Studies on *Aspergillus flavus* on tiger nut (*Cyperus esculentus*) incubated at different regimes of light

ABSTRACT

An experiment was carried out to ascertain the effect of light regimes (continuous day light, continuous darkness and alternative day light and darkness) on the growth of *A. flavus* on tiger nut at temperature of 28^{o}C and 30^{o}C. The experiment was a 2 x 3 factorial laid out in a completely randomized design (CRD) replicated 10 times. *A. flavus* was isolated from naturally infected tiger nuts obtained from different sellers at Delta park, Abuja park and Choba campus, University of Port Harcourt, Rivers State, Nigeria. The isolation and sub-culturing were done using Malt Extract Agar (MEA). A tiger nut-based-medium (15ml/Plate) was centrally inoculated with 6-day-old inoculums of *A. flavus* and incubated appropriately. Assessment of fungal growth was done daily for 10 days and mycelia diameter and mycelia growth rate obtained from the data. Result from the statistical analysis showed that the effect of light regime and temperature on the mycelia diameter of *A. flavus* was highly significant (P<0.001) and the interaction between light regime and temperature was also significant (P<0.0.01). Growth rate also showed significant (P<0.001) main effect of temperature and light regime and the light regimes x temperature was also significant (P=0.03). The highest mycelia growth rate and mycelia diameter occurred in continuous day light, followed by continuous darkness and finally alternative day light and darkness, with the maximum at 30^{o}C. Thus, storage of tiger nut should not be under continuous day light or at temperature >28^{o}C.

Keywords: *Aspergillus flavus*, *Cyperus esculentus*, Malt Extact Agar, Mycelia diameter, growth rate.

INTRODUCTION

Tigernut (*Cyperus esculentus*) is a consumable perennial grass-like plant of the sedge family (Turesson et al., 2010). Tiger nut is referred to by different names like nut sedge, earth almond, palatable galingale and ground almond (Defelice 2002; Sanchez-zapata et al., 2012). The three most populated ethnic groups in Nigeria which are Hausas, Igbos, and Yorubas called tiger nut tubers 'Aya', 'Ofio' and 'Imumu', respectively (FAO, 1988). A generally satisfactory name given to tiger nut in Southern Nigeria is 'Aki Hausa' which in a real sense portrays a nut that is to a

great extent cultivated and marketed by the Hausas that dominate Northern Nigeria (Bamishaiye and Bamishaiye, 2011; Udeozor and Amonorin, 2014).

It is generally believed that *Cyperus esculentus* is a nut considering the literal interpretation but according to Onyerinde and Olalusi (2013), he reported that *Cyperus esculentus* is not a nut but actually a tuber because it shares more characteristics with other tubers than nuts and therefore considered as a tuber. The various varieties of tiger nut that are in cultivation are: 'black, brown and yellow' (Umerie et al., 1997). From these three varieties, just two of them i.e yellow and brown varieties are found in most nearby market in Nigeria. Out of these varieties, the yellow variety is desired over others on account of its intrinsic properties like enormous size, appealing colour and fleshier nature; it additionally produces more milk during extraction, contains lower fat and higher protein and less anti-nutritional factors especially polyphenol (Okafor et al., 2003). Cultivation of tiger nut takes place in some West African countries such as, Nigeria, Cameroon, Senegal, Guinea and Ghana etc. In Nigeria, tiger nut is cultivated mostly in the northern region and the tuber is accessible in the market throughout the entire year (Bamishaiye and Bamishaiye, 2011). Tigernut is likewise filled in the center belt of Nigeria (Nwanguipke, 2010). Tiger nut has a lot of health benefits and has been recognized long ago as one of the best nutritional crops used to augment diets due to its richness in calcium and iron for body growth and development (Oladele & Aina 2007). Significant consumption of tiger nut has reduced established instances of different health related issues, for example, cancer, obesity and heart related diseases and furthermore really good for newborn babies, older people and athletes (Martinez, 2003). *Cyperus esculentus* was reported to help in forestalling heart coagulation and stimulates blood circulation, responsible for forestalling and treating urinary tract and bacterial infection, help with lessening the risk of tumor growth (Adejuyitan et al., 2009). Tigernuts are valuable to diabetics and those

