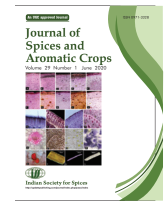
Journal of Spices and Aromatic Crops
Vol. 29 (1) : 72-77 (2020)
doi : 10.25081/josac.2020.v29.i1.6154

Indian Society for Spices



Impact of water activity and temperature on the growth and aflatoxin production by two toxigenic isolates of *Aspergillus flavus* on ginger

V C Okereke

Department of Crop and Soil Science, University of Port Harcourt,
Choba, PMB 5323, Port Harcourt, Nigeria.
E-mail: chykeoky@yahoo.com

Received 07 April 2020; Revised 08 June 2020; Accepted 09 June 2020

Abstract

Ginger may be affected by aflatoxin-producing *Aspergillus flavus* during storage. This study evaluated the interaction between different temperatures and water activity (a_w) levels on the growth and aflatoxin production (AFB_1) of two toxigenic isolates of *A. flavus* previously isolated from ginger (AFg) and turmeric (AFt) on ginger medium over a 10-day period. Results showed that regardless of the temperature, no growth was observed at 0.85 a_w and at 20°C and 0.9 a_w, suggesting limits for growth could vary. *A. flavus* had optimum conditions for growth at 0.98–0.995 a_w and 30–35°C in both isolates, while the level of AFB_1 production varied considerably among the isolates. Generally, AFt isolate produced higher concentration of toxins than AFg. Maximum AFB_1 production by both isolates was at 0.98 a_w but at different temperatures. This result could therefore be useful in the post-harvest handling of ginger and ginger-based products in stores against *A. flavus* colonisation and subsequent AFB_1 production.

Keywords: aflatoxin, *Aspergillus flavus*, isolate, lag phase, temperature, water activity

Aspergillus flavus is one of the most important spoilage and toxigenic fungi contaminating food products and may produce aflatoxin B_1 (AFB_1), which could cause cancer in humans (Liu & Wu 2010). Its growth and mycotoxin production has been affected by several factors such as fungal isolates, temperature, water activity (a_w) and post-harvest handling conditions (Magan *et al*. 2004; Park *et al*. 2005; Makun *et al*. 2007; Nguyen *et al*. 2007). Under environmental stress such as high temperature and limited water conditions, this organism produces higher amounts of aflatoxins (Payne *et al*. 1988; Craufurd *et al*. 2006; Kebede *et al*. 2012). In literature, it has been shown that the optimum temperature and a_w for growth and AFB_1 production by *A. flavus* range between 16 and 31°C and 0.82–0.99 a_w (Pitt & Hocking 2009). The mycelial growth is particularly affected by the incubation temperature and a_w

(Cairns-Fuller *et al*. 2005; Pardo *et al*. 2006; Akbar & Magan 2014). Moreover, in some regions of the world with high temperature and limited water, the contamination of food products with *A. flavus* is becoming a threat to food security (Magan *et al*. 2011). In Nigeria for instance, the rain forest ecological zone is characterized by high temperature and high relative humidity which encourages the growth of *A. flavus*. This zone accounts for large consumption of spices such as ginger, turmeric, garlic, coriander, red chilli etc. and also spice-based products. Due to the growing need for ginger in everyday living of the people of this zone, there is a need to examine the interacting effects of temperature and water activity and their influence on the level of colonization and AFB_1 production on these products especially under the changing climate. This will aid decision making process on the most appropriate management measures to be used in reducing the possible hazards relating to aflatoxin contamination in food products especially spices. The objective of this study was therefore to evaluate the impact of water activity ($a_{w)}$ and temperature on lag phase prior to growth, growth rate and aflatoxin production of two toxigenic isolates of *Aspergillus flavus* on ginger commonly consumed in the region.

Two aflatoxin producing isolates of *A. flavus* previously isolated from ginger (AFg) and turmeric (AFt) respectively, found to produce AFB_1 were used in the study. Confirmation of toxin production was done on a mycotoxin conducive Yeast Extract Sucrose agar medium (YES, Oxoid Ltd., UK) as used by Okereke & Godwin-Egein (2018) and the quantification was done using HPLC.

