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# Research Article
# Effect of Water Activity on Lag Phase and Mycelia Growth of *Aspergillus flavus* Isolated from Ginger

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## Abstract

**Background and Objective:** The incidence of mycotoxigenic moulds in spices in Southern Nigeria has increased under the climate change. The objective of the study was to examine the effect of different water activities on lag phase prior to growth and growth rate of three toxigenic and a non-toxigenic strain of Aspergillus section Flavi on ginger-based medium at 28±2°C. **Materials and Methods:** About 6-day old *mycelia* of *Aspergillus flavus* was used as inoculum and 2 µL of inocula of each of the *A. flavus* strains centrally inoculated into each of the plates. The experiment was a 5×4 factorial in completely randomized design (CRD) replicated thrice. **Results:** Statistical analysis indicated that $a_w$ significantly ($p<0.001$) affected lag phases and growth rates in all the strain studied. The lag phases significantly increased with imposition of water stress. The shortest average lag phase (1.2 days) was at 0.98 and 0.995 $a_w$ while the longest (2.5 days) was at 0.90 $a_w$. Overall, the growth of aflatoxigenic strains was similar over the 0.90 - 0.98 $a_w$ ranges but optimal condition was found to be at 0.98 $a_w$. Non-aflatoxigenic strain had maximum growth at 0.95 $a_w$. Under severe $a_w$ stress (0·85 $a_w$), no growth responses were observed, with all the strains unable to grow within the 12 days incubation period. The combined factors (strain x$a_w$) showed significant ($p<0.001$) interaction for growth rate while there was no evidence of statistical interaction ($p = 0.2$) effect on the lag phases prior to growth of strains. **Conclusion:** The result from this study could be used in decision-making process in relation to $a_w$ during ginger production and also contribute to optimal strategy selection in the control of aflatoxin contamination in ginger in the store.

Key words: Toxigenic and a non-toxigenic strain, mycotoxigenic moulds, *Aspergillus flavus*, ginger, lag phase, water activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Ginger (*Zingiber officinale*) is perishable in nature and the main causes of spoilage are poor handling, growth of spoilage micro-organisms, action of naturally occurring enzymes, chemical reactions and structural changes during storage[1]. Post harvest losses of ginger are high but can be substantially minimized by processing and proper storage at harvest. Large amount of ginger are being wasted annually due to fungal contaminations and some are capable of producing toxic metabolites. FAO[2] reported that 25% of the world's agricultural commodities were contaminated with mycotoxin. These toxic metabolites are detrimental to both human and animal health when ingested through contaminated foods and feeds and inhaled or absorbed through contact by the skin. *Aspergillus flavus* and *Aspergillus parasiticus* are the major producers of aflatoxins. They are well known producers of aflatoxin ($B_1$, $B_2$, $G_1$ and $G_2$), Somjaipeng and Ta-uea[3]. Ginger is predisposed to aflatoxin infection through damages caused by insects feeding on them, rough harvesting and poor handling which result in injury to the skin and flesh of the rhizome and improper conditions in storage. Several factors affect the growth of *A. flavus* and production of aflatoxin, these factors include temperature, water activity, fungal strains, microbial ecology and post-harvest handling methods[4-7]. High temperature and drought stress conditions result to relatively high aflatoxin production by *A. flavus*[8,9]. Temperature and water activity are also important factors that affect the mycelial growth[10-12]. Mycotoxin producing fungal species is high in occurrence in the humid tropics because environmental conditions are favourable to their survival. The combined effects of high temperature and water activity in the region leads to the contamination by aflatoxigenic fungi in major agricultural products and this poses a serious threat to food security. The economic damages arising as a result of aflatoxin contamination is beyond losses of crops in the field and in storage, there is loss of revenue as affected crops are rejected. Most of the time, qualitatively and quantitatively, contaminated produce are consumed, thereby endangering the health of both citizens and animals[13]. However, the level of water activity ($a_w$) in plants plays a major role in the activities of fungi and this is affected by changing climate conditions[14]. This study was therefore aimed at evaluating the effect of storage moisture conditions (water activity $a_w$) levels at 28±2°C on lag phase prior to growth and growth rate of three toxigenic (AFG4, AFG8 and AFG13) and a non-toxigenic (AFG9) strains of *Aspergillus* section *Flavi* on ginger-based medium.

