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Influence of Certain Intrinsic and Extrinsic Factors on the Growth of Some Filamentous Fungi Isolated from Wheat

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Abstract: This study investigated the experimental conditions favouring the growth of some xerophilic moulds on wheat using standard microbiological techniques. Results obtained reveals acidic pH range to significantly influence the growth of the studied fungal isolates (Fvalue = 48.247, $p<0.05$). The peak growth was observed at 96hours ($F=65.01$, $P<0.05$) while room temperature favors fungal growth more than incubation at both 30 and 35°C (Fvalue=17.977, $p<0.05$) on wheat. Results obtained have shown that filamentous fungi will grow on contaminated wheat to produce secondary metabolites if kept at room temperature for more than 48hours provided the wheat has an optimum acidic pH range.

Key words: Filamentous Fungi · Wheat · Intrinsic And Extrinsic Factors

INTRODUCTION

Filamentous fungi are one of the three groups of fungi belonging to the Kingdom Eumycota. The other two groups are mushrooms and yeast [1, 2]. Of these three groups, filamentous fungi comprise of an important class of significant commercial relevance. They are also vital for the maintenance of the ecosystems [3]. Some of these organisms act as plant pathogens causing severe crop losses and post harvest food spoilage [3, 4]. These fungi are known as common saprophytes and can be present in different environments, fields and warehouses [5]. Among the aspergilli, it is the section circumdati (the yellow aspergilli), which are well known as OTA producers [6]. They have been studied especially on cereals, where *Aspergillus ochraceus* (formerly known as *A. alutaceus*) and *Penicillium verrucosum* were considered the major cause of toxin presence [5]. Until 1998, *A. ochraceus* and *P. verrucosum* were also believed to be the relevant fungi for OTA presence in grapes but OTA producing *Aspergillus carbonarius* and *A. niger* were identified in dried vine fruits in 1999 [6].

The presence of Ochratoxin A in several foods [7-9] and its accumulated effect such as immunotoxicity, neurotoxicity, genotoxicity and possibly carcinogenicity has been documented [6]. As a result, the International Agency for Research on Cancer has classified it in group 2B as a human carcinogen [8]. The principal effect of this toxin is nephrotoxicity . It has implication in a human kidney disorder known as Balkan Endemic Nephropathy (BEN). Ochratoxin A has been suspected to be a risk factor for testicular cancer [10]. It disturbed cellular physiology in multiple ways [11].

Wheat is globally the leading source of vegetable protein in human food, having a higher protein content than either maize (corn) or rice. It is one of the first cereals known to have been domesticated [12]. Wheat grain is a staple food used to make flour for leavened, flat, steamed breads, cookies, cakes, pasta, noodles and couscous and for fermentation to make beer. Many different alcoholic spirits including grain whiskey and vodka and recently biofuel. It is a concentrated source of vitamins and minerals [13].

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In Nigeria, wheat is used as food and the raw wheat can be powdered into flour, germinated and dried creating malt; crushed or cut into cracked wheat, parboiled (or steamed) dried, crushed and branned into bulgar, or processed into semolina, pasta, or roux. Wheat is major ingredient in foods such as bread, porridge, cracker and biscuits [12].

Fungal growth is strongly affected by a number of conditions, including water activity (a_w) and temperature. These factors are not only important in fungal growth, but also in metabolite secretion. Therefore, there is need for developing of rapid and multivariate techniques for rapid assessment of microbiological quality of food products [14]. Such innovation in food analysis will require more knowledge on how the intrinsic factors of the food together affect the viable counts of microorganism in it. Hence, this study was carried out to give a scientific report on how pH, titrable acidity and moisture contents of garri circulating in Ogun State, Nigeria can be related to the viable microbial counts of this food.

MATERIALS AND METHODS

Collection of Garri Samples: Wheat samples used for this study were purchased from some selected markets in Lagos State, Nigeria. Laboratory size samples suitable for intrinsic factors and microbiological analyses were obtained according to the method described by Pomeranz and Meloan [15].

Intrinsic and Extrinsic Factors Analysis: The pH of each dispensed wheat sample suspension of 2% strength was determined at room temperature (29±2°C) using electrodes of a pH meter (Hanna instruments) placed directly into each suspension. The pH meter with accuracy of 0.1 was first standardized using buffer solution of pH 4 and 9. The determination was performed in duplicate to find the mean pH of the sample. This was performed in order to delineate the three pH range viz; acidic, basic and alkaline pH.

Statistical Analysis: Analysis of variance was used for comparing the observed means while the level of significance was set $P< 0.05$.

RESULTS

Table 1 depicts the effect of pH on the growth of some selected fungal isolates grown on wheat. As shown in the table, acidic pH significantly influence the growth of the studied fungal isolates (F value = 48.247, $p<0.05$) (Table 2) while the least fungal growth was observed at neutral pH. This observation was further represented in Figure 1 below.