A standard media of 5% milled ginger agar (50 g of ginger powder + 10 g of technical agar + 0.16 g of chloramphenicol + 1000 mL of distilled H_2O) was used in the study. Water activity treatment levels of 0.85, 0.90, 0.93, 0.95, 0.982 and 0.995 were derived by adding increasing amounts of glycerol and verified with the a_w meter (Aqualab, Decagon devices, Inc., USA). The inocula of *A. flavus* was prepared from 6-day-old cultures (6-day-old mycelia + 9 mL sterile water supplemented with 0.05% (w/v) Tween 80) grown on Malt Extract Agar (MEA) at 25°C. After the solidification of the media in the plates, they were centrally inoculated at one point with 2 µL of the inoculum of each of the isolates of *A. flavus* for each treatment. After inoculation, the Petri plates were sealed with parafilm tape and kept in closed polyethylene bags at the tested incubation temperatures (20, 25, 30, 35 and 37°C). Each treatment was carried out in triplicate.

Assessment of fungal growth rate was done daily during the 10-day incubation and colony diameter was measured in two directions at right angles to each other (Marin *et al*. 1996). Lag phase (days, λ) and mycelia growth rate (mm/day, µ) for each combination treatment were calculated from linear regression slopes of the growth curves by equalling the regression line formula to the original inoculum size (diameter, mm). This was derived mathematically as follows;

$$y = ax + b$$

Where, y=original inoculum size (2 mm); a=growth rate; x=lag phase

After 10 days of incubation, plugs were taken from each plate using a 4 mm cork borer into Eppendorf tubes and stored at -20°C for aflatoxin analysis. The procedure of Abdel-Hadi *et al*. (2010) was used in the aflatoxin extraction. Extraction was done with 0.75 mL of 100% methanol by shaking thoroughly for 1 h at 150 rpm at 25°C in an orbital shaker. The extract was transferred to Eppendorf tubes and completely dried at 45°C in a speed vacuum in the dark. Samples were dissolved in 1 mL of methanol: water (50:50), vortexed and filtered with 0.22 µm filter (Kromega, Jaytee Biosciences Ltd., UK) into sylinazed HPLC vials using 1 mL syringes

(Terumo Medical Corporation, UK). The HPLC used was an Agilent 1200 Series system (Agilent, Berkshire, UK) with a fluorescence detector (FLD) (Millipore Waters, Corporation Massachusetts USA), at excitation and emission wavelength of 365 and 440 nm respectively. The flow rate of the mobile phase (methanol/water/acetonitrile, 30/60/15, v/v/v) was 1 mL min^{-1} and the run time was 12 min. Separation was achieved through the use of a C_{18} column (Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm) preceded by a Phenomenex Gemini C_{18} 3 mm, 3 µm guard column.

The experiment was laid out in 5 × 6 factorial completely randomised design (CRD), replicated three times for each isolate. Statistical analysis was performed using Genstat 16th Edition; VSN industrial Ltd, UK for normally distributed data. Comparisons were considered significantly different at 5% probability level and below for all single and interacting treatments.

Results showed that when limited water conditions and temperature changes were imposed, the time prior to growth (lag phase) increased in both isolates. Longest initial lag phase was observed at 20°C at 0.93 a_w (> 5 days), while the shortest lag phase was at 0.982–0.995 a_w at 20–37°C (<1 day) for the two isolates (Fig. 1).

Overall both *A. flavus* isolates had similar environmental behaviour in relation to the imposed treatments of a_w and temperature with maximum growth occurring approximately at 0.95–0.995 a_w and 30–37°C. Some workers have suggested that *A. flavus* can grow slowly at high temperature (40°C) (Somjaipeng & Ta-uea 2016) and the tolerance of these isolates to 37°C in the present study was influenced by the water activity of the medium. With regards to the effect of the imposed temperatures, the observed higher growth rate of the two isolates of *A. flavus* at optimum temperature of 30–35°C at almost all a_w levels used is supported by the fact that the tolerance of the fungus to low a_w occurs at optimum incubation temperature (Aldred 1999; Magan 2007; Somjaipeng & Ta-uea 2016). Tolerance to low a_w was not isolate dependant as the two isolates showed optimal growth at 30–35°C. Also, regardless of the incubation temperature, no growth was observed at 0.85 a_w and 0.9 a_w at 20°C, suggesting limits for *A. flavus* growth could vary in ginger and ginger-based products (Fig. 2).

