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Assessment of the effect of water activity on lag phase and growth rate of *Aspergillus flavus* isolated from dried chili and chili powder

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Abstract
This study was aimed at analysing the effect of water activity on the growth rate and lag phase of *Aspergillus flavus* isolated from dried chili and chili powder samples. Isolates are grown on agar containing 3% chili powder extract. The diameter of each isolate in different water activities was measured for 12 days on a 24 hour interval. Analysis showed that different water activities have an impact on the growth rate and lag phase of *A. flavus*. All the isolates showed good growth on a range of 0.95–0.99 a_w, while media with 0.88 a_w didn't show any *A. flavus* growth. Favorable growth conditions may allow mold to produce hazardous mycotoxins.

Keywords: Water activity, chili, chili powder, *Aspergillus flavus*

1. Introduction
Mycotoxins are low-molecular-weight secondary metabolites. *Aspergillus*, *Fusarium*, and *Penicillium* are major genera that produce different mycotoxins (Reverberi *et al.*, 2010) [11]. Mycotoxins can adversely affect humans, animals and huge economics loss across the whole globe.

Aspergillus sp. is the group of mold that produces the most toxic mycotoxins, namely aflatoxin. *A. flavus* and *A. parasiticus* are two important species. Major type of aflatoxins includes G2, G1, B2 and B1. Among all the aflatoxins, aflatoxin B1 is carcinogenic and teratogenic (Organization and Cancer, 1993) [8]. Mycotoxins such as ochratoxins are produced by some *Aspergillus* genus, such as *A. niger* (Merla *et al.*, 2018) [7].

Chili fruits are consumed world-wide. It can be affected by *Aspergillus sp*. During harvesting or post-harvest practices such as picking, drying, or transporting. Aflatoxin B2, B1 and ochratoxin contamination are frequently reported in dried chili (Iqbal *et al.*, 2017; Wikandari *et al.*, 2020) [4, 13].

Unbound water available in food materials is known as "water activity". This water can be used by microorganisms for their growth (Bellı *et al.*, 2004) [1]. Water activity is one of the most important environmental factors that influence fungal growth on food. The aim of this study was to analyze the effect of a_w on the growth rate and lag phase of *A. flavus* isolates from dried chili and chili powder.

2. Materials and Methods
2.1. Fungal culture
Aflatoxigenic isolates of *A. flavus* AF1, AF2, AF3, AF4 and AF5 isolated from dried chili and chili powder were obtained from the Centre for Post-Harvest Technology, TNAU, Coimbatore and used in this experiment. All fungal isolates were maintained on Potato Dextrose Agar (PDA).

2.2. Chili powder extract agar (3%)
30 g of chili powder in distilled water boiled for 30 minutes. The solution was filtered through a triple-layered cheese cloth. By using distilled water, the total volume was made up to 1 L and 1.5% agar was added (Marín *et al.*, 2009) [6].

A standard calibration graph was plotted using 5%, 10%, 15%, 20%, 25%, and 30% of glycerol media (Fig. 1). The water activity (a_w) of the chili medium was adjusted by adding

glycerol to reach the desired level (0.99, 0.95, 0.91, and 0.88). The water activity of the medium was measured by a water activity meter (AquaLab PRE V3.65). The measurements were performed in triplicate at 25 °C.