Table 3 depicts the influence of time of incubation on the growth and survival of the studied filamentous fungi. All the filamentous fungi grew on the wheat media after 48hours period. The peak growth was observed at 96hours and that was also significant ($F=65.01$, $P<0.05$)

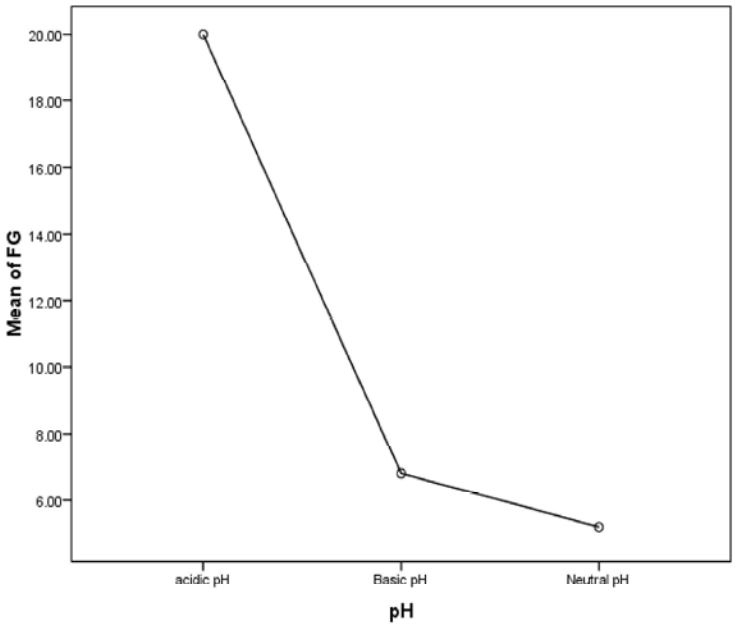

Fig. 1: Effect of pH on fungal growth

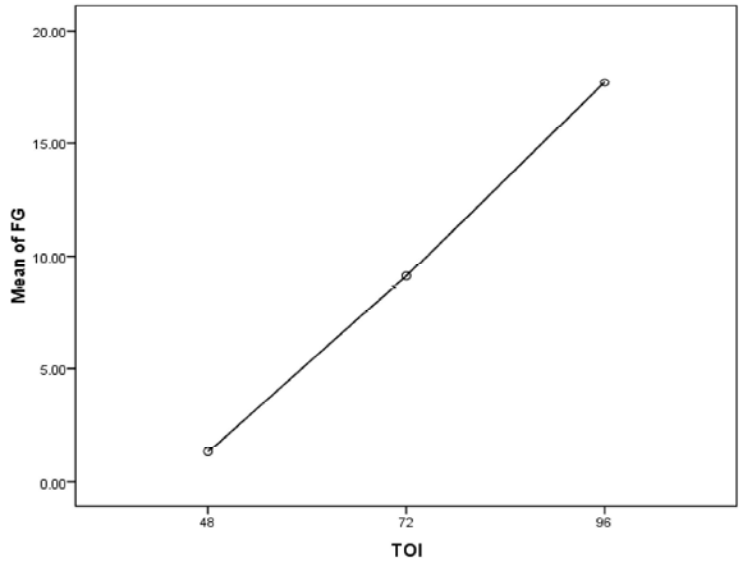

Fig. 2: Effect of time of incubation on fungal growth

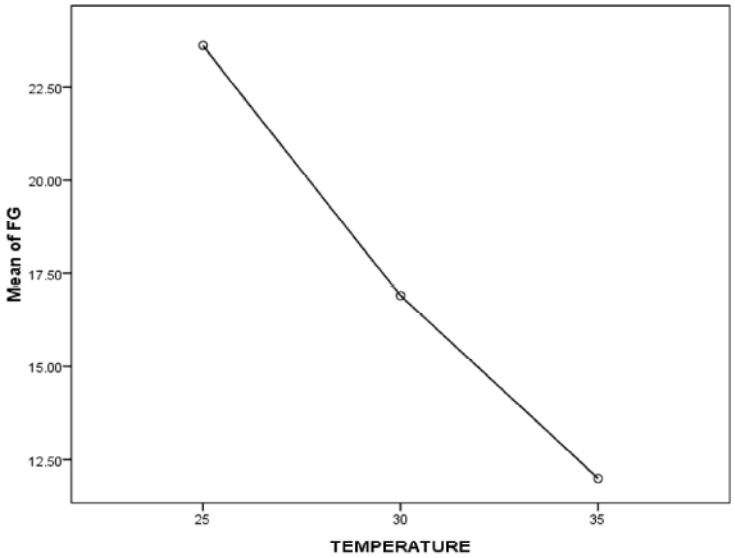

Fig. 3: Effect of temperature of incubation on fungal growth

Table 1: Effect of pH on the growth of filamentous fungi in wheat

					95% Confidence Interval for Mean			
FG	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Acidic pH	10	20.0000	4.29470	1.35810	16.9278	23.0722	10.00	24.00
Basic pH	10	6.8000	3.35989	1.06249	4.3965	9.2035	.00	11.00
Neutral pH	10	5.2000	3.35989	1.06249	2.7965	7.6035	.00	10.00
Total	30	10.6667	7.63085	1.39320	7.8173	13.5161	.00	24.00

