be used for preservation of some fungi, especially the yeasts. Prolonged storage in the oil medium did not affect the morphological characteristics of the yeasts. The dermatophytes were less favored by this storage method and so alternatives should be sought that best suite their maintenance.

Mycology in Nigeria is still at its developmental stages and fungal cultures are required for teaching, as well as fundamental and applied mycological researches. Many times peculiar isolates have been lost as a result of inadequate preservation due to lack of proper facilities or numerous problems with existing ones. Nigeria has many unexplored resources for microbiological development, atili oil being one. Atili oil is cheap and readily available, and the procedure for its use for fungal culture preservation is easy. In our laboratory fungal species were maintained by means of periodical sampling with attendant problems. With this knowledge, atili oil preservation will be explored. The procedure will however be extended for a longer period of time to determine for how long fungi can be stored in atili oil and still remain viable, retaining their typical species characteristics.

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