The level of AFB_1 production by the two AF-producing isolates varied significantly (P<0.001) and isolate AFt produced higher concentration of toxins than isolate AFg (Fig. 3). Maximum AFB_1 production by both isolates was obtained at 0.98 a_w but at different temperatures, emphasizing the importance of temperature in the *A. flavus* colonization of food products. For isolate AFg, the highest AFB_1 production of 40 ng g^{-1} of agar was obtained at 20°C, while AFt, had maximum production of 244 ng g^{-1} of agar at 30°C. Lahouar *et al.* (2016) observed maximum AFB_1 production of 266 ng g^{-1} at 0.97–0.99 a_w by *A. flavus* in sorghum seeds. Giorni *et al*. (2007), Abdel-Hadi *et al*. (2010) and Mousa *et al*. (2011) also observed that the optimal conditions for AFB_1 production by *A. flavus* ranged from 25–30°C and 0.96–99 a_w. This variation in the optimal conditions including the one found in the current study has been attributed by many workers to the culture media used and the nature of the fungal isolate (Klich *et al*. 2007; Gallo *et al*. 2016). Analysis of variance showed that the main effect of a_w and temperature and a_w × temperature effect were significant (P<0.001). Lag phases and growth rate were different especially for 0.90 and 0.93 a_w at 20–25°C. No difference was found between 0.982 and 0.995 a_w at all the tested temperatures. For each isolate, there was significant (P<0.001) influence of a_w and temperature on AFB_1 production as the optimum conditions for AFB_1 production varied considerably.

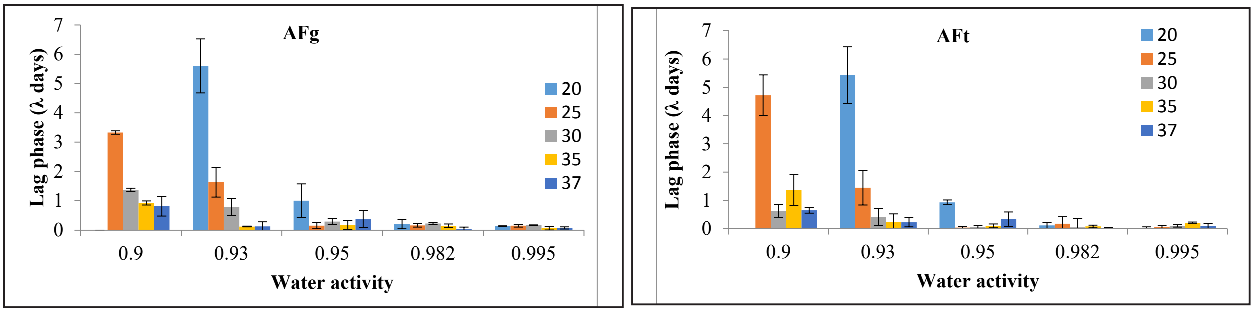


Fig. 1. Effect of temperature regimes and water activity (a_w) levels on the lag phases prior to growth of two isolates of *A. flavus* (AFg & AFt) on ginger-based medium after 10 days of incubation

