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GROWTH ASSAY OF *Histoplasma capsulatum* var *duboisii* ON DIFFERENT CAVE SOIL EXTRACTS

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ABSTRACT

Studies were carried out to assay the colonial growth patterns of an isolate of *Histoplasma capsulatum* var *duboisii* on different cave - soil agars and assess the effect of these soil samples on the growth of the fungus.The isolate of *H. capsulatum* var *duboisii* used for the study was from a case of African histoplasmosis diagnosed from University of Calabar teaching hospital, Calabar - Nigeria. The growth was assessed using the colony size method. Modified soil extract agars were prepared from soil extracts collected from three bat-infested caves in Cross River State of Nigeria namely Agwagune, Bukulum, Wula and garden soil. The colonial growth patterns of *H. capsulatum* var *duboisii* showed a mean growth temperature of 30°C. The fungus also showed a better growth pattern on Bukulum soil extract agar with a peak colonial growth diameter of 38mm, followed by garden (35mm). Agwagune (32mm) Wula (29mm). Generally, the soil extracts showed no remarkable effect on the growth and survival of the fungus when compared with garden soil, thereby questioning the relevance of bat guano to the existence of this fungus in nature.

KEYWORDS: Growth assay, *Histoplasma capsulatum* var *duboisii*, caves, soil extracts

INTRODUCTION

Histoplasma capsulatum var *duboisii* is a dimorphic fungus that exists in two morphological forms i.e. yeast and mould. It is the causative agent of African histoplasmosis, systemic mycoses that exhibits two main clinical manifestations viz cutaneous and disseminated disease.

Isolation of the causative fungus was first reported from pooled soil samples in a Kenyan village (Al-Doory and Kalter, 1967). Also a study by Gugnani *et al* (1994) reported isolation of this fungus from soil admixed with bat guano and in the intestinal content of a bat found in a sandstone cave in a rural area of Anambra state of Nigeria. However, previous studies examining bats and soils from bat infested sandstone caves in Cross River state yielded no isolate of *H. capsulatum* var *duboisii*, although positive cases have been reported in this locality(Ekanem *et al*, 1998).

This study is to establish the optimum growth temperature and assess the effect of various soil samples on the growth of *H. capsulatum* var *duboisii* isolate from a case of African histoplasmosis diagnosed in the University of Calabar teaching hospital, a tertiary and reference hospital located South East of Nigeria.

MATERIALS AND METHODS

Location: Soil samples were collected from three bat–infested caves and a garden. One cave was located at Agwagune in Biase local government area (LGA) and two in Bukulum and Wula in Boki LGA of Cross River State of Nigeria. Agwagune in Biase LGA is located west central of Cross River State while Bukulum and Wula in Boki LGA are located north east of the state. The cave soils had sand and stones in them and the caves were hardly accessible, being located in very rough terrain.

Collection of Soil/bat guano samples: Soil admixed with bat guano were collected at a depth of about 10cm from each location above. Each soil sample was collected with the aid of a hand augur and put into a clean wide-mouth screw cap plastic container previously disinfected with alcohol and allowed to dry.

Media: Sabouraud dextrose agar (Biotec, UK) (SDA) and modified soil extract agar were media used for the study. A modification of soil extract agar as described by Emmons *et al* (1977) was prepared by using soil extract in place of distilled water to prepare sabouraud dextrose agar. The soil extract was prepared for each of the four soils above with garden soil acting as the control. They were labeled as follows- Agwagune-A, Bukulum-B, Wula-W and garden-G.

Soil extract preparation: Fifty grams of each soil sample was suspended in 120ml of tap water and autoclaved for 3 hours at 121°C. The supernatant was filtered through a Whatman no. 2 filter paper while still hot. After filtrations more water was added to make the total filtrate volume 100ml. This was then used to prepare the sabouraud dextrose agar in Petri plates.

Test Organism: The test organism was a clinical isolate of *H. capsulatum* var *duboisii* from a 12year old male patient from Biase LGA of Cross River State.

Growth Assay on SDA and Modified Soil Extract Agar: The colony size method as described by Fawole and Oso(1988) was used to assess the growth of the organism. Five-millimeter diameter growth of a subculture growth on SDA of the test organism was inoculated centrally into freshly prepared duplicate SDA and modified soil extract agar plates with the aid of a sterile cork borer. The plates were incubated at room temperature (26-28°C) and 35°C. The diameter of each colony growth was measured with the aid of a calibrated ruler every two days until cessation of growth was observed.

