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# Growth response of *Botryodiplodia theobromae* to some environmental factors and its implication on yam storage

**A. A., Markson [*1], G. Omosun[2], B. E. Madunagu[1], A. C. Amadioha[3], S. E. Udo[4], E. J. Umana[1], U. Ekaluo[5]**

**ABSTRACT**

Successful yam storage has always been mitigated by activities of microorganisms. One of such is *Botryodiplodia theobromae* Pat. This fungus is a serious rot pathogen of many tuber crops. It causes dry rot of yam in the field and in storage. This study investigated some environmental factors that influence its growth *in vitro*. The factors considered were temperature and nutrient sources (carbon and nitrogen). Results of this study revealed that the growth of *B. theobromae* was supported within the temperature range of $20^0$C and $30^0$C. Below the lower limit, there was no initiation of growth and above the upper limit; there was significant decline in growth. At $40^0$C, there was a total cessation of growth. Of the five carbon sources tested, sucrose gave the highest growth (110mg mycelial dry weight) while Carboxymethyl Cellulose (CMC) recorded the least mycelial dry weight of 70mg. Urea was the nitrogen source that supported the growth (100mg) of the test pathogen best, followed by potassium nitrate. Ammonium nitrate recorded the lowest growth value which did not differ from the 40mg weight recorded in the control experiment.

## INTRODUCTION

*Botryodiplodia theobromae* Pat. is a coelomycete, a facultative parasite and one of the pathogens causing rot of various plant parts such as fruits, stems and tubers (Amadioha and Markson, 2007). Cultures of this fungus produce initially white, fluffy and feathery mycelia which become grey and eventually black growing in a radial pattern from the centre of the plate outwards. The mycelia are hyaline, septate, producing brown coloured, one-celled conidia borne singly at the tip of each conidiophore (Markson, 2010a). It has been reported as the most virulent in post harvest deterioration of yam tuber (Okoro and Nwankiti, 2004). Damages resulting from attacks by *B. theobromae* are in the form of cell wall maceration, starch depletion, reduction of nutrient elements and necrosis (Markson *et al.*, 2010a and b).

Environmental factors are key determinants of the level of activity and survival of organisms. Important among such factors are nutrients and temperature. The amount of nutrient and the level of temperature to which an organism is exposed determine its level of growth. Kim *et al.*, (2005) reported on the influence of temperature on the growth of six isolates of *Sphaeropsis pyritrescens* causing rot of pear. They discovered that the fungus could grow within the temperature range of $-3^0$C and $25^0$C but at $35^0$C growth was inhibited. At $-3^0$C the mycelial growth was relatively slow (0.28mm$d^{-1}$ in average) and appeared fluffy, and at 0, 5 and $25^0$C, the fungus produced thin mycelium in somewhat circular colonies. They recorded optimum growth at temperature of $20^0$C with an average growth of 7.3 mm d-1. In a similar study to assess the effect of temperature on four rot pathogens of cowpea in Akwa Ibom state,

Asuquo (1997) observed that *B. theobromae* could initiate growth at temperatures below $20^0$C in culture. He reported a growth decline from $30^0$C and a total cessation of growth at $40^0$C.

The effect of some nitrogen sources on two pathogens; *Rhizopus oryzae* and *Rhizoctonia bataticola* was investigated by Amadioha (1995). He reported potassium nitrate as the best source of nitrogen for the growth of both pathogens. On the influence of carbon source on the growth of *Geotrichum candidum*, sucrose was reported as the most effective in supporting the growth of the organism (Akinyosoye and Akinyanju, 1989).

Many investigations on the effect of temperature have been focused on the direct influence of temperature on the physiological activity of the stored products and not on the activity of the storage microbes causing damage in storage. This paper therefore presents the influence of temperature and nutrient sources on the activity of the rot pathogen, *Botryodiplodia theobromae* as a means of modifying environmental conditions to discourage the survival of pathogens in storage rather than the direct approach of control using chemicals which are not environmental friendly.

