Evaluation of Growth of Toxigenic Strain of Aspergillus flavus on Turmeric-Based Media at Different Water Activities

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Abstract

Climatic conditions of the rain forest ecological zone of Nigeria favour the growth of *Aspergillus flavus* and subsequent aflatoxin production. This study was carried out to investigate the effect of water activity (0.85, 0.9, 0.95, 0.98 and 0.995 a_w) on the lag phase prior to growth and mycelial growth of a toxigenic strain of *A. flavus* on turmeric-based media at 28±2°C after 12 days incubation period. Four different varieties; UT14, UT25, UT35 and UT46 obtained from National Root Crop Research Institute (NRCRI), Abia State and used in the preparation of the different media. A toxigenic strain of *A. flavus* isolated from turmeric in Port Harcourt was used in the experiment. The experiment was a 5 x 4 factorial laid out in completely randomized design (CRD), replicated three times. Results showed that regardless of the variety, no growth was observed at 0.85 a_w at 12 days of incubation. The shortest lag phase prior to growth was obtained at 0.995 a_w and the longest obtained at 0.90 a_w. Optimal growth was observed at 0.98 a_w in three varieties (UT14, UT25and UT46), UT35 had marginal optimal growth at 0.995 a_w. Minimal growth of the *A. flavus* strain was obtained at 0.90 a_w at 28±2°C in all varieties. This result could be useful in the storage of turmeric, as this food product could be stored at moisture content of < 85%.

Key words: Toxigenic, *Aspergillus flavus*, Lag phase, Strain, Turmeric, Water activity

Introduction

Contamination of food products with *Aspergillus flavus* can occur during production, storage, processing, transportation or marketing (Bankole *et al*., 2006). This could have great effect on the quality of the products. In addition to the colonization of food products, many strains of fungal specie can produce significant quantities of aflatoxin which, when consumed are toxic to mammals (Agrios, 2005). The incidence of this fungus increases in the presence of insects (Adedire, 2001) and some types of stress such as drought, temperature (Craufurd *et al*., 2006; Kebede *et al.*, 2012) and poor storage condition.

Generally, excessive moisture conditions and high temperatures of storage increase the occurrence of *A. flavus* and subsequent aflatoxin production. Key ecological factors of water activity (a_w) and temperature influence the colonization of food commodities by spoilage fungi (Magan *et al*., 2010; Akbar and Magan, 2014). Studies have shown that under high temperature stress when combined with drought conditions, higher amounts of aflatoxins are produced by *A. flavus* (Jone *et al*., 1980; Payne *et al.*, 1988; Craufurd *et al*., 2006; Kebede *et al.*, 2012). The combination of high temperature and water stress in some regions of the world could increase the contamination of mycotoxigenic fungi in major commodities and thus becomes a serious problem in food security and food nutritional quality worldwide (Magan *et al*., 2011). Large amount of turmeric are being wasted annually due to poor post-harvest handling which results to fungal colonization and subsequent toxin contamination, thus making the products unfit for human consumption. The level of fungal mycelial growth is influenced by the level of water activity and temperature of the store (Cairns *et al*., 2005; Pardo *et al*., 2006; Akbar and Magan, 2014), thus the need to determine the optimum water activity for growth of *A. flavus* on turmeric. The objective of the study was therefore to evaluate the effect of storage moisture conditions at 28±2°C on the lag phase prior to growth and growth of a toxigenic *strain of Aspergillus flavus* on different varieties of turmeric used as media.