## MATERIALS AND METHODS

**Experimental site:** The experiment was carried out in the Department of Crop and Soil Science Laboratory, University of Port Harcourt, Nigeria (6.55° 0.2' N latitude, 4.54° 10.02' E longitudes) from March-August during the 2018 cropping season.

**Sample collection:** A variety of ginger (*Zingiber officinale*) (UG1), a high yielding genotype was obtained from the National Root Crops Research Institute (NRCRI), Umudike, Nigeria was used in the experiment. The samples were cleaned to remove soil particles and air dried and ground into powder using a blender and stored properly at room temperature.

**Source of *Aspergillus flavus* strains:** Four strains of *A. flavus* [three toxigenic (AFG4, AFG8 and AFG13) and one non-toxigenic (AFG9) strain] were used in the experiment. The strains were isolated from naturally infected ginger and deposited at the Laboratory of Department of Crop and Soil Science, University of Port Harcourt, Nigeria. The toxigenicity of these strains was confirmed on Coconut agar medium (CAM)[15].

**Sub culturing of *Aspergillus flavus* using malt extract agar (MEA):** The MEA medium was prepared according to the manufacturer's instructions, autoclaved for 45 min at 121°C and 0.16 g of chloramphenicol/liter of medium was added to the melted medium to inhibit bacterial growth before pouring 15 mL into 90 mm sterile plates. When agar plates were solidified, *A. flavus* (6 day old mycelia+9 mL sterile water supplemented with 0.05% (w/v) Tween 80) were one-point centrally inoculated and incubated at 28±2°C. Sub-culturing was made to obtain pure cultures.

**Preparation of ginger-based medium and inoculation:** A standard medium of 2.5% milled ginger agar (25 g of ginger powder+10 g of technical agar+0.16 g of chloramphenicol+1000 mL of water) was used in this experiment. The $a_w$ of media was modified by adding increasing amounts of glycerol to obtain $a_w$ 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 culture was prepared by autoclaving at 121 for 45 min, shaken vigorously prior to pouring 15 mL into 90 mm sterile Petri dishes when culture solidified at room temperature, 2 µL of inocula of each

Table 1: p-values for the lag phase (λ, days) and growth rate (days) of *A. flavus* strains on ginger-based medium at different water activities

| Parameters | Lag phase | Growth rate |
|---|---|---|
| Strain | $0.064^{ns}$ | <0.001*** |
| Water activity ($a_w$) | <0.001*** | <0.001*** |
| Strain x $a_w$ | $0.2^{ns}$ | <0.001*** |

ns: Not significant, ***: Very highly significant

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 dishes. Incubation of fungi was done at 28±2°C.

**Fungal growth assessment and data collection:** 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[16]. 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 was by equaling the regression line formula to the original inoculum.

**Experimental design and statistical analysis:** A factorial design with two (5×4) factors: Water activity and *A. flavus* strains was applied. Each treatment, water activity x *A. flavus* strain combination was carried out in triplicate. Analysis of data was done using Genstat 16th Edition, VSN industrial Ltd, UK for normally distributed data. Comparisons were considered significantly different at 5% probability level for all single and interacting treatments.

## RESULTS

**Effect of water activity ($a_w$) levels on the lag phase prior to growth of *Aspergillus flavus* strains:** Figure1(a-d) showed the effect of different $a_w$ levels on the lag phase prior to growth initiation of the four strains of *A. flavus* examined. The result showed that as water stress (drier conditions) was imposed, the time prior to growth increased. This was particularly evident in the toxigenic strains of AFG4 and AFG8 (Fig. 1a and b). At the incubation temperature of 28±2°C, the shortest lag phase was at 0.98 - 0.995 $a_w$ and the longest lag phase was at 0.9 $a_w$ in all the *A. flavus* strains examined. Table 1 showed that, there were no significant ($p = 0.064$) differences among the four strains of *A. flavus* in relation to lag phases prior to growth. The lag phase prior to growth ranged from 1.3-2.9, 1.2-2.7, 1.2-2.1 and 1.0-2.2 days in isolate AFG4, AFG8, AFG9 and AFG13, respectively, (Fig. 1a-d).