## MATERIALS AND METHODS

### Sample collection and pathogen identification

Symptomatic and asymptomatic tubers of white yam (*Dioscorea rotundata* Poir) were sampled from open market stalls in three markets in Calabar, Cross River State, Nigeria. The markets were Akim, Marian and Watt.

*Corresponding author.
[1]Department of Botany, University of Calabar, Calabar, Nigeria.
[2]Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.
[3]Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Nigeria.
[4]Department of Biological Sciences, Cross River State University of Technology, Calabar, Nigeria.
[5]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.

Tissues about 5mm in diameter from the symptomatic and asymptomatic white yam tubers were removed following surface sterilization with 70 % ethanol for 10s, blotted dry with sterile paper towel, and plated onto chloramphenicol-amended Potato Dextrose Agar (PDA). After three days of incubation at 28$^{o}$C, microbial growth was assessed by microscopy. Cultures of the isolates were transferred to new PDA-containing plates, from where axenic cultures were generated (Gevens *et al*., 2008). Identification of the isolates were based on morphological characteristics, described in the 1987 illustrated genera of fungi by Barnett and Hunter and with literature on identification of pathogenic fungi by Rossman *et a*l, (1997). Confirmation was made by comparing with cultures identified by International Mycological Institute, Egham, UK.

**Pathogenicity test**

The confirmation of the pathogenicity of isolates from white yam was done using axenic cultures of the isolates to inoculate three white yam tubers per pathogen with 5mm-diameter mycelial agar discs of a 4-day-old culture. On appearance of symptoms, the tissues at the margins of the healthy and diseased parts were surface-sterilized, excised and plated onto PDA for incubation at 28$^{o}$C for four days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates.

**Effect of temperature on the growth of *Botryodiplodia theobromae* in culture**

The effect of temperature on the mycelial growth of *B. theobromae* was carried out in incubators set at temperatures between 20 and 40$^{0}$C at 5$^{0}$C interval. Twenty millilitres of sterilized nutrient broth (NB) was dispensed into each 100ml conical flasks and inoculated with a 5mm diameter of a four day old mycelia plus agar disc of *B. theobromae*. The incubation was for seven days at 20, 25, 30, 35 and 40$^{0}$C. The mycelia were harvested and filtered using a pre- weighed filter paper. The filter paper containing the mycelia were dried to constant weight at 80$^{0}$C for 24 hours in an oven allowed to cool and reweighed. The weight of the mycelia was determined by subtracting the weight of the filter paper from the weight of filter paper plus weight of mycelia (Amadioha, 2000).

Wm = Wfm –Wf.

Where Wm = weight of mycelia

Wfm = weight of filter paper + weight of mycelia

Wf = weight of filter paper

**Effect of carbon sources on growth of *Botryodiplodia theobromae* in culture**

This test was done using a carbon-free sterilized basal medium (Akintosoye and Akinyanju, 1989). The medium contained 0.1g of $NH_4NO_3$; 11.5g of $KH_2PO_4$; 0.5g of Mg $SO_4.7H_2O$; 0.1g of $CaCl_2.H_2O$; 0.01g of NaCl; 2ml of stock micronutrients solution containing 1.2g of $FeCl_3.6H_2O$; 0.2g of $MnCl_2.4H_2O$; 1.6g of $ZnSO_4.7H_2O$; 0.4g of $CoCl_2.6H_2O$; 0.4g of KI; 0.4g of $H_2SO$; 0.2g of $CuSO_4$ and distilled water to make up one litre. A 1% (w/v) of glucose, sucrose, starch, pectin, carboxmethyl cellulose (CMC) were sterilized separately and incorporated into the medium as the carbon source. Twenty millilitres of each of the sterile carbon source was aseptically added to 20ml portion of the sterile basal medium in a 100ml Erlenmeyer flask. Each flask was inoculated with a mycelial disc (5mm diameter) of the test fungus and incubated at 30$^{0}$C for 10 days. Thereafter, the mycelia were filtered, oven-dried at 80$^{0}$C for 24 hours and weighed using a Bitec electronic weighing balance (Amadioha and Markson, 2007).