Materials and Methods

The experiment was carried out at the Department of Crop and Soil Science Laboratory, University of Port Harcourt, Nigeria (6.55º 0.2' N latitude, 4.54º 10.02' E longitude). Four (4) varieties of turmeric {UT46 (high yielding), UT14 (moderate yielding) and UT25 and UT35 (low yielding)} were obtained from the National Root Crop Research Institute (NRCRI), Umudike, Nigeria, and used for the experiment. The samples were cleaned, chopped into pieces, air dried, ground into powder and stored properly. A toxigenic strain of *Aspergillus flavus* confirmed using Coconut agar medium (CAM) (Lin and Dianese, 1976) was used in the experiment. The *A. flavus* strain was maintained on Malt Extract Agar (MEA) medium. A standard medium of 2.5% milled turmeric agar (25 g of tumeric powder + 10 g technical agar + 0.16g chloramphenicol + 1000 ml of water) of each variety was prepared. The a_w of the media were modified by adding increasing amounts of glycerol to obtain the following a_w treatment levels of 0.85, 0.90, 0.95, 0.98 and 0.995. These were checked with a_w meter (Aqualab, Decagon devices, Inc., USA). The media were prepared by autoclaving at 121℃ for 45 minutes, shaken vigorously prior to pouring 15 ml into 90 mm sterile plates when media solidified at 25±2°C. 2 µl of inocula of each of the *A flavus* strains previously prepared from 6-days old mycelia + 5 ml sterile water supplemented with 0.05% (w/v) Tween 80 were centrally inoculated into each of the plates. Incubation was at 28±2°C. Fungal growth assessment was done daily for 12 days; measurement of growth was done in two directions (i.e. horizontal and vertical) at right angles to each other (Marin *et al*., 1996). Data was used for the determination of lag phase (λ) in days prior to growth and growth rate (mm/day). Data was fitted using linear model. Growth rate was calculated from the slope of the regression graph while lag phase by equaling the regression line formula to the original inoculum. Analysis of data was done using Genstat 16th Edition; VSN Industrial Ltd, UK. Comparison of data means was considered at 5% probability level.

Results

Regardless of the variety, no growth was observed at 0.85 a_w after 12 days of incubation. The strain of *Aspergillus flavus* exhibited varying degrees of growth at the different water activity levels. Increased water activity resulted in decrease in the lag phase prior to growth of the fungus. Statistically, the main effect of a_w, variety and a_w and variety interaction were all significant ($P<0.001$) (Table 1). On the average, the shortest lag phase prior to growth of *A. flavus* in the various media was at 0.995 a_w (1.2 days) and the longest at 0.90 a_w (< 0.5 days) (Fig. 1). Fig. 2 shows that with the exception of UT35, the fungus had optimal growth at 0.98 a_w. *Aspergillus flavus* on UT35 had

marginal optimum growth at 0.995 a_w. Furthermore, the *A. flavus* strain exhibited the fastest growth rate on UT14 medium (1 mm/day), followed by UT35 (0.7 mm/day), UT46 (0.6 mm/day) and UT25 (0.5 mm/day) with the least growth rate.

Table 1: P values for the lag phase (?? days) and growth rate of *A. flavus* strain on turmeric-based media at different water activities (a_w)

Parameter	Lag phase	Growth rate
Variety	< 0.001***	< 0.001***
Water activity (a_w)	< 0.001***	< 0.001***
Variety x a_w	< 0.001***	< 0.001***