**Effect of water activity on the growth rate of *Aspergillus flavus* strains:** Figure 2 showed the effect of $a_w$ on the relative growth rates of the different strains of *A. flavus* examined. The pattern of mycelia growth for the toxigenic strains (AFG4, AFG8 and AFG13) in the ginger-based medium was similar with optimum mycelia growth obtained at 0.98 $a_w$ (Fig. 2a,b and d). The values were 6.61 mm/day in AFG13, 8.6 mm/day in AFG8 and 8.8 mm/day in AFG4. For the non-toxigenic strain (AFG9), the optimum mycelia growth was obtained at 0.95 $a_w$ with a value of 8.2 mm/day (Fig. 2c). However, water activity at 0.9 recorded the lowest mycelia growth in all strains. Statistics showed that in relation to the growth rate, strain and water activity interaction was significant ($p<0.001$) (Table 1).

## DISCUSSION

The *A. Flavus* grew on Nigerian ginger products and could cause accumulation of aflatoxin in these products. It was therefore, useful to determine the optimal ecological conditions for growth and aflatoxin production by this pathogen. This would help in proposing for control strategies to minimize mycotoxin contamination within the HACCP framework in the ginger-based industries. Magan *et al.*[17] and Akbar and Magan[12] had stated that factors such as temperature and water activity are key ecological factors that influence and determine colonization of food products by both toxigenic and non-toxigenic spoilage fungi. This study showed that although the lag phase profile for all the strains was quite similar, AFG9 and AFG13 had shorter lag phase when compared with AFG4 and AFG8. Moreover, once AFG4 and AFG8 growth was initiated, they grew faster than AFG13 at the incubation temperature of 28±2°C. The shortest average lag phase (1.0 days) was at 0.98 and 0.995 $a_w$ while the longest (2.9 days) was at 0.90 $a_w$. However, growth rates was optimum at 0.98 $a_w$ and 0.95 $a_w$ for toxigenic and non-toxigenic strains, respectively. This result points to the fact that the growth of non-toxigenic strains was optimal under drier conditions and this needs to be confirmed *in vivo*. The implication of this is that under drought stress occasioned by climate change, there could be high colonization of ginger by non-toxigenic strains of *A. flavus* with little or no toxin production. Previous studies on *A. flavus* growth on groundnuts had suggested $a_w$ optima of 0.99 $a_w$ at