RESULTS

The colonial growth pattern of *H. capsulatum* var *duboisii* at room temperature (26-28°C) compared favourably with that at 35°C on SDA with peak growth diameters of 33mm each. The organism did not grow very well at 37°C on SDA and recorded a 12mm colonial diameter at peak growth (Fig 1). Similarly, the growth pattern of *H. capsulatum* var *duboisii* on garden (G) (Fig.2a) and Wula (W) (Fig. 2b) soils extracts at room temperature compared favourably with growth at 35°C. This pattern was observed especially with Agwagune (A) soil extracts (Fig. 2d) which showed higher peak growth diameters at room temperature than at 35°C. As indicated in Figs. 2a to 2d the fungus showed a better growth pattern at room temperature with Bukalum soil extract recording a peak growth diameter of 38mm

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Fig. 2c) followed by Garden, Agwagune and Wula soil extracts with peak growth diameters of 35mm (Fig. 2a), 32mm (Fig. 2d) and 29mm (Fig. 2b) respectively. The soil extracts generally showed no remarkable effect on the growth and survival of the organism.

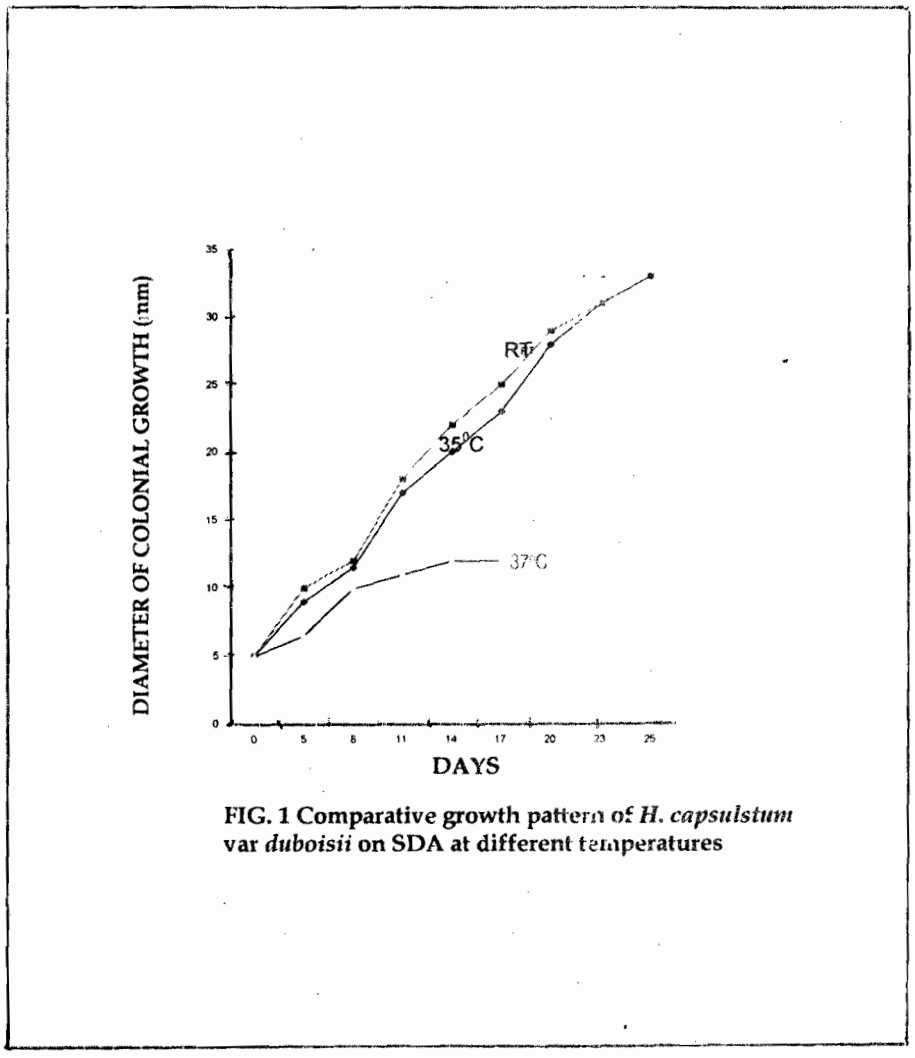

FIG. 1 Comparative growth pattern of *H. capsulstum* var *duboisii* on SDA at different temperatures

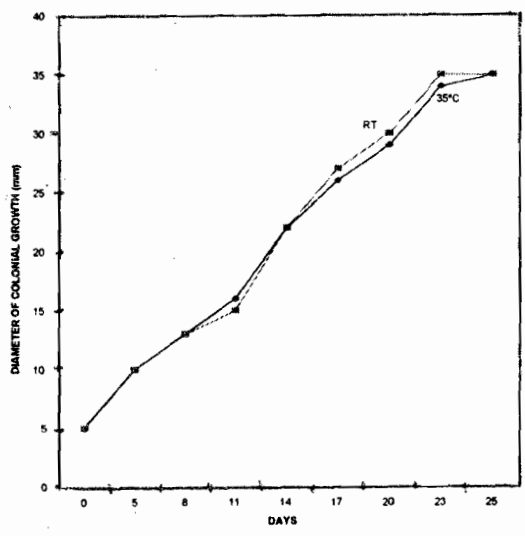

FIG. 2a. Comparative growth patterns of *H. capsulatum* var *duboisii* at RT (26-28°C) and 35°C on Garden soil extract agar medium.