trying to reduce cholesterol or get fit, the extremely high fiber content joined with an enjoyable taste makes them ideal for healthy consumption (Dianne, 1960). Tiger nut can be eaten as appetizer which can be ready by soaking in water for few minutes. It can likewise be eaten broiled, dried, baked and can be made into an invigorating drink called "Horchata De Chufas" or tigernut milk. The processes involved in production of tigernut milk include: fresh tigernuts, sorting and washing, grinding/milling, extraction/sieving, pasteurizing, homogenizing, bottling, cooking, tigernut milk (Udeozor, 2012). It is used as a flavouring agent for ice cream and biscuits (Cantalejo, 1997). *Aspergillus flavus* is a saprophytic and pathogenic fungi with a cosmopolitan distribution. It is the most widely reported food borne fungus and is one of the dominant species found on stored products, particularly grains. According to Duran et al., (2009), he reported that *Aspergillus flavus* as developed an extraordinary ability among *Aspergillus species* to colonize plant seeds. A. flavus grows on crops before harvest and during storage (Saini and Kaur, 2012). The presence of *A. flavus* infection rises in the presence of insects and many other diseases which include stalk rot, drought, severe leaf damage, and less ideal storage condition (Agrios, 2005). The growth of A. flavus is seriously affected by environmental condition such as relative humidity and temperature (Giorni et al, 2012). *A. flavus* prevails more in a drier condition where the temperature is high while as the temperature decreases it growth and sporulation reduce. According to Agrios, (2005), he reported that *A. flavus* had rapid growth at 30–55 °C, slow growth at 12–15 °C, and almost ceases growth at 5–8 °C. Light plays a vital role in the growth of *A. flavus.* Shehu and Bello, (2011), reported that *A. flavus* growth is supported by continuous light (day light) than in darkness.

MATERIALS AND METHODS

Sample collection

Samples of tiger nuts were purchased from the three campuses of the University of Port Harcourt namely; Choba campus, Delta park and Abuja park. Samples were collected during the dry season (January- February 2019) and were thoroughly washed with distilled water and rinsed. The samples were air dried and then ground into to powder with a kitchen blender and properly stored in an air-tight container at room temperature.

Fungi isolation and identification

Tiger nuts were chopped into bits and surface sterilized by immersing into 2% hypochlorite for 2 minutes to destroy the micro-organism at the surface of the sample. The sterilized tiger nuts were rinsed three times with distilled water and air dried on a filter paper for 20 minutes. The chopped particles of tiger nuts were inoculated into sterile plates containing Malt Extract Agar (MEA) medium. Each plate containing five pieces of tiger nuts slice was incubated at room temperature. Sub culturing was done to obtain pure cultures on Malt Extract Agar (MEA). The identification of *A. flavus* was done microscopically, based on the morphology of spores and conidial, colony growth, with reference to Pitt & Hocking (2009).

Sub- culturing of *Aspergillus flavus* using malt extract agar (mea) medium to obtain a pure culture

Aspergillus flavus was sub-cultured using Malt Extract Agar (MEA). The MEA medium was prepared by dissolving 50grams of commercially manufactured MEA in 1000ml of distilled water and chloramphenicol of about 0.16g was added, the mixture was homogenized by stirring it mechanically to ensure uniformity. The medium was sterilized by autoclaving for about 15minutes at 121^{o}C. 15ml of the hot mixture was poured into 90mm Petri dishes and allowed to solidify at 28^{o}C ± 2^{o}C. The isolated *A. flavus* was inoculated on the medium and left to incubate at 28^{o}C ± 2^{o}C. Repeated sub culturing was done to get a pure culture.

Preparation of tiger nut based medium and inoculation

A standard medium of 2.5% milled Tiger nut (25g of tiger nut powder + 1000ml of distilled water + 20g of Agar agar + 0.16g of Chloramphenicol) was used for this experiment. The mixture was autoclaved at 121^{o}C for about 15minutes, shaken vigorously prior to pouring 15ml into 90mm sterile petri-dishes and allowed to solidify at room temperature. The *Aspergillus flavus* that was previously cultured was centrally inoculated into the plates, using 5ml sterile water supplemented with 0.05% Tween 80 and incubated at room temperature.

Treatment application

2.5% tiger nut based medium (25g grounded tiger nut + 20g of technical agar + 0.16g of chloramphenicol + 1000ml of distilled water) was used. A- 6 -day old inoculum of *A. flavus* was centrally inoculated into each of the prepared plate. The inoculated plates were incubated in three regimes of light (darkness, day light and alternating day light and darkness) at two different temperatures: 28^{o}C and 30^{o} C for 10 days.

Fungal growth assessment and data collection

Mycelia growth (colony diameter) measurement was done daily for 10 days; the measurement was carried out in two directions (horizontal and vertical) at right angles to each other with the aid of a meter rule and a marker pen and the mean was recorded. Data was fitted using linear model. The growth rate was calculated from the slope of the regression graph.