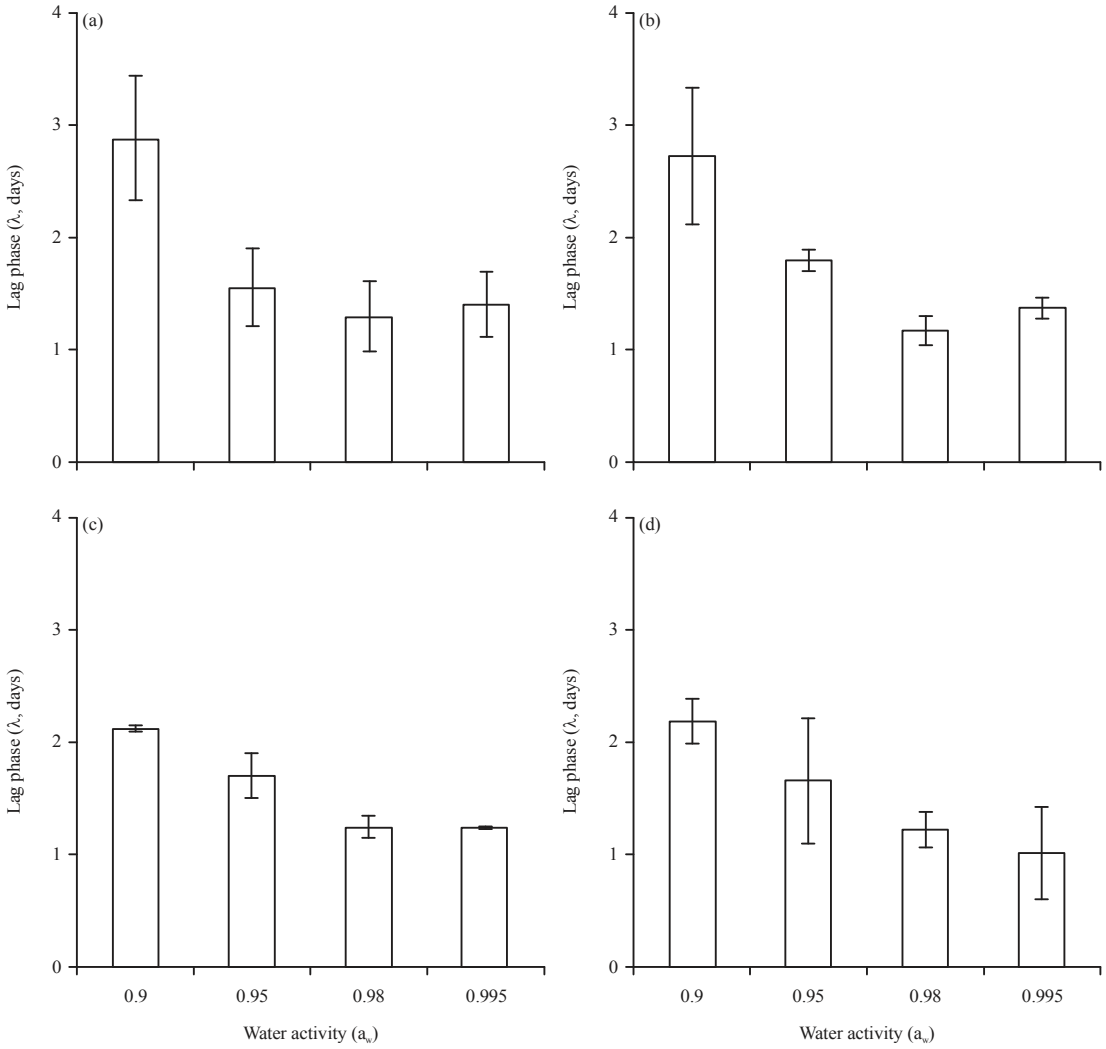


Fig. 1(a-d): Effect of different water activities ($a_w$) on the lag phase prior to growth of four strains of *A. flavus*, a: AFG4, b: AFG8, c: AFG9 and d: AFG13 on a ginger-based medium at 28±2°C after 12 days of incubation
Bars indicate standard deviation of the mean

temperature[18,19] 25-30°C. Mousa *et al.*[20] observed that the optimal growth of *A. flavus* was at 0.90-0.92 $a_w$ at 30°C in polished and brown rice. Castano *et al.*[21] in their study showed optimum growth and toxin production by *A. flavus* at 25-30°C. Also Gallo *et al.*[14] in their study observed that maximum accumulation of fungal biomass by *A. flavus* was obtained at 0.96 $a_w$ and 28°C on almond medium. The authors attributed differences in optimal growth conditions of *A. flavus* found in literatures to the type of medium used.

With 30°C being the optimum temperature associated with the agro climatic zone of the experimental site, this predisposes food products to high risk of *A. flavus* contamination. A number of authors have detailed the optimum and marginal conditions of $a_w$ for growth of *A. flavus*. They found that the lowest $a_w$ for growth was about 0.85-0.88 $a_{w22}$ and the highest[12,3] at >0.95 $a_w$. Both the toxigenic and non-toxigenic strains of *A. flavus* used in the current study exhibited similar boundaries for growth in relation to $a_w$ at 28±2°C incubation temperature. Generally, *A. flavus* was able to grow over a wider range of $a_w$ levels and appears to be more tolerant of drier condition (0.9 $a_w$). The strains were not able to grow at 0.85 $a_w$ at 28±2°C over the 12 day incubation period, thus confirming the findings of Lahouar *et al.*[23]. The authors had reported no growth of *A. flavus* at 0.85-0.88 $a_w$ at 15-37°C on sorghum seeds after 28 days of incubation. The current study therefore, provides useful information on the optimal $a_w$ conditions necessary for growth and inhibition of both toxigenic and non-toxigenic *A. flavus* in ginger, although an *in vivo* study is required to validate this report.

