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Effects of Water Activity on the Radial Growth of *Aspergillus niger* on Solid Medium

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Authors' contributions

This work was carried out in collaboration between both authors. Author AAM conceived and designed the study. Authors BA and AAM carried out the bench work. Author BA managed the literature searches and wrote the first draft of the manuscript. Author AAM analyzed the data of the study, agreed with manuscript results and conclusions, made critical revisions and approved final version. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The effects of water activity (a_w) on the radial growth of *Aspergillus niger* on Malt Extract Agar was investigated using sodium chloride and glycerol mixtures. Varied concentrations of each solute was used to modify the water availability of the medium. The Petri plates were incubated at 31 ± 2℃ representative of the Ghanaian ambient temperature. The inhibitory effects showed by two solutes on the growth of the *A. niger* was qualitatively similar but showed quantitative differences. The growth rate was erratic with no clear cut generalized response. On the whole, growth was better on glycerol-modified medium compared with the growth on the sodium chloride modified medium. Growth was optimal at the range of 0.904-0.947a_w on the glycerol-modified medium and 0.800-0.821a_w on the sodium chloride medium. Growth was severely retarded at 0.768a_w when the water availability was adjusted with seventy-five grams (75 g) sodium chloride. Glycerol used to modify

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water availability of a medium may serve as carbon and energy sources to promote growth of fungi. On the other hand, high concentration of sodium chloride as solute to control water activity of a medium may be toxic to the organism hence the differences in the growth pattern observed. Microorganisms react not only to water activity but also the solute adjusting the water availability.

Keywords: Water activity; Aspergillus niger; sodium chloride; glycerol; radial growth.

1. INTRODUCTION

Water availability is a factor that can be controlled to prevent or reduce fungal growth. For growth to occur, a certain level of water needs to be available. The water that is available to support fungal growth is commonly expressed as water activity (a_w). Water activity compares the physical properties of water in a material with that of pure water to generate a scale from 0 -1. Pure distilled water has a value of 1.0 and as the value moves closer to 0, more and more solutes are present in the water and less and less water is available for an organism to use, thereby preventing the growth of many fungi [1].

Fungi have in the course of evolution diversified to exploit a wide variety of habitats. Different species hence require different conditions of growth Different fungi have different water activity requirements. These varying requirements allow them to be grouped into three broad categories. Fungi which require a_w higher than 0.9 are known as hydrophilic fungi, whereas those that grow between 0.8 and 0.9 are known as mesophilic fungi. Finally, fungi that can grow at a_w below 0.8 are known as xerophilic fungi [2].

Fungi follow a pattern of growth and development which is much less predictable than for plants and animals. A typical pattern of growth follows a response to the environmental factors such as water availability [3] and need sufficient nutrient to grow [4].

An earlier work done [5] revealed that lowering the water activity of a medium has threefold effects; (1) Prolongation of the lag phase including the time required for germination of conidia, (2) reduction of growth rate and (3) reduction of the total amount of growth. These responses are independent of the type of solute used in lowering the water activity of the medium.

Parra et.al. [6], investigated the effects of interactions of water activity (0.99-0.90a_w), temperature (20, 30 and 35℃), solutes for adjusting the water activity (glycerol, sodium chloride) on growth and sporulation of a wild-type strain of *Aspergillus niger* and two genetically modified lysozyme strains for the first time. The data in that study indicates that optimum growth rates were achieved by the three *Aspergillus* strains in the range of 097-0.95a_w. However, the growth rate of the genetically-modified strains were similar to each other but significantly different from the wild-type strain. Interestingly, the study reported higher tolerance to lower water activity of the genetically- modified *Aspergillus* strains than the wild type when the medium was amended with sodium chloride., The best growth was attained at 30℃ when glycerol was the solute used to adjust the water availability in the medium. There were variations in optimum water activity, temperature and sporulation for the three *Aspergillus* strains used in the study.

The current study sought to ascertain the growth pattern of *Aspergillus niger* van Tieghan under different water activities created using sodium chloride and glycerol mixtures on Malt Extract agar incubated at 31 ± 2℃ representative of the Ghanaian ambient temperature.

The *Aspergillus niger* used in this investigation was isolated from cowpea from a local market in the Accra metropolis. Previous work by various investigators revealed the highest percentage isolation of the genus *Aspergillus* among other genera from maize [7,8] Recently, [9] isolated four fungal genera; Aspergillus, *Fusarium, Penicillium* and *Mucor* from maize, cowpea, peanuts and bambara beans from two popular markets and shopping malls in the Accra metropolis. Of these, *Aspergillus* species predominated with 31.9%; The paper attributed the high percentage isolates to poor storage conditions such as temperature, relative humidity among others.

