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Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus*

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Abstract: This study was carried out to evaluate the in vitro use of pH factor in the control of *Aspergillus parasiticus* which is a notable causative agent of food rot and plant mycotoxins. Under laboratory condition, *A. parasiticus* was innoculated in Potato Dextrose Broth media of varied pH ranging from 4.0 to 10.0 and incubated for 7 days at room temperature. Within the pH range of 4.0 to 10.0, the mean mycelial dry weight produced ranged from 355.67mg to 302.73mg while the spore production ranged from 4.5 x 10^7 to 2.8 x 10^7. There was no significant difference at 95% confidence limit between the mycelial dry weight means except at pH 10.0. The spores formed at pH of 5.0 and 7.0 were significantly different from other pH; with highest number of spores formed at pH of 5.0 and the lowest at pH of 10.0. The lowest mycelial weight and spore formation recorded at pH 10.0 indicate that higher alkaline medium is not suitable for development of *A. parasiticus*. It was therefore concluded that certain alkaline medium can be used to inhibit the mycelia growth and sporulation of *A. parasiticus* in order to prevent it from damaging our crops.

Keywords: *Aspergillus parasiticus*, pH, Mycelia, Sporulation

1. Introduction

Much as the Aspergillus fungi play very important economic role in the fermentation industries, they are notorious for various plant and food secondary rot, with the consequence of possible accumulation of mycotoxins [1, 2]. They can contaminate agricultural products at different stages including pre-harvest and post-harvest handling. Reported annual loss of 20 – 30% in crops that is due to pests and decay by the phytopathogenic fungi have an immediate and significant impact on people's livelihood [3]. Changes due to spoilage by Aspergillus species can be of sensorial, nutritional and qualitative nature such as; pigmentation, discoloration, rotting, development of off-odours and off-flavours. However, the most notable consequence of their presence is mycotoxin contamination of foods and feeds. Because they are opportunistic pathogens, most of them are encountered as storage moulds on plant products [4]. Various mycotoxins have been identified in foods and feeds contaminated by Aspergillus species, the most important being the aflatoxins, and ochratoxin 'A' [5].

It has been shown that pH, water activity (w_a) and temperature are important criteria for understanding the ecology of spoilage fungi, especially mycotoxigenic species [6]. Reports have shown that growth of fungi could be affected by 'Hydrogen ion concentration' (pH) in a medium in which it grows, either directly by its action on the cell surfaces or indirectly by its effect on the availability of nutrients. However, acid/alkaline requirement for growth of fungi is quite broad, ranging from pH 3.0 to more than pH 8.0, with optimum around pH 5.0 if nutrient requirements are satisfied [7]. Reference [8] also reported that growth of *Aspergillus carbonarius*, isolated from wine and table grapes was influenced better at pH 4.0 and 7.0 than at pH 2.6, regardless of water activity(w_a) level. In general, Aspergillus species are more tolerant to alkaline pH while Penicillium species appear to be more tolerant to acidic pH [9]. Studies on pH reveal that fungi grow at pH 3.0 - 8.0, with maximum production of dry mycelial weight and sporulation at pH 5.5 and pH 6.5 respectively, in liquid media [10, 11]. In general, a neutral to weak acidic environment was suitable for mycelial growth, with optimum pH 5.0 –7.0 and pH 5.0 –8.0 for conidial production [12]. For this reason, in-vitro studies of

mycelia growth and sporulation of *A. parasiticus* at varying pH level was investigated to understand growth and sporulation of this organism in the food context, so as to control the quality of foods and food product from formulation to storage.

2. Materials and Methods

2.1. Collection of Fungi

The stock of *A. parasiticus* used in this study was obtained from the Department of Biological Sciences, Federal University of Technology, Minna, Niger State. The fungus was activated by sub-culturing on potato dextrose agar (PDA) in Petri dishes.

2.2. Preparation of Potato Dextrose Broth Media of Varying pH Levels

Potato dextrose broth (PDB) was prepared using modified procedure of [13]. 300g of peeled Irish potato (*Solanum tuberosum*) was boiled in 250ml of distilled water and filtered through a muslin cloth. 20g of glucose and 0.05g of chloramphenicol were added to the filtrate and the volume was made up to one liter with distilled water. The prepared medium was distributed in aliquot of 50ml in twenty one sterile 250ml conical flasks. Sets of three flasks were adjusted by adding either hydrochloric acid (0.1M HCl) or sodium hydroxide (0.1M NaOH) [10] to get the required pH of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. The pH was measured using electrical pH meter (Jenway 3305) before sterilization in autoclave at 121ºC.

2.3. Inoculation and Mycelial Development of A. parasiticus in the Broth Media

A two millimeter disc of the culture of *Aspergillus parasiticus* was transferred into each flask. The inoculated flasks were incubated on bench top for seven (7) days at room temperature (approximately 27 ± 3^0C). The culture media were decanted and, each flask medium was separately filtered using known weighted Whatman filter paper No.1 to recover the mycelia after washing several times with distilled water. The mycelia were oven dried at 80^0C until constant dry weight. The dried mycelia were weighed (g) using electrical mettler balance [14]. The average of the triplicates was recorded.

2.4. Preparation of Potato Dextrose Agar Media of Varying pH Levels

Seven (7) different pH levels of Potato dextrose agar (PDA) were prepared using the procedure described above with the addition of 20g agar (a solidifying agent) and made up to one liter with distilled water. The pH of each medium was adjusted by adding either hydrochloric acid (0.1M HCl) or sodium hydroxide (0.1M NaOH) to get the required pH values [10]. The pH level was measured using electrical pH meter (Jenway 3305) before sterilization in autoclave at 121ºC. Aliquot of 25ml of each of the sterilized media was poured in 10cm diameter sterile Petri dishes. Each pH treatment was in triplicate. The media were allowed to cool and solidified.