Table 2: Analysis of Variance of the pH effect on growth of fungi

FG	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1319.467	2	659.733	48.247	.000
Within Groups	369.200	27	13.674		
Total	1688.667	29			

Table 3: Influence of time of incubation on the growth of filamentous fungi in wheat

					95% Confidence Interval for Mean			
FG	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
48	10	1.3000	1.41814	.44845	.2855	2.3145	.00	3.00
72	10	9.1000	3.90014	1.23333	6.3100	11.8900	3.00	16.00
96	10	17.7000	3.71334	1.17426	15.0436	20.3564	12.00	26.00
Total	30	9.3667	7.48554	1.36667	6.5715	12.1618	.00	26.00

Table 4: Analysis of variance of the effect of time of incubation on fungal growth

FG	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1345.867	2	672.933	65.099	.000
Within Groups	279.100	27	10.337		
Total	1624.967	29			

Table 5: Effect of temperature of incubation on the growth of xerophilic moulds studied

					95% Confidence Interval for Mean			
FG	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
25	10	23.6000	5.18973	1.64114	19.8875	27.3125	16.00	31.00
30	10	16.9000	3.95671	1.25122	14.0695	19.7305	11.00	23.00
35	10	12.0000	3.74166	1.18322	9.3234	14.6766	6.00	18.00
Total	30	17.5000	6.39908	1.16831	15.1105	19.8895	6.00	31.00

Table 6: Analysis of Variance on the effect of temperature of incubation on fungal growth

FG	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	678.200	2	339.100	17.977	.000
Within Groups	509.300	27	18.863		
Total	1187.500	29			

(Table 4). When the mean growth of the filamentous fungi was represented on graph, it produces a straight line graph showing an exponential growth rate ranging from the least time of incubation to a final incubation period of 96 hours.

Table 5 represents the effect of temperature of incubation on the growth of xerophilic moulds grown on wheat. As shown in the table, room temperature favors fungal growth more than incubation at both 30 and 35°C. This observation was found to be significant when compared using ANOVA test (Fvalue=17.977, $p<0.05$) (Table 6). On plotting the mean fungal growth against time, an inverse straight line was gotten with the growth rate decreasing from the highest temperature of incubation through 30°C. The growth rate reaches exponential phase at 96hours.

DISCUSSION AND CONCLUSION

Filamentous fungi are ubiquitous contaminants of pre and post harvest foods including the ready to eat foods [7-8, 17, 18] . Since these fungi were isolated from wheat, it would be important to investigate the experimental conditions favoring the growth of these xerophilic moulds in vitro. In this study, acidic pH significantly influence the growth of the studied fungal isolates (Fvalue = 48.247, $p<0.05$) while the least growth was observed at neutral pH. This observation was not surprising because the observed pH values in our study incidentally falls within the range that favors the growth of fungi in food [9]. According to Ogunledun [9], when microbes are placed in environments below or above neutrality, their ability to proliferate depends upon their ability to bring the environmental pH to a more optimum range. When placed in acidic environment, the cells must either keep H^+ ions from entering as rapidly as they enter. Such key cellular compounds as DNA and ATP require neutrality [16]. Also, when most microbes grow in acidic media, their metabolic activities result in the media or substrates becoming less acidic while those that grow in high pH environments tend to effect a lowering of pH [14].

From the response of the fungal isolates to the length of the time of incubation, the peak growth was observed at 96hours and that was also significant ($F=65.01$, $P<0.05$). When the mean growth of the filamentous fungi was represented on graph, it produces a straight line graph

showing an exponential growth rate ranging from the least time of incubation to a final incubation period of 96hours. This findings may not be unconnected to the fact that maximum growth rate for xerophilic fungi is reached after 72 hours.

When the impact of temperature on the studied fungal growth was evaluated, room temperature favors fungal growth more than both 30 and 35°C incubation. This observation was found to be significant when compared using ANOVA test (Fvalue=17.977, $p<0.05$). When the mean fungal growth was plotted against time, an inverse straight line was obtained with the growth rate decreasing from the highest temperature of incubation through 30°C. O Brian *et al*. [19] reported temperature range of 25-30°C as the best favoring proliferation as well as toxin production in *Aspergillus flavus.* In conclusion, it could be inferred that acidic pH, room temperature as well incubation period of up to 96hours favors the growth of the studied filamentous fungi on wheat.

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