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### ABSTRACT

The influence of temperature and relative humidity on the culture of species of fungi isolated from rotting sweet potato tubers for delineation of physical control measure was investigated in this study. The fungi were cultured at five temperature regimens: 27±3°C (i.e., room temperature, 'Control'), 35, 40, 45 and 50°C; and six Relative Humidity (R.H.) levels: 32, 50, 74, 85, 97 and 100%, for a period of eight days. The growth of the fungi increased till 40°C, after which significant ( $P < 0.05$ ) drop in growth was recorded. However, significant ( $P > 0.05$ ) differences in growth of the fungi were limited to the first three temperature regimens tested. With respect to relative humidity, all the five fungi tested recorded their least growth at the lowest relative humidity (i.e., 32.50%), *Aspergillus niger*, *Rhizopus oryzae* and *Mucor racemosus* achieved their maximum growth at 50% R.H. (i.e., 46±28.07, 53.38±23.37 and 59.63±28.09mm, respectively); while *Fusarium oxysporum* and *Scopulariopsis brevicaulis* grew optimally at 85% R.H. (56.43±28.83 and 28.38±18.02mm, respectively). These results suggest significant shifts in the optimal temperature and R.H. for the growth of some of the fungi. The findings of this study should guide the development of better storage facilities for potato tubers, through the modification of micro-climatic conditions.

**Key words:** Fungi, Growth, Relative Humidity, and Temperature

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### INTRODUCTION

Sweet potato (*Ipomoea batatas*) is one of the most important food crops, yet only 5% of its world production is contributed by Africa (FAO, 1992). A major limitation to optimum production of sweet potato in Africa in general and Nigeria in particular, has been the huge postharvest losses often associated with the tubers under storage conditions (George, 1988; Abubakar, 1990). In Kenya, postharvest soft-rotting of sweet potato

tubers was rated as a major production constraint (Mutuura *et al.*, 1992). According to Kihurani *et al.* (2008), certain storage environmental conditions are known to directly or indirectly predispose sweet potato tubers to postharvest microbial deterioration. Walker (1998) observed that the progression of sweet potato soft-rot diseases was fastest when a high humidity was combined with a temperature of 80°F. For example, the optimum temperature for the growth of *Erwinia* sp, a major causal pathogen of potato soft-rot, has been put at

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between 85° and 120°F, with the maximum been slightly over 100°F. Similar observations have been made with respect to the influence of temperature and R.H. on soft-rot microbial pathogenic activities in other crops. Bhat *et al.* (2010a&b) observed higher growth of microbial pathogens of soft-rot at 30°C, and storage temperatures ranging from 30 to 35°C as the most favourable for their development on cabbage. Also, Farrar *et al.* (2000) reported temperatures between 30 and 37°C as optimum for soft-rot development in different vegetable crop species. However, Raju *et al.* (2008) observed enhancement of soft-rotting ability of the bacterial *Erwinia carotovora* subsp. *carotovora* at much lower temperatures between 20 and 30°C, with severity of soft-rotting increasing with higher relative humidity. The highest extent of soft-rotting was recorded when samples were incubated at 35°C and 100% relative humidity. At the other extreme, soft-rot diseased tubers placed in dry atmospheric conditions became dehydrated quickly thus, halting the progression of the disease (Walker, 2004). In Minna, the heavy postharvest losses of sweet potato have been attributed to poor prevailing storage environmental conditions. Yet, information on the influence of environmental factors on the activities of sweet potato soft-rot microbial agents is very scanty and, perhaps, explains the delay in the development of cost-effective postharvest storage strategies for the crop in the country. This study was, therefore, carried out to determine the influence of temperature and relative humidity on growth activities of fungi associated with sweet potato soft-rotting.

## MATERIALS AND METHODS

### Collection of Sweet Potato Tubers

Samples of Soft-rot diseased sweet potato tubers were collected from different

locations within Minna Emirate, Nigeria. Collected tubers were individually preserved in labeled sterile polythene bags before transportation to the Laboratory for analyses.

### Isolation of Micro-organisms

The isolation of fungal microbes associated with soft-rot in the diseased-tubers was carried out following standard procedures. The tubers were first washed under running tap water and then surface sterilized with 70% alcohol. Thereafter, they were cut into 2x2x2mm cubes, under sterile conditions, and each piece of tuber cube was aseptically placed on potato dextrose agar (PDA) medium. The inoculated plates were incubated at room temperature (27±3°C). The fungal colonies that developed were subcultured onto PDA, to obtain pure cultures.

### Identification of Isolated Micro-organisms

The fungal isolates were identified using morphological characteristics according to the methods of Barrow and Feltham (1992) and Garrette (1978).

### Effect of Temperature on Growth of Fungal Isolates

Pure cultures of the fungal isolates were subsequently inoculated onto PDA media and incubated at five temperature regimen namely, 27±3°C (i.e., room temperature, 'Control'), 35, 40, 45 and 50°C. Each temperature treatment had three replicates and the whole experiment was allowed to stand for eight days. Growth extent of the isolates was estimated at 24-hr intervals, using the Linear Measurement technique.