2.5. Inoculation and Sporulation of A. parasiticus in the Agar Media

Two millimetre discs of the culture of *Aspergillus parasiticus* were obtained from the growing edges of cultured PDA colonies using sterilized cork borer. The agar plugs were transferred to the centre of PDA plates (one plug per plate) at different pH levels and incubated at temperature of approximately 27 ± 3^0C for seven (7) days [15]. Each treatment was in triplicate.

The spores in each treated Petri dish was washed in 20 ml sterilized water by the use of camel hair brush into test tube. 0.2ml drop of this solution was placed in the haemocytometer and mounted for counting on compound light microscope. Each treatment was in triplicate [14]. The number of spores per ml was computed using the formula below:

$$\text{Number of spores per ml} = \frac{\text{average spores counted} \times (25 \times 10^4) \times \text{20(dilution factor)}}{}$$

2.6. Statistical Analysis

Data obtained were analysed using one way Analysis of Variance (ANOVA) and the group means compared by Duncan Multiple Range Test (DMRT) using the Statistical Package for Social Science (SPSS) version 16.0. (2007).

3. Results

3.1. Effect of Different pH Levels on Dry Mycelial Weight of Aspergillus parasiticus

Results of this study showed that *A. parasiticus* grow more in acid medium and also in a neutral medium as well as weak alkaline medium (pH 4.0 – 8.0) (Table 1). The highest mean of dried mycelial weight (355.67mg) was obtained in pH 4.0 broth medium. This was followed by 353.3mg in pH 7.0. The lowest mycelia dry weight (302.73mg) was obtained in pH 10.0 broth medium. This value was significantly different ($p < 0.05$) from all other values (Table 1).

3.2. Effect of Different pH Levels on Sporulation of Aspergillus parasiticus

The effect of pH on spore formation of *A. parasiticus* is presented in Table 2. This shows that among the treatment combinations, pH 5.0 produced the highest spores per ml (8.33 × 107), followed by pH 7.0 (7.67 × 107). The lowest spores' formation of 2.83 × 107 was recorded at pH 10.0.

Table 1. *Mean and Standard Error of dry mycelial weight of Aspergillus parasiticus cultured at different pH levels*

pH	(X ± SD)mg
4.0	355.67 ± 3.024^{a}
5.0	352.97 ± 2.511^{a}
6.0	352.90 ± 2.751^{a}
7.0	353.30 ± 27.214^{a}
8.0	350.30 ± 8.810^{a}
9.0	330.77 ± 21.795^{a}
10.0	302.73 ± 17.043^{b}

*Values are Mean ± SD of triplicate (3) dried weight of *Aspergillus parasiticus.* Values with the same superscript are not significantly different ($p > 0.05$) tested by DMRT**.**

Table 2. *Mean and Standard Error of spore count per ml ofAspergillus parasiticus cultured at different pH*

pH	(X× 10^7 ± SD)
4.0	4.500 ± 0.500^{ac}
5.0	8.333 ± 0.764^{b}
6.0	4.833 ± 0.577^{c}
7.0	7.667 ± 1.528^{b}
8.0	4.333 ± 0.764^{ac}
9.0	3.333 ± 0.288^{ad}
10.0	2.833 ± 0.288^{d}

*Mean values of triplicate (3) sporulation of *Aspergillus parasiticus*. Values with the same superscript are not significantly different ($p > 0.05$) tested by DMRT.

4. Discussion

The assessment of mycelia development and sporulation of *A. parasiticus* showed that the fungus is tolerant of acidic and neutral conditions while, these characteristics are significantly suppressed by alkaline condition. These observations confirm the report that fresh and dried mycelia pellets are significantly influenced by pH of media [14]. Filamentous fungi are generally known to be tolerant to acidic pH and most of them have an optimum pH between 5.0 and 6.0 for cellular growth and several metabolic activities [16]. However, the range of pH for growth in *A. parasiticus* with regards to dry mycelia weight and sporulation seem to be wide; spanning from pH 4.0 to pH 9.0 and pH4.0 to pH8.0 respectively. The difference in the dry mycelia weight of *A. parasiticus* between pH4.0 and pH 10.0 was 14.89%. Again, this study has obtained result similar to the report by [17] at pH 10.3 on *Alternaria solani* (42.8%), *Phytophthora capscici* (17.4%) and *P. cinnamomi* (12.6%) respectively. At pH 11.7, the growths in these fungi were completely inhibited.

The result of spore production of *A. parasiticus* at varying pH levels was also similar to the report by [12], though who quoted pH 5.0 – 8.0 been favourable for conidial production of *Diplocarpon mali*. This result also confirmed the statement of [18] who reported that reducing the pH to an acid condition (pH 4.5) do not affect spores production stage. This affirmed the statement reported by [19] that the higher the alkalinity in media the lower the colony counts per ml. This shows that although certain alkaline medium (pH 8.0 & 9.0) might favour the spore formation of *A. parasiticus*, higher pH values from 10 tend to hinder its sporulation. This will eventually reduce contamination of foodstuff and food produce by this toxigenic fungus, thus, avoiding its health hazard. Therefore, certain alkaline medium can be explored to inhibit the mycelia growth and sporulation of the fungi in order to prevent its damages to our crops.

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