**Effect of nitrogen sources on growth of *Botryodiplodia theobromae* in culture**

The basal medium used in this study was a nitrogen-free sterilized basal medium containing, 10g glucose; 0.01g NaCl and 2ml micronutrients solution per litre of distilled water. A 0.3% (w/v) solution of each of the following sources: aspartic acid, $KNO_3$, urea, glutamic acid and $NH_4Cl$, was sterilized separately and 20ml of each of these was added aseptically to 20ml of the sterile basal medium in a 100ml Erlenmeyer flask. Each flask was inoculated with a 5mm diameter fungal disc and incubated at 30$^{0}$C for 10 days. Thereafter, the fungal mycelia were filtered, oven-dried at 80$^{0}$C for 24 hours and weighed using Bitec electronic weighing balance (Amadioha and Markson, 2007).

## RESULTS AND DISCUSSION

**Sample collection and pathogen identification**

Following isolations of rot-causing pathogens of yam, three isolates (*Botryodiplodia theobromae, Rhizopus stolonifer* and *Penicillium expansum*) were obtained. Of the three, one was very virulent. On PDA, the virulent isolate was *B. theobromae*. The colony was initially white, fluffy and feathery, becoming grey and eventually black. The growth was radial in pattern from the centre of the plate outwards. Literature on identification of pathogenic fungi (Rossman *et al*., 1997) corroborates these observations and the appearance of this fungus (*B. theobromae*), fitted the description of *Botryodiplodia* Pat. (=*Lasiodiplodi theobromae* (Pat.) Griff and Maubl.) given by Marley (1998). The true identity of this fungus was confirmed by comparing their cultures with that identified (IMI 347961) by International Mycological Institute, Egham, UK.

**Koch's postulates and pathogenicity test**

The *B. theobromae,* isolate was pathogenic on the three (3) white yam tubers used for the test. Symptoms of decay (rot) caused by *B. theobromae* was observed as dry black rot.

**Effect of temperature on the growth of *Botryodiplodia theobromae* in culture.**

The effect of temperature on mycelial growth of *B. theobromae* was investigated using the mycelial dry weight method. The result is presented in Fig. 1. At $20^0$C the test pathogen recorded a dry weight of 0.98mg which increased to 1.08mg at $30^0$C and then declined as the temperature increased indicating the sensitivity of the pathogen to temperature above $30^0$C. The increase in growth with rise in temperature above minimum up to the optimum is attributed to the positive effect of temperature on the many metabolic processes that control growth (Jacome and Schun, 1993). Beyond $30^0$C, there was a sharp growth decline; and at $40^0$C, the growth ceased. Cessation of growth at this temperature level suggests a possible negative effect of high temperature on the enzyme systems of the test pathogen. One of such temperature-incited negative effects on enzymes is denaturation. In a similar study assessing the effect of temperature on four rot pathogens of cowpea in Akwa Ibom state, Asuquo (1997) reported a similar growth pattern for *B. theobromae* attributing it to a bridge in enzyme activity. The eventual rapid decline in growth of the pathogen beyond the optimum temperature could result from the effect of high temperature on the physical state of the cytoplasm (Jacome and Schun, 1993). Several storage methods are employed in yam storage. Such methods include above-ground methods (heaping and covering with tarpaulin, storing on shelves, in barns) and the underground pits (Ezeike, 1995; Osunde, 2008). In each of these methods, different levels of temperatures are maintained. Air temperatures of $25^0$C and $35^0$C have been reported for pit and barn respectively. In the present investigation, temperatures between $20^0$C and $30^0$C favour the growth of *B. theobromae*, hence storage in soil pits should not be used as it will encourage *B. theobromae* attacks on the tubers and as such should be discouraged. However, if it is modified to support elevated temperatures within the range of $30^0$C and $35^0$C, it can be considered. Storage in barns is recommended as air temperatures will not support the growth of *B. theobromae*. Of the numerous plant disease management methods, physical methods are the least employed (Arinze, 2005). The influence of temperature of above $30^0$C on the growth of *B. theobromae* could be exploited as an effective physical control therapy for the management of tissue rot of yam incited by this fungus especially if applied at the point of initiation of infection.