### Effect of Relative Humidity on Growth of Fungal Isolates

Inoculated plates of the fungal isolates were placed individually on copper wire gauze in the upper chamber of desiccators with the lower chamber containing 250ml saturated solution each of the following salts:

Magnesium chloride, Calcium nitrate, Potassium nitrate, Ammonium sulphate, Potassium sulphate and distilled water (i.e., control), to give relative humidity of 32.5, 74, 85, 97.5 and 100%, respectively. Also, each R.H. treatment had three replicates and the whole experiment was monitored for fungal growth at 24-hr interval for eight days at room temperature (i.e.,  $27.00 \pm 3.00^\circ\text{C}$ ).

**Data Analysis**  
Mean daily growth of the fungal isolates at different temperature and R.H. treatments were calculated. For each parameter (i.e., temperature and R.H.), differences in the mean daily growth values for each fungal isolates, among the different treatments were compared for statistical significance using the Chi-square tested, at  $P = 0.05$  level of significance.

## RESULTS

The influence of temperature on growth of the fungal isolates is presented in Figure 1. Generally, growth activities of the isolates increased till  $40.00^\circ\text{C}$ , after which significant ( $P < 0.05$ ) drop in growth was recorded at higher temperatures tested. However, few exceptions to this general observation were recorded. For example, while the growth activity of *Fusarium oxysporum* dropped from  $55.29\text{mm}$  at  $27 \pm 3^\circ\text{C}$  to  $48.75\text{mm}$  at  $35.00^\circ\text{C}$ , *Scopulariopsis brevicaulus* achieved its highest growth at  $27 \pm 3^\circ\text{C}$ . Also, significant differences in mean weekly growth of the isolates were limited to the first three temperature regimens tested (range =  $27 \pm 3$  to  $40^\circ\text{C}$ ), while such differences at  $45^\circ\text{C}$  were not significant. At

the highest temperature investigated, the fungal isolates had more-or-less equally very poor growth, with the exception of *Aspergillus niger*.

The growth of all fungal isolate equally responded significantly ( $P < 0.05$ ) to increase in Relative Humidity (R.H.), though with different patterns of such responses. While all their fungi recorded their least mean growth at 32.50% R.H. (range =  $6.25 \pm 1.39\text{mm}$  in *Trichoderma viridae* to  $28.25 \pm 4.36\text{mm}$  in *Mucor racemosus*), *A. niger*, *R. oryzae* and *M. racemosus* achieved maximum growth at 50 R.H. (i.e.,  $46 \pm 23.07$ ,  $53.38 \pm 23.37$  and  $59.63 \pm 28.09\text{mm}$ , respectively). On the other hand, *F. oxysporum* and *S. brevicaulus* grew optimally at 85.00% R.H. ( $56.43 \pm 28.83\text{mm}$  and  $28.38 \pm 18.02\text{mm}$ , respectively). Distinctly, the growth of *T. viridae* were highest at the last three R.H. (i.e., 85.00 - 100.00%), with insignificantly different ( $P > 0.05$ ) growth rate (range =  $21.00 \pm 14.50\text{mm}$  at 97.50% R.H. to  $21.25\text{mm}$  at 85.00 and 100.00% R.H.). On the whole, three of the fungi namely, *F. oxysporum*, *S. brevicaulus* and *T. viridae* continued to grow very well at extremely high R.H. of 97.50 and 100.00%. At the other extreme, i.e., low R.H. of 32.50%, the growth of *S. brevicaulus* and *T. viridae* were seriously impeded (i.e.,  $8.50 \pm 2.07$  and  $6.25 \pm 1.39\text{mm}$ , respectively), while the other fungi achieved more than 20mm mean growth.

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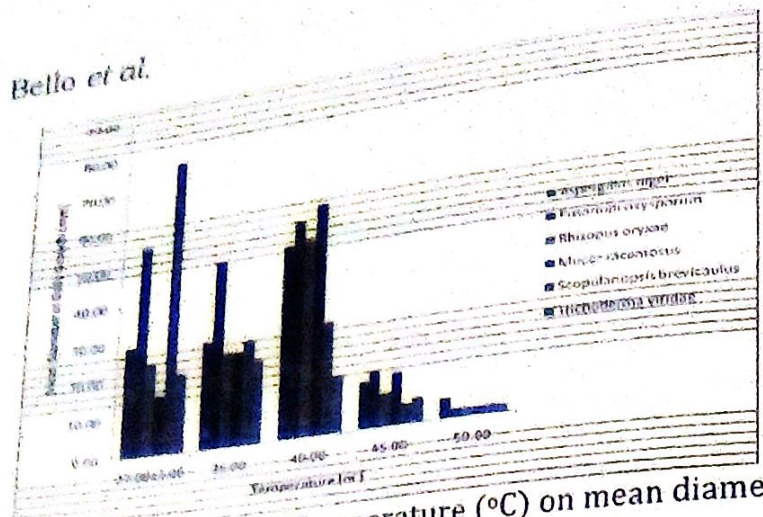