Mathematically: $Y=ax + b$

Where, y = original inoculum size (mm)

a = growth rate (mm/day)

b = intercept

x = lag phase (days)

Experimental design and data analysis

The experiment was a 2 x 3 factorial laid out in a completely randomized design (CRD) replicated 10 times. Analysis of Variance (ANOVA) was used in the data analysis and means separated using Least Significant Difference at 5% probability level.

RESULT

Analysis of variance (ANOVA) showed that the main effect of light regime ($P<0.001$), temperature ($P<0.001$) and light regime x temperature ($P<0.001$; $P=0.032$) had significant influence on the growth rate and mycelia diameter of *Aspergillus flavus* (Table 1).

Table1: P value for mycelia diameter (mm) and growth rate (mm/day) *A. flavus* on tiger nut-based medium

Parameters	Mycelia Diameter (mm)	Growth Rate (mm/day)
Light	< 0.001 ***	< 0.001 ***
Temperature	< 0.001 ***	< 0.001 ***
Light x Tempt.	< 0.001 ***	0.032*

*= significant; *** = very highly significant

Effect of temperature on mycelia diameter of *Aspergillus flavus*

From Table 2 below, it was observed that temperature had effect on the mycelia diameter of *Aspergillus flavus* on tiger nut based medium. The mycelia diameter of *Aspergillus flavus* was observed to be higher at temperature of 30^oC than at 28^oC. There was significant difference ($P<0.001$) at both temperatures (28^oC and 30^oC) in the mycelia diameter of *Aspergillus flavus* (Table 1).

Effect of light regime on mycelia diameter of *Aspergillus flavus*

The result gotten from the study shows that the three light regimes (continuous day light, continuous darkness and alternative day light and darkness) had effect on the mycelia diameter of *A. flavus* on tiger nut based medium. From table 2, the highest mycelia diameter of *A. flavus* was observed in continuous day light, followed by continuous darkness while alternative day light and darkness had the least mycelia diameter. The three light regimes showed significant difference ($p < 0.001$) in the mycelia diameter of *A. flavus* (Table 1).

Effect of interaction between light regime and temperature on the mycelia diameter of *A. flavus*

The result showed that interaction between light regimes and temperatures had effect on the mycelia diameter of *A. flavus*. From Table 2, there was increase in mycelia diameter in the different light regimes (continuous day light, continuous darkness and alternative day light and darkness) at both temperatures (28^{o}C and 30^{o}C). The mycelia diameter of *A. flavus* in continuous day light at 30^{o}C was found to be higher than in continuous darkness and alternative day light and darkness at the same temperature. The mycelia diameter in continuous darkness at 30^{o}C was found to be higher compared to alternative day light and darkness. At 28^{o}C, the highest mycelia diameter of *A. flavus* occurred in continuous day light, followed by continuous darkness and finally alternative day light and darkness. Generally, the mycelia diameter *A. flavus* in all the three light regimes (continuous day light, continuous darkness and alternative day light and darkness) at 30^{o}C was higher than at 28^{o}C in the same light regimes. Table 1 shows that there was significant difference ($P < 0.001$) in the mycelia diameter of *A. flavus* in the light regime and temperature interaction.

Table 2: Effect of light regimes on the mycelia diameter of *Aspergillus flavus* on tiger nut-based medium at 28°C and 30°C

Parameters	28°C	30°C
Day light	84.55	85.00
Darkness	82.95	84.95
Light x darkness	79.20	84.85
LSD 0.05 (Tempt)	0.345	
LSD 0.05 (Light)	0.4225	
LSD 0.05 (Day light x Tempt)	0.5975	

Growth rate of *Aspergillus flavus*

Three light regimes (continuous day light, continuous darkness, and alternative day light and darkness) were employed to investigate the growth rate of *A. flavus* at two degrees of temperature (28°C, and 30°C).

Effect of temperature on the growth rate of *A. flavus*

Table 3 indicates that temperature had effect on the growth rate of *A. flavus*. The maximum effect on the growth rate was observed at the temperature of 30°C whereas the minimum effect was seen at 28°C. In other words, the growth of the *A. flavus* was higher at 30°C than at 28°C. From Table 1, it was observed that both temperatures (28°C and 30°C) had significant difference ($p < 0.001$) in the growth rate of *A. flavus.*

Effect of light regimes on the growth rate of *Aspergillus flavus*

From Table 3, it was observed that light regimes (continuous day light, continuous darkness, and alternative day light and darkness) had effect on the growth rate of *Aspergillus flavus.* The highest growth rate of *Aspergillus flavus* occurred at continuous day light, followed by

continuous darkness, while the least growth occurred under alternative day light and darkness. Significant difference (P<0.001) was observed in the growth rate of *A. flavus* in the three light regimes (Table 1).