*** = very highly significant.

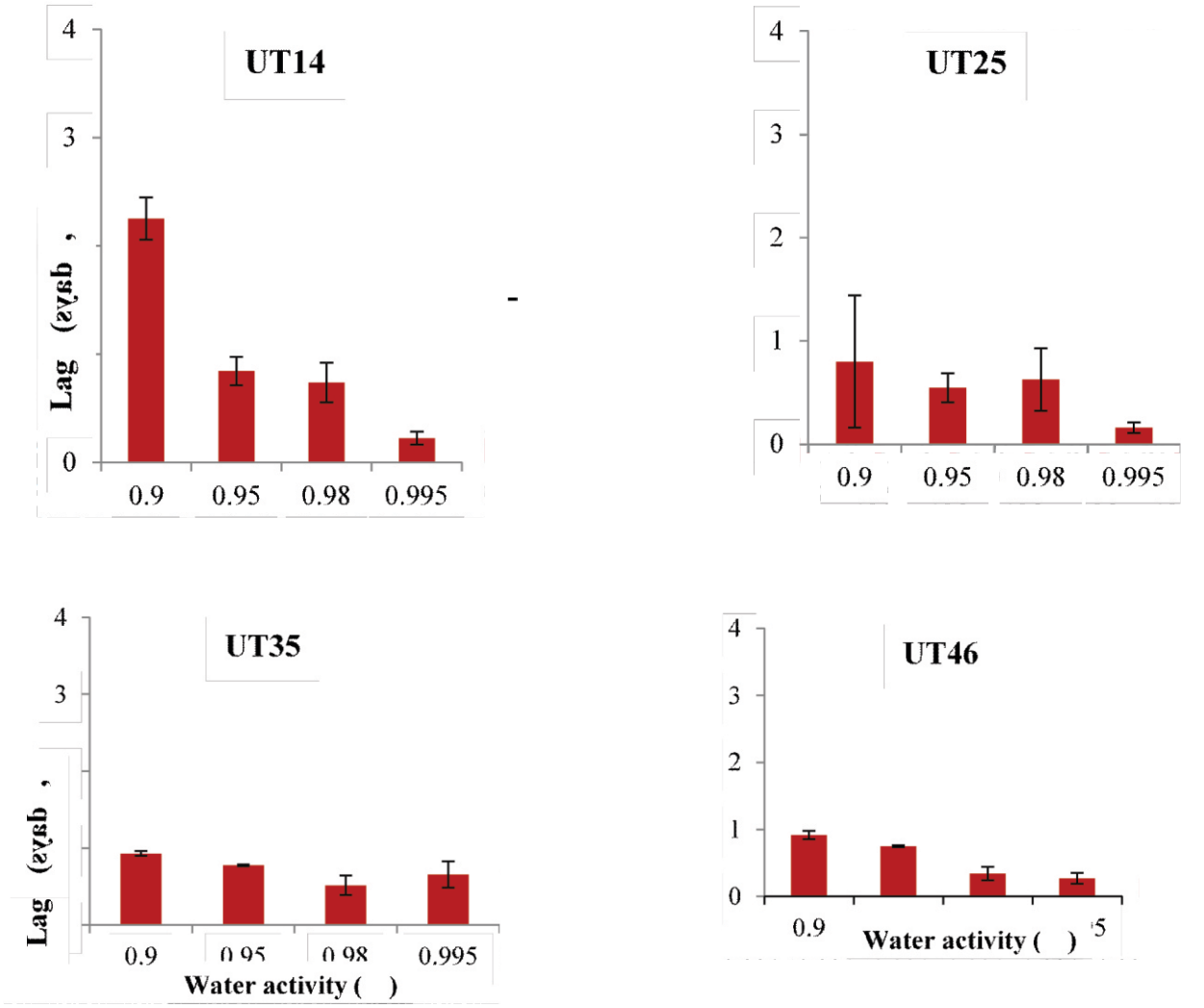
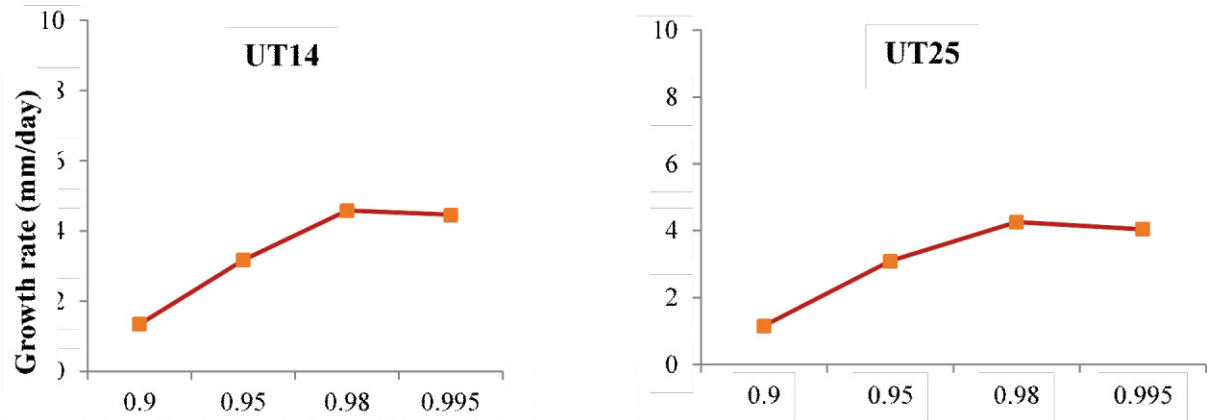


Figure 1: Effect of different water activities (a_w) on the lag phase prior to growth strain of *A. flavus* on turmeric-based media at 28±2°C after 12 days of incubati