The health hazards associated with the consumption of contaminated maize grains by toxigenic moulds such as *Aspergillus* species is well-known. Some produce fungal toxins called *aflatoxin, ochratoxin* which are of global health concern [10,11]. Quite apart from this, *A. niger* demonstrates oligotrophy capable of growing in nutrient-depleted environment [12].

The current study sought to ascertain the optimum growth conditions of the local species of *Aspergillus niger* under different water activity (a_w) provided by sodium chloride and glycerol mixtures against the Ghanaian ambient temperature of 31 ± 2℃. This information would serve as a springboard for more investigative works to make policy- makers on food safety give useful advice on proper methods of drying cereal/grains and storage under appropriate temperature and relative humidity.

2. MATERIALS AND METHODS

2.1 Materials

Aspergillus niger van Tieghan used for the study was isolate from cowpea (*Vigna unguiculata Walp*) bought from a local market in Accra metropolis.

Malt Extract Agar (CM, 72) was the mycological medium used for the study. Anhydrous sodium chloride (EMD, SX0420-1), and glycerol (Wagtech, GL2879) were used to adjust the water activity of the medium.

Unless otherwise stated all Laboratory work was carried out at the Microbiology Laboratory of Accra Technical University, Accra-Ghana.

2.2 General Methods

2.2.1 Sterilization of glassware

All glassware used for the study were washed in soapy water and rinsed with tap-water. Petri dishes were packed into appropriate canisters after they had dried and then placed in a pre-heated hot-air oven at 160℃ for one hour.

2.2.2 Preparation of media

The Potato Dextrose Agar used for the isolation of the *Aspergillus niger* on cowpea was prepared as follows;

Two hundred (200) grams of peeled and sliced Irish potato were boiled in 500mL distilled water, strained through cheese cloth and made up to 1000 mL; twenty (20) grams glucose and fifteen (15) grams agar were added. The mixture was homogenized by heating briefly on a hot plate thereafter sterilized in an autoclave at 121℃ for 25 minutes.

The Malt Extract Agar was prepared following the manufacturer's instructions.

Briefly, 48 g of the powder was weighed and dissolved in 1L distilled water then heated in a water bath for 5mins to homogenize. Thereafter sterilized in an autoclave for 10 mins at 121℃. Care was taken not to overheat the medium

2.2.3 Isolation of *Aspergillus niger* on cowpea using serial-dilution method

A 10 g sample of the grains was weighed and transferred aseptically into 100ml 0.1% Peptone in 250 mL Erlenmeyer flasks and then shaken in Gallenkamp Model Orbital shaker at 140rev/min for 30 min. From this stock suspension serial dilution method was employed up to 10^{-3} and spores were raised in the prepared Potato Dextrose Agar.

The plates were incubated at 28-31℃ until fungi grew (5-10 days).

2.2.4 Identification of *Aspergillus niger* on PDA plates

The fungus was identified by its colour, morphological and cultural characteristics as outlined by Samson and Van Reenen-Hoekstra (1988).

2.2.5 Adjustment of water activity in malt extract agar using sodium chloride and glycerol

A 2.5 g, 5.0 g and 7.5 g of sterilized anhydrous sodium chloride were weighed separately and added into three 250 mL sterile conical flasks containing 100 mL of prepared Malt Extract Agar. The conical flasks were labeled, 25 g/L, 50 g/L, and 75 g/L respectively. The control flask received no addition of sodium chloride.

Similarly, 160 g/L, 190 g/L, and 220 g/L of glycerol on Malt Extract Agar were prepared for water activity (a_w) determination.

2.2.6 Water activity determination of malt extract agar amended with different weights of sodium chloride and glycerol

The water activity (a_w) of the medium was measured using a Rotonic Hydrolab 2 (Rotonic AG, Bassersdorf, Germany).

A 8 g for each treatment of the medium was weighed using Satorius Portable analytical balance, (PT 600, Satorius GMBH Germany), into a sample vial and was transferred into the

chamber of the Rotonic Hydrolab 2 for water activity measurement. The procedure was repeated three times for each treatment and the average values were calculated.

2.2.7 Inoculation of *Aspergillus niger* onto treated malt extract agar

About 20 mL of the treated medium was poured aseptically into appropriately labeled sterilized Petri dishes and left to solidify in a Lamina flow cabinet. Two diameters were drawn to intersect at the centre of the bottom of each sterilized Petri dish. A flamed inoculating needle was used to pick a 3 mm discs of pure culture of *Aspergillus niger* and then placed at the intersection point of the lines drawn beneath the plates. Three replicates were poured for each treatment. The plates were incubated at 30℃ for the fungi to grow. Measurements of the radial growth were taken along two diameters with a transparent meter rule for each plate for seven consecutive days.