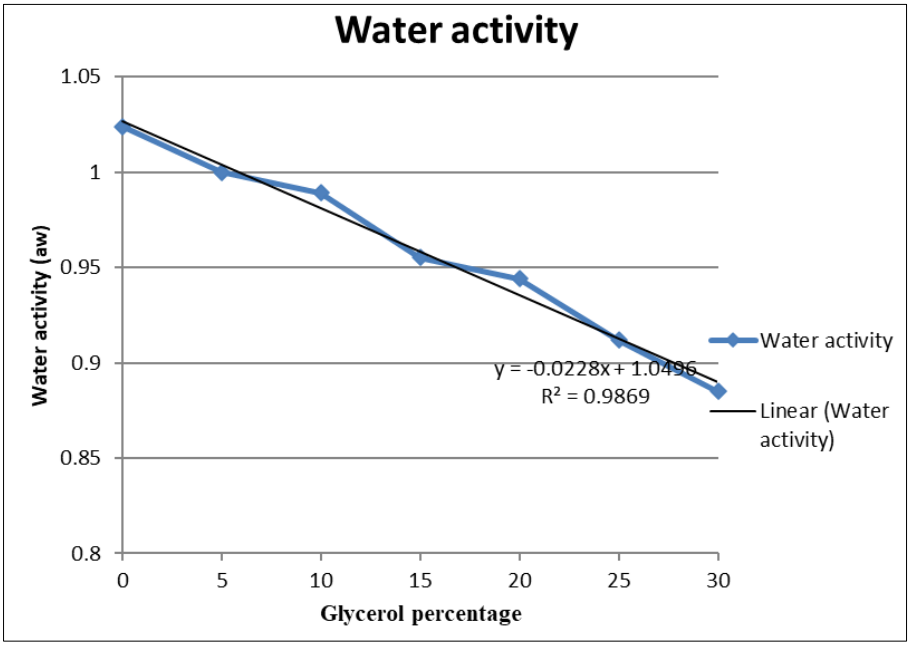

Fig 1: Calibration curve between water activity and glycerol concentration

2.3. Assessment of fungal growth

A. flavus isolates grown on PDA plates at room temperature for 6 days. A 6mm agar plug was removed from each isolate using a sterile cork borer. Plugs are placed at the centre of chili powder extract agar of different water activity values. Inoculated plates were incubated at room temperature. For 12 days, the diameter of the colony was measured every 24 hours.

3. Results and Discussion

3.1 Effect of a_w on *Aspergillus flavus* growth rate

Laboratory experiment for 12 days on different water activity conditions for growth of *A. flavus* revealed that, if water activity is higher fungal growth also will be higher. 0.99 a_w showed maximum average growth rate of *A. flavus* on chili powder extract agar (14.58 mm/day). Agar with 0.88 a_w didn't show any *A. flavus* growth up to 12 days of experiment (Fig. 2). Scanning electron micrographs of Conidia and spores of *A. flavus* isolate grown on chili powder extract agarshown in Fig. 3&4.*A.flavus* growth on maize extract agar medium showed maximum fungal growth at 0.99a_w (Bernáldez *et al.*, 2017) [2]. In his study, (Chuaysrinule *et al.*, 2020) [3] showed effect of water activity on *A. flavus* growth on chili powder based agar medium and found that the most suitable conditions for *A. flavus* were higher than 0.97a_w.*A. flavus* growth was reduced while water activity was below 0.90 a_w (Lasram *et al.*, 2016) [5]. Meat products based agar media for comparative analysis of *A. flavus* and a_w didn't observe any mold growth at 0.85a_w (Peromingo *et al.*, 2016) [10].