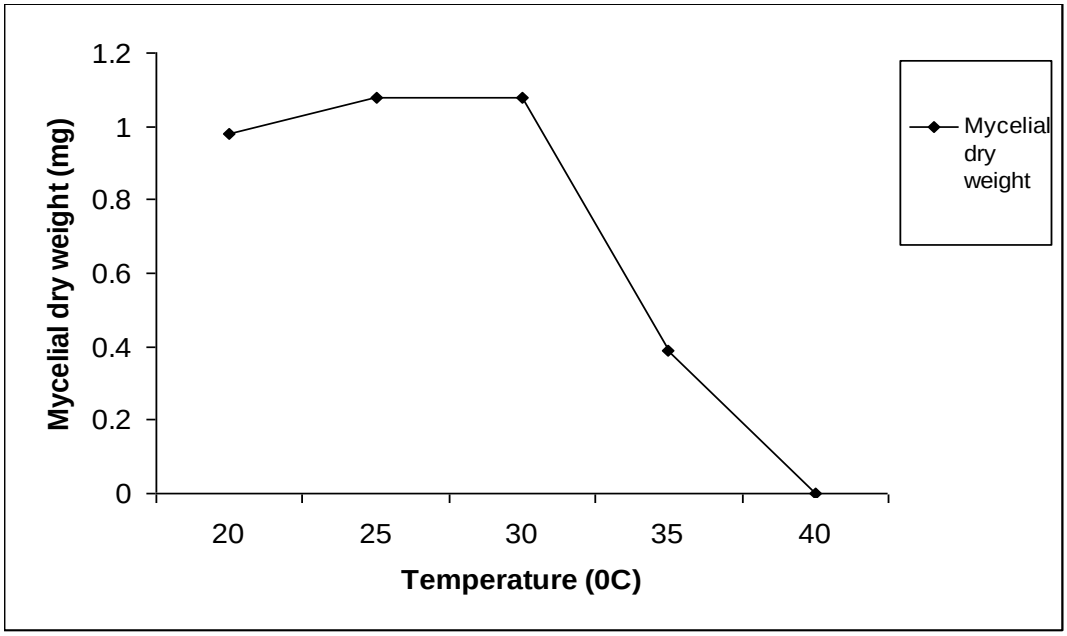


Fig. 1. Effect of temperature on the growth of *Botryodiplodia theobromae* in culture.

**Effect of carbon sources on the growth of *Botryodiplodia theobromae* in culture.**

The effect of carbon sources on the growth of *B. theobromae* in liquid medium is presented in Fig. 2. All the treatments were significantly different ($P<0.05$) from the control experiment. The medium containing sucrose as the only carbon source recorded the highest mycelial dry weight (110mg). This was closely followed by glucose (100mg), then starch (90mg). CMC and pectin each recorded 75mg mycelial dry weight whereas 25mg dry weight was recorded in the control experiment. Akinyosoye and Akinyanju (1989) also reported sucrose as a better carbon source for the growth of a related fungus- *Geotrichum candidum.* The preference of sucrose by *B.*

*theobromae* is likely a function of the chemical structure of the compound (sucrose) compared with other sources of carbon. The findings of this study could be exploited to protect yam from invasion by *B. theobromae* and related pathogens through bio-modification of yam tissues for availability of the carbon source that is least preferred by the prevalent pathogens in the storage environment.