Effect of interaction between light regime and temperature on the growth rate of *A. flavus*

Table 3 indicates the interaction between light regime (continuous day light, continuous darkness, and alternative day light and darkness) and temperature (28^oC and 30^oC) had effect on the growth rate of *A. flavus.* In the interaction, the growth rate of *Aspergillus flavus* was observed to be higher at 30^oC in all the light regimes (continuous day light, continuous darkness, and alternative day light and darkness) than at 28^oC in the same light regimes. The highest growth rate of *A. flavus* at both 28^oC and 30^oC occurred in continuous day light, followed by continuous darkness and then alternative day light and darkness. There was significant difference (P = 0.032) in the growth rate of *A. flavus* in light regimes and temperature interaction, (Table 1).

Table 3: Effect of light regime on the growth rate of *Aspergillus flavus* on tiger nut-based medium at 28^oC and 30^oC

Parameters	28^oC	30^oC
Day light	10.66	11.95
Darkness	10.34	11.32
Light x darkness	9.54	10.41
LSD 0.05 (Tempt)	0.0465	
LSD 0.05 (Light)	0.0569	
LSD 0.05 (Light x Tempt)	0.0805	

DISCUSSION

This study was carried out to ascertain the effect of light regimes (continuous day light, continuous darkness, and alternative day light and darkness) and temperature (28^{o}C and 30^{o}C) on the growth of *A. flavus* on tiger. The presence of *Aspergillus flavus* from tigernuts in this present study, is in agreement with previous studies of Shamsuddeen and Aminu (2016) who established the presence of *Aspergillus flavus* on freshly consumed tigernuts in Kaduna, Nigeria. From the study, it was shown that light and temperature, has effect on the growth rate of *A. flavus* and the interaction between light and temperature also have a great effect on the growth of *A. flavus*. The result has pointed out that *A. flavus* can grow on different regimes of light ranging from day light, darkness and alternative day light and darkness. A previous study by Shehu and Bello. (2011) had earlier reported that the highest growth of *A. flavus* occurred in continuous day light than in continuous darkness, alternative light and darkness in some cereals. The result obtained from these researchers has agreed with the result of this current study. From the result of this study, it was observed that *A. flavus* can grow at temperature of 28^{o}C and 30^{o}C in day light, darkness and alternative day light and darkness but the highest growth rate was observed at 30^{o}C in all the light regimes. In other words, *A. flavus* grows well in elevated temperatures above >28^{o}C. Agrios (2005), stated that *A. flavus* had rapid growth at 30–55 °C, slow growth at 12–15 °C, and almost ceases growth at 5–8 °C. Oladirin and Iwu, (1993) also reported that the optimum growth of *Aspergillus flavus* occurs at 30^{o}C, therefore in most cases favoured by hot conditions, which is in line with the present work. Mycelia diameter of *A. flavus* from the study was observed to be significant at the different regimes of light (continuous day light, continuous darkness, and alternative day light and darkness) and temperature (28^{o}C and 30^{o}C). The highest colony diameter of *Aspergillus flavus* was observed in continuous day light, followed by continuous

darkness and finally alternative light and darkness at temperature of 30^{o}C when compared to 28^{o}C in the same regimes of light on tiger nut based medium. This study showed that light regimes and temperatures had different effects on the growth of *Aspergillus flavus.* Generally, the result points to the fact that *Aspergillus flavus* can grow in 28^{o}C and 30^{o}C in different light regimes, best in continuous day light. This fungus appears to be tolerant to higher temperature with an optimal growth at 30^{o}C. This implies that higher temperature with continuous day light regime favoured the colonization of tiger nut by *Aspergillus flavus.* This is in line with the works of Monica and Lenda, (2002) who stated that *A. flavus* can grow in temperature as higher as 37^{o}C. Therefore, the study provides useful information necessary to growers and seller of tiger nut on the storage and management of the fungus against microbial losses.

CONCLUSION

From the study, light regimes (continuous day light, continuous darkness, and alternative day light and darkness) and temperature (28^{o}C and 30^{o}C) had significant influence on the growth of *Aspergillus flavus.* The fungus grew in all the light regimes but the highest growth was observed in continuous day light. Under lower temperature of 28^{o}C reduced growth rate of *A.flavus* was observed when compared to 30^{o}C. Therefore the spoilage of tiger nut by *A.flavus* will be reduced when stored at temperature < 30^{o}C and in a light regime that is not continuous day light.

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