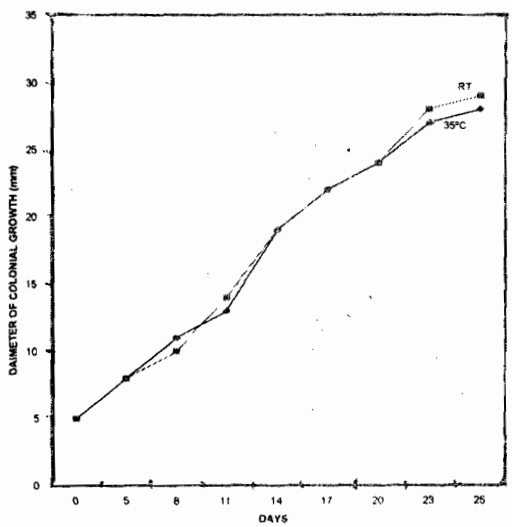

FIG. 2b. Comparative growth patterns of *H. capsulatum* var *duboisii* at RT (26-28°C) and 35°C on Wula soil extract agar medium.

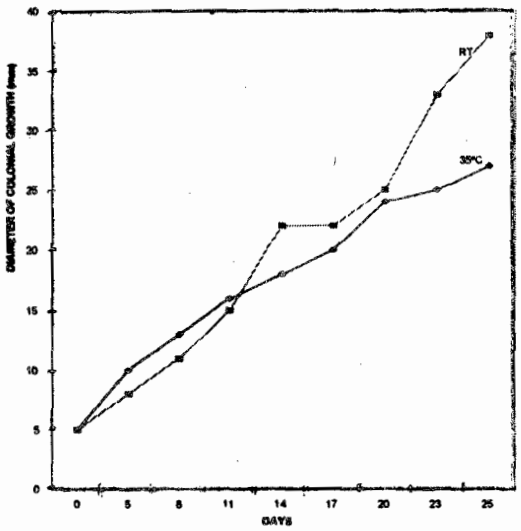

FIG.2c. Comparative growth patterns of *H. capsulatum* var *duboisii* at RT (26-28°C) and 35°C on Bukulam soil extract agar medium.

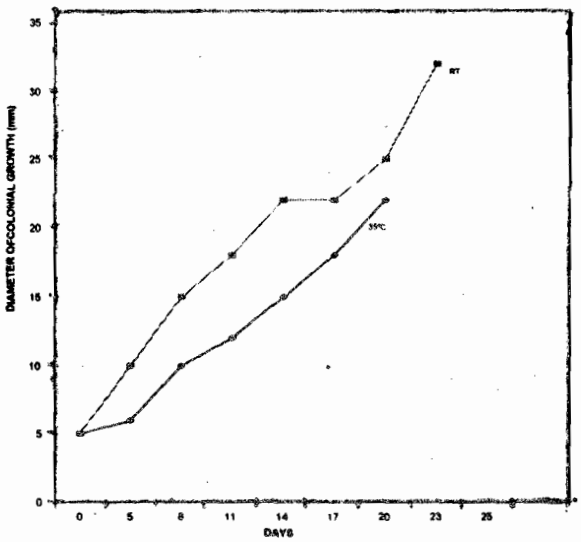

FIG. 2d. Comparative growth patterns of *H. capsulatum* var *duboisii* at RT (26-28°C) and 35°C on Agwagune soil extract agar medium.

DISCUSSION

Much of the epidemiology of African histoplasmosis is still unknown or speculative and soil is suggested to be the habitat of *H. capsulatum* var *duboisii* especially in areas contaminated with bat or fowl droppings (Basset *et al*., 1962; Cockshott and Lucas, 1964; Aubury and Lecamus, 1986). The study of Gugnani *et al* (1994) marked a major breakthrough in the isolation of *H. capsulatum* var *duboisii* from its natural habitat-soil and linked its association with bat guano. Long before now, the first discovery of this fungus from soil in Kenya(Al-Doory and Kalter, 1967) was later questioned by De Vroey(1972) who had an unsuccessful attempt at isolating this fungus from soil. Due to the difficulty in isolating this fungus from soil, researchers have shied away thereby creating a big gap between Al-Doory and Kalter's isolation and Gugnani *et al*'s discovery. A gap that spans for a period of almost 30years (1967-1994).

The optimum growth temperature range of this isolate was between 26 – 35°C with mean temperature at 30°C. Most diagnostic procedures recommend 25-30°C as temperature for isolating the mould form of *H. capsulatum* var *duboisii* (McGinnis, 1980; Larone *et al*., 1999). This study shows that a temperature of 30°C is most appropriate for cultivation of this organism in our environment.

The use of soil extracts from cave soils with bat guano did not influence the growth pattern of the *H. capsulatum* var *duboisii* isolate as the growth curve of the organism on media prepared from these soil extracts measured favourably with that grown on garden soil extract which was used as the control. Therefore, presence or absence of bat guano in soil may not have any effect on the nutritional requirements and existence of *H. capsulatum* var *duboisii* in its natural habitat even though this organism has been isolated from and associated with such soils.

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