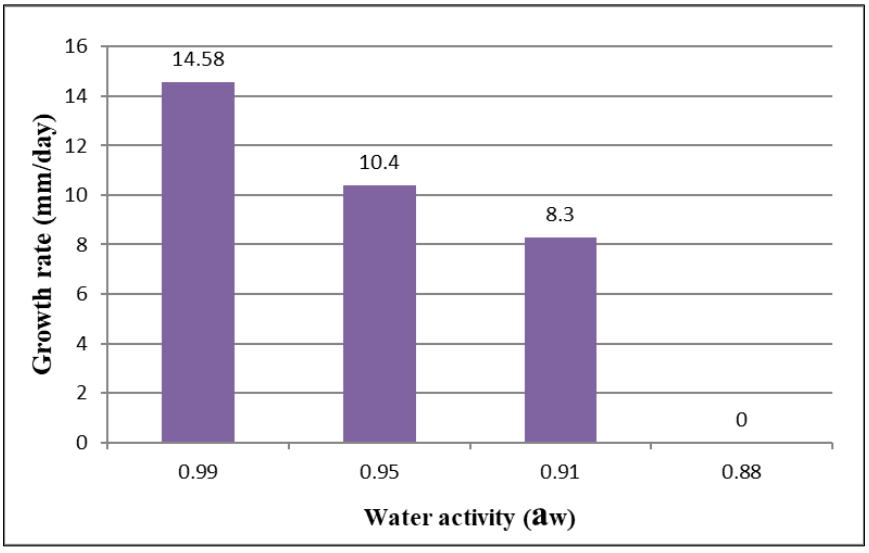

Fig 2: Growth rate of *Aspergillus flavus* on different water activity condition

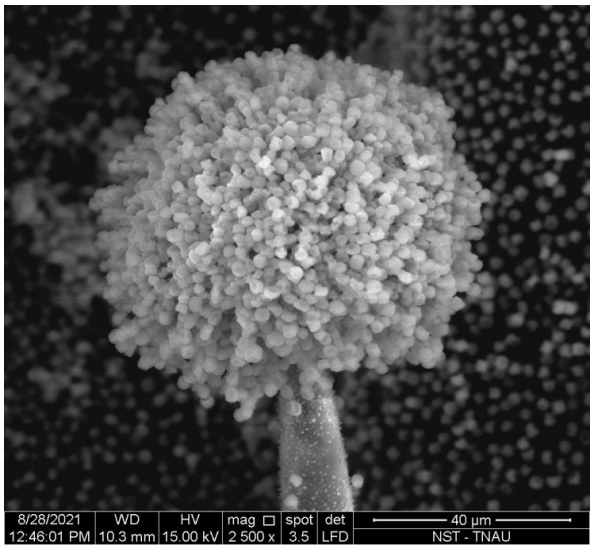

Fig 3: Scanning Electron Micrograph of *A. flavus* conidia

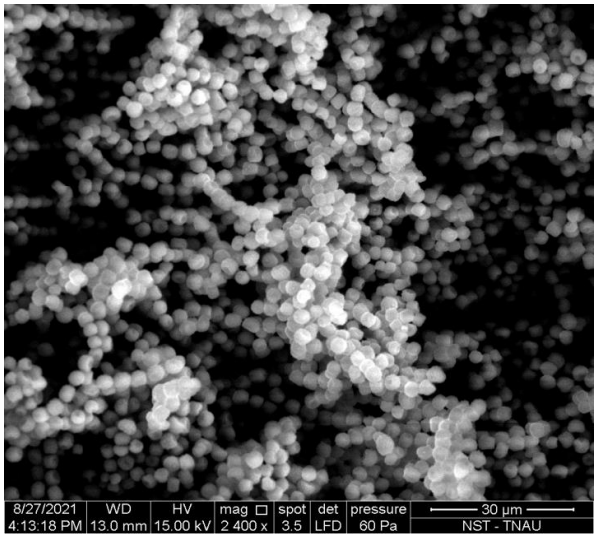

Fig 4: Scanning Electron Micrograph of *A. flavus* spores

3.2. Effect of a_w on *Aspergillus flavus* lag phase

The effect of different water activity levels on the average of lag phase before the growth of five isolates is shown in Table 1. This showed that when water activity decreases, a longer lag phase was observed. The shortest average lag phase of *A. flavus* was in 0.99a_w. The lag phase of *A. flavus* in 0.88a_w continued up to the twelfth day of the experiment.

Table 1: *Aspergillus flavus* growth in different water activity conditions

Days	0.99 (a_w)	0.95 (a_w)	0.91(a_w)	0.88 (a_w)
1	+	-	-	-
2	++	-	-	-
3	++	+	-	-
4	+++	++	-	-
5	+++	+++	-	-
6	++++	+++	-	-
7	++++	++++	-	-
8	++++	++++	+	-
9	++++	++++	+	-
10	++++	++++	++	-
11	++++	++++	++	-
12	++++	++++	++	-

(-) no growth, (+) growth zone ≤ 20%, (++) growth zone ≤ 50%, (+++) growth zone ≤ 80%, (++++) growth zone ≤ 100%

Isolates from stored rice grains, *Aspergillus spp*. showed a shorter lag phase of 1 day at a water activity range of 0.95–0.98 (Somjaipeng and Ta-uea, 2016) [12]. An average shorter lag phase of 1.2 days was found in a range of 0.99 to 0.98 a_w, whereas an average longer lag phase of 2.5 days was reported in *A. flavus* in ginger based medium (Omolola *et al*., 2018) [9].

4. Conclusion

The effect of water activity on the lag phase and growth rate of *A. flavus* was studied. The study showed that water activity has a huge impact on mold growth. All the isolates showed a high growth rate and a short lag phase in a range of 0.95–0.99 a_w. Considering this relationship, controlling the growth of *A. flavus* during the post-harvest operations can be possible in chili and chili powder. Harvested products can be stored in a lower water activity atmosphere, which will prevent mold growth and mycotoxin production.

5. Acknowledgments

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