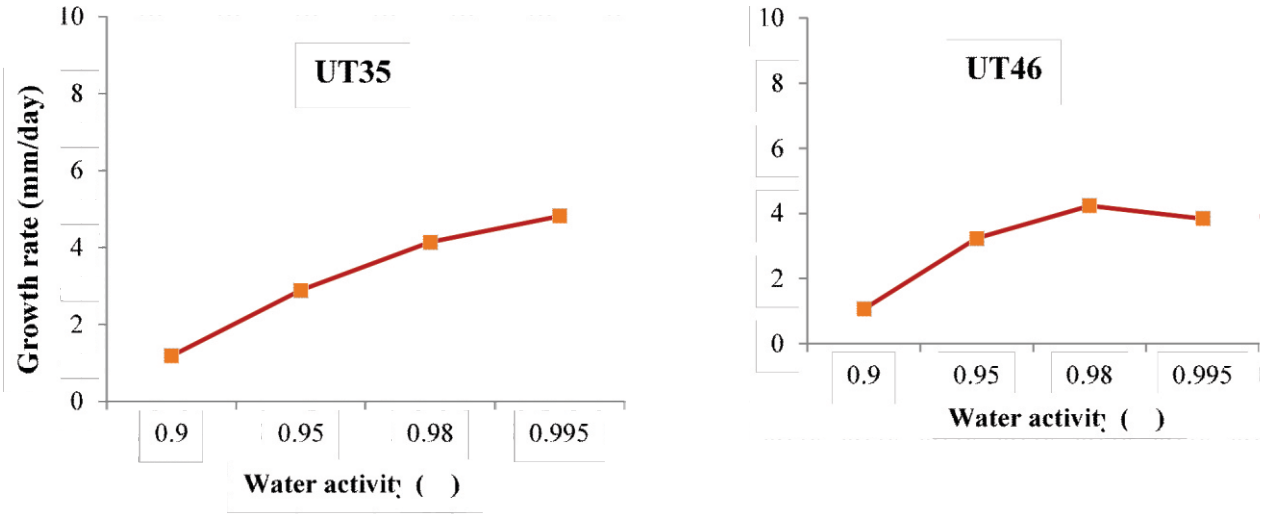


Figure 2. Effects of different water activities (a_w) on the growth rates of *A. flavus* on turmeric-based media at 28±2°C after 12 days of incubation.

Discussion

The present study investigated the growth of a toxigenic strain of *A. flavus* on different varieties of turmeric used as growth media. *A. flavus* could grow on turmeric-based media at 28±2°C. Jeswal & Kumar (2014 and 2015) reported the occurrence of *Aspergillus* spp in both stored ginger and turmeric samples. Rawat *et al*. (2014) also found that *A. niger, A. flavus, Mucor* and *Rhizopus* species were the dominant mycoflora in sampled spices including ginger and turmeric. The strain of *A. flavus* used in the current study was not able to grow at 0.85 a_w at 28±2°C over the 12 day incubation period and this is in line with the findings of some authors. Akbar and Magan (2014) reported no growth *A. flavus* at a water activity level of 0.85 a_w regardless of the temperature, while Labouar *et al.,* (2016) observed no growth of *A. flavus* at 0.85 - 0.88 a_w at 15 – 37°C. Also Akbar and Magan (2014) reported lag phase prior to growth of *Aspergillus* spp at <1 day at 0.95 – 0.98 and 25 – 37°C and optimum growth at 0.98 a_w at 30-35°C. Their result is similar to that obtained from this study. The current finding has revealed that the different varieties of turmeric could react differently to the growth of *A. flavus* at the various water activity levels. These differences could be attributed to the difference in genotype. The result also points to the fact that the growth of the toxigenic strain of *A. flavus* was optimal at 0.98 a_w, although this needs to be validated *in vivo.* Generally, *A. flavus* was able to grow over a wider range of a_w levels and appears to be tolerant of drier condition (0.90a_w). The implication is that under drought stress occasioned by climate change, there could be high colonization of turmeric by toxigenic strains of *A. flavus* with variation in optimum conditions of on turmeric varieties. The mycelial prediction of *A. flavus* at different a_w levels and incubation temperature employed in the study might be useful in the determination appropriate conditions for drying and storage of turmeric to prevent aflatoxin contamination.

Conclusion

The findings from the current study explain the effect a_w could have on the growth and colonization of *Aspergillus flavus* which could occur during post-harvest handling and storage of turmeric. It showed *A. flavus* grows on turmeric: no growth occurred at 0.85 a_w; increased water activity resulted in decrease in the lag phase prior to growth and different varieties of turmeric reacted differently to the growth of *A. flavus* at the various water activity levels. This information could be used in decision-making process in relation to a_w during turmeric production and also contribute to optimal strategy selection in the control of aflatoxin contamination in turmeric. Turmeric should be dried immediately after harvest at least below 85% moisture content level and stored under appropriate conditions in order to minimize contamination with *A. flavus*.

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