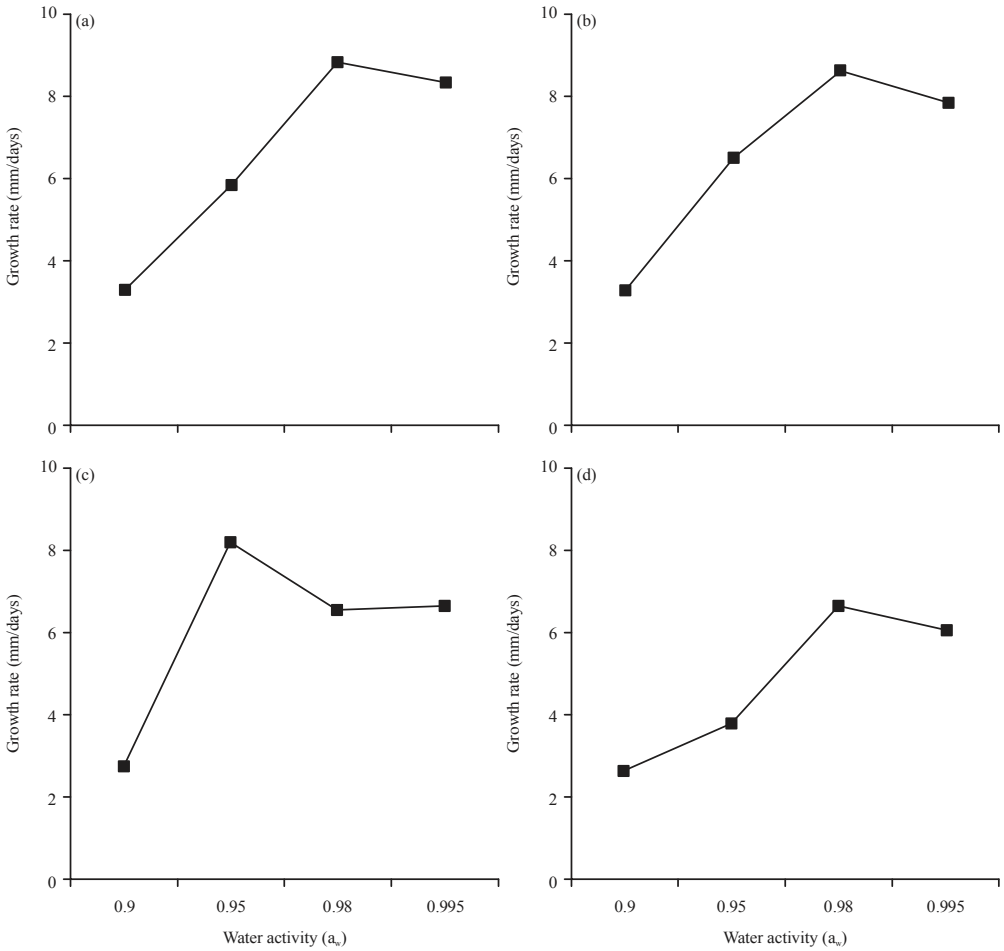


Fig. 2(a-d): Effect of different water activities ($a_w$) on the relative growth rate of four strains of *A. flavus*, a: AFG4, b: AFG8, c: AFG9 and d: AFG13 on a ginger-based medium at 28±2°C after 12 days of incubation

## CONCLUSION

This study provided growth patterns of *Aspergillus flavus* in response to different water activity ($a_w$) level at optimum temperature of 28±2°C on a ginger agar medium. The optimum condition for growth was revealed. *Aspergillus flavus* grows over a wider range of $a_w$ levels and is tolerant of drier conditions (0.9 $a_w$). Overall, growth did not occur at 0.85$a_w$ at 28±2°C. This is a useful information that could explain the effect of $a_w$ on the growth and colonization of *Aspergillus flavus* which occur during post-harvest handling and storage of ginger.

## SIGNIFICANCE STATEMENT

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 ginger. This information could be used in decision-making process in relation to $a_w$ during ginger production and also contribute to optimal strategy selection in the control of aflatoxin contamination in ginger in the store. Ginger 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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