2.3 Statistical Analyses

Data obtained was stored in Microsoft Excel and the analysis by the Statistical Package for the Social Sciences (SPSS), version 20. Data was summarized by determining the means, median, minimum and maximum values of the Zone diameter. A P-Value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Optimal radial growth of *Aspergillus niger* isolated from cowpea (*Vigna unguiculata*) was investigated on Malt Extract agar with different water activities created by the addition of different weights of Sodium chloride and Glycerol. The results obtained are shown in Figs. 1,2,3 and 4 below.

3.2 Discussion

Investigations by [5,13] revealed that growth of filamentous fungi is dependent on thermodynamic factors such as water availability and temperature.

A search through pertinent literature shows that quite a number of studies have been done to ascertain the optimum water activity (a_w) and temperature range for growth and sporulation of different strains of *Aspergillus niger.*

In a work carried out by [6] on three strains of *A. niger* consisting of a wild-type and two genetically modified lysozyme strains grown on Malt Extract Agar which a_w were amended with sodium chloride (NaCl) and glycerol at 20, 30 and 35℃. The data in that study indicate that the use of glycerol to modify the water availability of

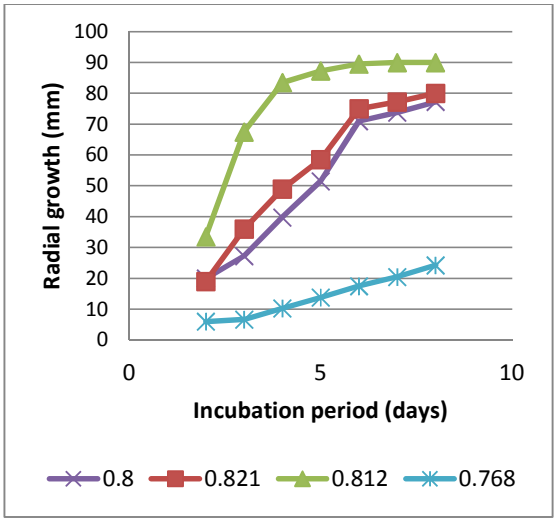

Fig. 1. Effect of water activity on the radial growth of *Aspergillus niger* on malt extract agar amended with varied concentrations (g/L) of sodium chloride

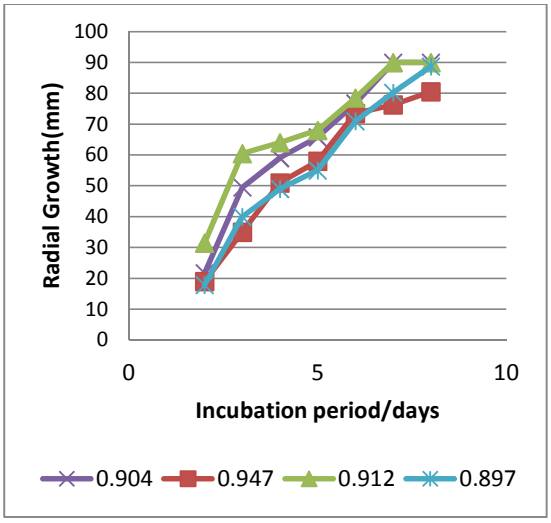

Fig. 2. Effect of water activity on the radial growth of *Aspergillus niger* on malt extract agar amended with varied concentrations (g/L) of glycerol

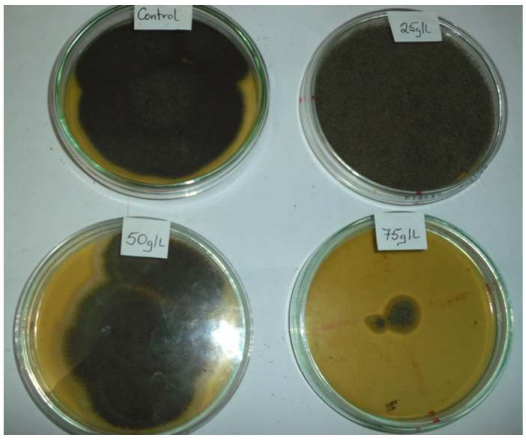

Fig. 3. Five- day old culture of *Aspergillus niger* growing on Malt Extract Agar amended with varied concentrations of sodium chloride
Top Left: control (a_w 0.800), Top Right: (a_w 0.812). Bottom Left: (a_w 0.821), Bottom Right: (a_w 0.768)