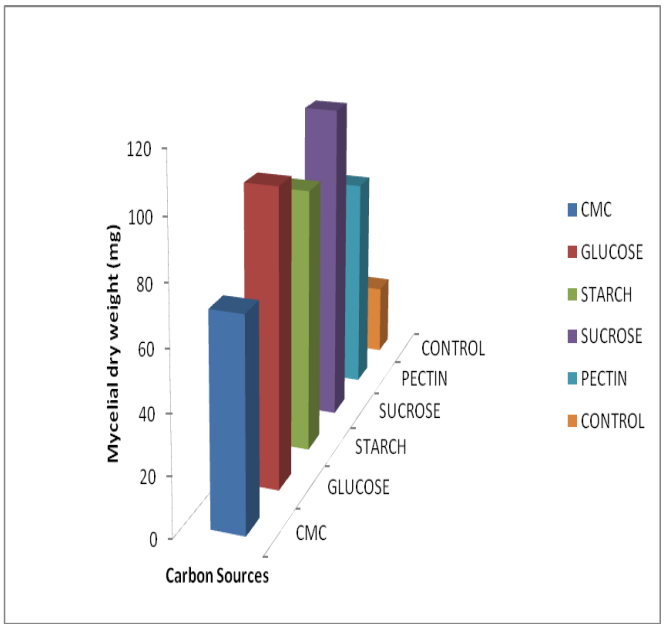


Fig. 2. Effect of carbon sources on the growth of *Botryodiplodia theobromae* in culture.

**Effect of Nitrogen sources on the growth of *Botryodiplodia theobromae* in culture.**

Nitrogen is an important component of various cellular structures. The supply of this nutrient element is therefore crucial to the survival and development of any biological system. The response of the various nitrogen sources on the test fungus revealed that all the nitrogen sources impacted on the growth of the pathogen, to varying degrees (Fig. 3). Urea was found to be the best nitrogen source (100mg) for the growth of *B. theobromae*. This was followed by potassium nitrate (90mg), aspartic acid (80mg) and glutamic acid (65mg). Ammonium nitrate (40mg) was the least effective in supporting the growth of the fungus in culture. A mycelial dry weight of 35mg recorded in the control experiment was however not significantly different ($P<0.05$) from that obtained with ammonium nitrate treatment. The results obtained in this study differed with that reported by Amadioha (1995) in a similar experiment carried out using potato tubers infected with two pathogens – *Rhizopus oryzae* and *Rhizoctonia bataticola* where potassium nitrate was the best nitrogen source and urea the least in supporting the growth of the two organisms. In this study, the test fungus recorded the highest growth in the medium containing urea as the source of nitrogen followed by potassium nitrate. The variation in these two results may be due to differences in preference of the nitrogen sources by the different pathogens in the two separate experiments.

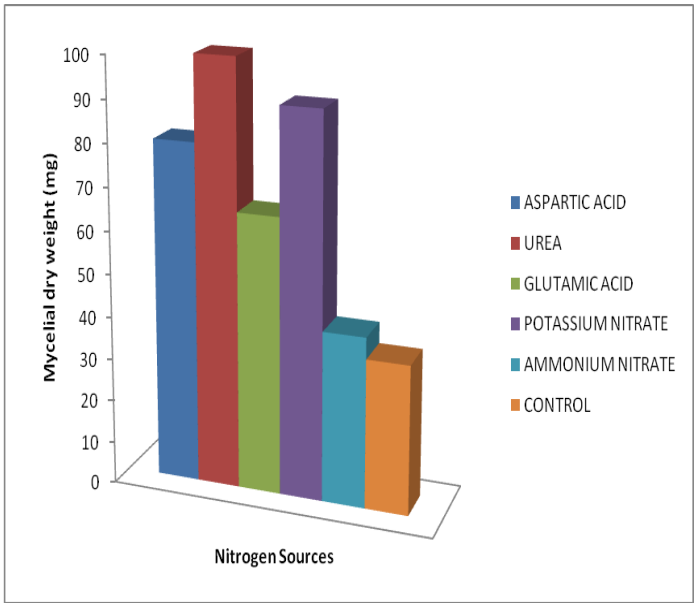


Fig. 3. Effect of Nitrogen sources on the growth of *Botryodiplodia theobromae* in culture.

Yam has, for ages, continued to contribute to the survival of humans and animals by providing a ready source of carbohydrate and raw materials for industries. However, the forms of carbohydrate (sucrose and glucose) preferred by the pathogens are those easily available on hydrolysis of starch. It is also seen that the ambient temperature range for yam storage coincides with that favourable for the growth and development of the pathogens. From the afore-going, it is necessary to develop new and novel storage methods which will ensure safe storage of yam and at the same time discourage the survival and proliferation of pathogens in storage.

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