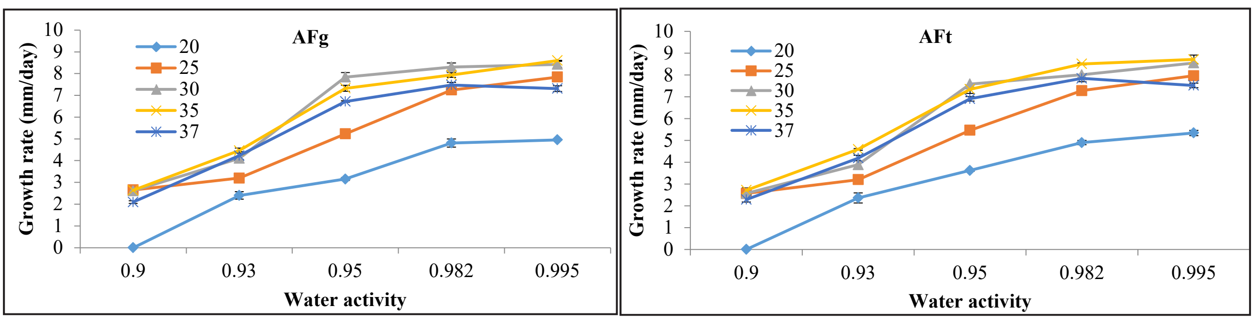


Fig. 2. Effect of temperature regimes and water activity (a_w) levels on the relative growth rates of two isolates of *A. flavus* (AFg & AFt) on ginger-based medium after 10 days of incubation

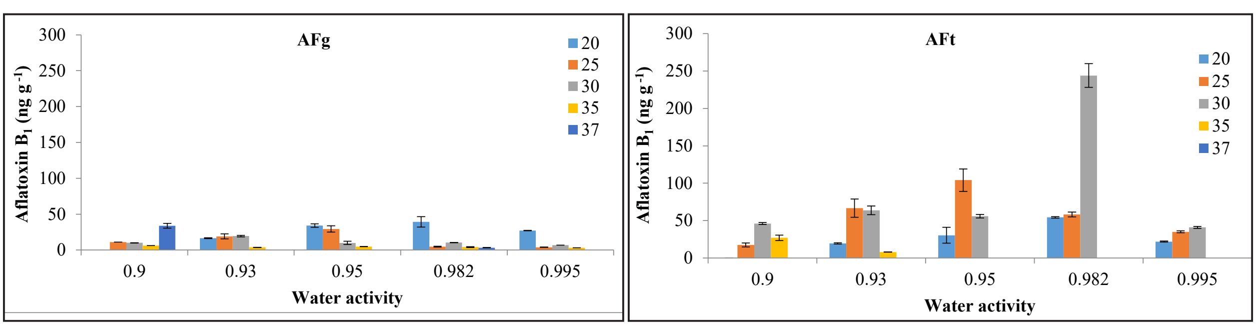


Fig. 3. Effect of temperature regimes and water activity (a_w) levels on AFB_1 production of two isolates of *A. flavus* (AFg & AFt) on ginger-based medium after 10 days of incubation

The ecophysiological growth pattern of two *A. flavus* isolates of Nigerian origin to interacting effect of temperature and water activity levels on ginger was evaluated in the current study. The optimum conditions for *A. flavus* growth was 0.95–0.995 a_w and 30–35°C for the two isolates, while the level of AFB_1 production among the isolates varied considerably. Both isolates produced maximum AFB_1 at 0.98 a_w, temperatures of 20°C and 30°C were optimum for isolates AFg and AFt, respectively. This result could therefore be useful in the post-harvest handling of ginger and ginger-based products in stores against *A. flavus* colonisation and subsequent AFB_1 production.

References

Abdel-Hadi A, Carter D & Magan N 2010 Temporal monitoring of the nor-1 (affl) gene of *Aspergillus flavus* in relation to aflatoxin B1 production during storage of peanuts under different water activity levels. J. Appl. Microbiol. 109: 1914–1922.

Akbar A & Magan N 2014 The impact of water and temperature interactions on lag phase, growth and potential ochratoxin A production by two new species, *Aspergillus aculeatinus* and *A. sclerotiicarbonarius*, on a green coffee-based medium. Int. J. Food Microbiol. 188: 116–121.

Alderd D, Magan N & Lane B S 1999 Influence of water activity and nutrients on growth and production of squalestatin S1 by a *Phoma* sp. J. Appl. Microbiol. 87: 227–241.