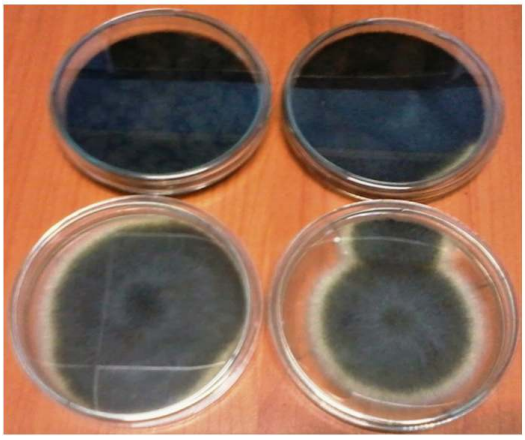

Fig. 4. Five - day old culture of *Aspergillus niger* growing on Malt Extract Agar amended with varied concentrations of glycerol
Top Left: control (a_w 0.947) Top Right: (a_w .0.912) Bottom Left: (a_w 0.904), Bottom Right (a_w 0.897)

the medium produced a better growth rate of the three A niger strains compared with NaCl amended medium. Furthermore, the study shows that a combination of aw, temperature and the type of solute used to modify the medium had the greatest effect on the growth rate of strain L11 containing the full-length hen egg white lysozyme than the other *A. niger* strains investigated.

Mitchell et. al. [14], working on *A. carbonarius* from different regions in Europe reported a range of 10-40℃ and 0.77-0.99a_w optimum at 35℃ and 0.99a_w. An *Aspergillus* sp isolated from gravevine grew optimally at 0.95a_w and temperature range of 30-35℃ [15].

Penicillium roquefortii strain isolated from cheese grew optimally at 0.97-0.98a_w with sporulation best at 0.96a_w [16,17].

In this study, *Aspergillus niger* van Tieghan isolated from cowpea (*Vigna unguiculata* Walp) was grown on Malt Extract Agar plates amended with glycerol and sodium chloride to adjust water availability of the medium and incubated at 31℃ ± 2 of the Ghanaian ambient temperature. Optimal growth was in the range 0.904-0.947a_w on glycerol-modified media. However, a range 0.812-0.821a_w was optimal on sodium chloride-modified media (Figs. 3,4). Generally, there was a significant association (p-value < 0.001), between the radial growth and the incubation period on both the glycerol-modified and sodium chloride-modified Malt Extract plates for the different water activities. Interestingly, *A. niger* was significantly severely depressed throughout the study period at 0.768a_w adjusted with seventy-five grams (75 g) of sodium chloride (P-value = 0.002, Figs. 1,3).

These findings agree with that of [6] which reported among others the poor growth of the three *Aspergillus niger* strains on the sodium chloride- modified Malt Extract agar used in their investigations. There was far better growth of the *A. niger* strains on the glycerol-modified Malt Extract plates. According to their report, glycerol used to adjust water availability of a medium can be utilized as a carbon and energy sources and can act directly as a compatible solute. On the other hand, high concentration of sodium chloride used to adjust water availability can be toxic to the growing microorganism. Findings which agree with the current study (Figs. 1,3).

There was no association between the various water activity and the radial growth of *Aspergillus niger* on the sodium chloride-modified Malt Extract plates (P-value = 0.175). Similarly, association was not found between the various water activity and the radial growth of *Aspergillus niger* on the glycerol-modified Malt Extract plates (P-value = 0.601). The inhibitory effects exhibited by the different solutes in the current study on the radial growth of *A. niger* was qualitatively similar but showed quantitative differences on the two treated media. The growth rate was erratic with no clear cut generalized response (Figs. 1,2). These findings contradict the work done by [18]. Thus, colony radial growth rate on

solid medium cannot always be used to determine the effect of water activity on fungal growth rate. Many factors such as the microorganism, type of medium, type of solute used in adjusting the medium among others might influence the growth rate of microorganism on solid and liquid media.

4. CONCLUSION

The growth rate of *Aspergillus niger* van Tieghan was better on the glycerol- modified Malt Extract agar compared with the growth rate of the same *A. niger* strain on sodium chloride-modified Malt Extract agar. There was severe retardation of growth of the *A.niger* on sodium chloride-modified Malt Extract agar at 0.768aw using seventy-five grams (75 g) of sodium chloride to adjust the water availability in the medium.

Microorganisms react not only to water activity but also the solute adjusting the water activity.

Minimum water activity values for growth vary with different solutes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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