Carins-Fuller V, Aldred D & Magan, N 2005 Water, temperature and gas composition interaction affect growth and Ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. J. Appl. Microbiol. 99: 1215–1221.

Craufurd P Q, Prasad P V V, Waliyar F & Taheri A 2006 Drought, pod yield, pre harvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger. Field Crops Res. 98: 20–29.

Gallo A, Solfrizzo M, Epifani F, Panzarini G & Perrone G 2016 Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Aspergillus flavus* on almond medium. Int. J. Food Microbiol. 217: 162–169.

Giorni P, Magan N, Pietri A, Bertuzzi T & Battilani P 2007 Studies on *Aspergillus* section *Flavi* isolated in northern Italy from maize. Int. J. Food Microbiol. 113: 330–338.

Kebede H, Abbas H K, Fisher D K & Bellaloui N 2012 Relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress. Toxins 4: 1385–1403.

Klich M A 2007 Environmental and developmental-factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. Mycosci. 48: 71–80.

Lahouar A, Marin S, Crespo-Semperem A, Said S & Sanchis V 2016 Effects of temperature, water activity and incubation time on fungal growth and aflatoxin B_1 production by toxinogenic *Aspegillus flavus* isolates on sorghum seeds. Rev. Argentina De Microbiol. 48: 78–85.

Liu Y & Wu F 2010 Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. Environ. Health Perspect. 118: 818–824.

Magan N, Sachis V & Aldred D 2004 Role of spoilage fungi in seed deterioration. In: Fungal Biotechnology in Agricultural, Food and Environmental Applications (Ed). Marcell Dekker. New York. Pp 311–323.

Magan N & Aldred D 2007 Post-harvest control strategies: Minimising mycotoxins in the food chain. Int. J. Food Microbiol. 119: 131–139.

Magan N, Medina A & Aldred D. 2011 Possible climate change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathol. 60: 150–163.

Makun H A, Gbodi T A, Akanya O H, SalakoE A & Ogbadu G H 2007 Fungi and some mycotoxins contaminating Rice (*Oryza sativa*) in Niger State, Nigeria. Afr. J. Biotech. 6: 99–108.

Marin S, Sanchis V, Teixido A, Saenz R, Ramos A J, Vinas I, Magan N 1996 Water and temperature relations and microconidal germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. Can. J. Microbiol. 42: 1045–1050.

Mousa W, Ghazali F M, Jinap S, Ghazali H M & Radu S 2011 Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. J. Appl. Microbiol. 111: 1262–1274.

Nguyen M T, Toziovanu M, Tran T I & Pfohl-Leszkowicz A 2007 Occurrence of aflatoxin B_1, citrinin and ochratoxin A in rice in five provinces of the Central Region of Vietnam. Food Chem. 105: 42–47.

Okereke V C & Godwin-Egein M I 2018 Mycotoxigenic *Aspergillus flavus* from ginger and turmeric consumed in the Niger Delta Region of Nigeria. J. Spices Arom. Crops 27: 151–157.

Pardo E, Malet M, MarAn S, Sanchis V & Ramos A J 2006 Effects of water activity and temperature on germination and growth profiles of ochratoxigenic *Penicillum verrucosom* isolates on barley meal extract agar. Int J. Food Microbiol. 106: 25–31.

Park J W, Choi S Y, Hwang H J & Kim Y B 2005 Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. Int. J. Appl. Microbiol. 10: 305–314.

Payne G, Thompson D, Lillehoj E, Zuber M & Adkins1988 Effect of temperature on the pre harvest infection of maize kernels by *Aspergillus flavus.* Photopathol, 78: 1376–1380.

Pitt J I & Hocking A D 2009 *Aspergillus* and related teleomorphs. In: Pitt J I & Hocking A D (Eds.), Fungi and Food spoilage (pp.91–99). Academic Press, London.

Somjaipeng S & Ta-uea P 2016 Evaluation of the effect of water activity and temperature on lag phase and growth rate of aflatoxigenic *Aspergillus* section *Flavi* strains isolated from stored rice grain. Agric. Agric. Sci. Proc. 11: 38–45.