Figure 1: Influence of Temperature (°C) on mean diameter of daily growth (mm) of soft-rot fungal isolates from diseased sweet potato tubers

Table 1: Influence of relative humidity (%) on mean diameter of daily growth (mm) of soft-rot fungal isolates from diseased sweet potato tubers

Test Isolates	Mean Growth of Fungi at Different Relative Humidities (%)					
	32.5	50.00	74.60	85.00	97.50	100.00
<i>Aspergillus niger</i>	25.25±15.69 <sup>a</sup>	46.00±23.07 <sup>c</sup>	36.13±37.31 <sup>b</sup>	27.63±35.31 <sup>a</sup>	28.00±36.08 <sup>a</sup>	29.00±37.50 <sup>a</sup>
<i>Fusarium oxysporum</i>	25.13±14.69 <sup>a</sup>	39.13±22.48 <sup>b</sup>	47.50±32.70 <sup>c</sup>	56.43±28.83 <sup>d</sup>	53.86±27.93 <sup>c</sup>	46.75±32.27 <sup>c</sup>
<i>Rhizopus oryzae</i>	22.50±14.05 <sup>a</sup>	53.38±23.37 <sup>b</sup>	23.13±36.54 <sup>a</sup>	24.00±37.50 <sup>a</sup>	23.63±36.15 <sup>a</sup>	23.63±36.15 <sup>a</sup>
<i>Mucor racemosus</i>	28.25±14.36 <sup>a</sup>	59.63±28.09 <sup>b</sup>	23.50±36.12 <sup>a</sup>	24.50±36.83 <sup>a</sup>	24.25±36.75 <sup>a</sup>	24.25±36.75 <sup>a</sup>
<i>Scopulariopsis brevicaulus</i>	8.50±2.07 <sup>a</sup>	20.75±14.35 <sup>b</sup>	25.88±16.98 <sup>b</sup>	28.38±18.02 <sup>c</sup>	27.50±17.35 <sup>c</sup>	27.34±17.35 <sup>c</sup>
<i>Trichoderma viridae</i>	6.25±1.39 <sup>a</sup>	14.53±8.07 <sup>b</sup>	18.25±11.65 <sup>b</sup>	21.25±13.98 <sup>c</sup>	21.00±14.50 <sup>c</sup>	21.25±15.00 <sup>c</sup>

\*Values followed by same superscript alphabet in a row are not significantly different at P = 0.05 level of significance

### DISCUSSION

To some extent the mean growth of the fungi increased with increasing temperature but succumbed at extremely high temperatures. For most of the species, the optimum temperature was 40°C. This finding confirms the popular belief that temperature is one of the major environmental factors influencing rot at temperatures ranging from 30 – 37°C. Even temperatures as low as 20 – 30°C were

the progression of sweet potato soft-rot disease during storage (Brown and Nelson 2003). Notably, the optimum temperature for the growth of most of the soft-rot fungi recorded in this study (i.e., 40°C) was relatively higher than reported elsewhere. Bhat *et al.* (2010a&b) and Farrar *et al.* (2000) observed optimal growth of microbial pathogens of soft-rot reported as optimally enhancing the rotting ability of microbial pathogens.

The optimum temperatures for the growth of microbial pathogens is often related to the prevailing temperature of the environment. This, perhaps, explains why relatively high temperatures for the optimum growth of the fungi. The mean temperature during the dry season in the region where postharvest storage of sweet potato is practiced, was about 31°C (Oyama et al, 2011)

The growth of the fungi also responded to growth to increase in relative humidity. However, all species had their optimum least weekly growth at the same R.H. (i.e. 72.50%). This is to be expected, as microbial pathogens generally require a minimum level of relative humidity to meet their enzymatic and metabolic activities. The results of this study showed that *F. oxysporum*, *F. verticillium* and *S. rotundum* grow optimally at very high R.H. (i.e. between 85 and 100%), which is consistent with those of Raju et al (2008). However, as noted with the influence of temperature on growth of the fungal isolates in this study, any of the fungi achieved their optimum growth at relative humidities well outside the range generally regarded as comfortable or closely related to that prevailing in the area in this study. *A. niger*, *R. oryzae* and *R. solani* achieved maximum growth at a low R.H. of 50%. However, the annual mean R.H. in Minna was recently reported to be 61.6% - 71.66% (Oyama et al, 2011) but, further buttressing the earlier observation that the sweet potato soft rot fungal pathogens in Minna have evolved and adapted to the prevailing temperature and R.H. in the area.

CONCLUSION

The growth of sweet potato soft rot fungal isolates in Minna, Katsina state significantly increased by varying temperature and relative humidity. However, while some of the fungi species grew optimally at

temperatures and R.H. considered comfortable for soft rotting sweet potato tubers, some of the fungi species achieved optimum growth at temperatures and relative humidities clearly outside the normal range, but closely related to prevailing environmental conditions in Minna state, suggesting microclimatically induced adaptation of the fungal species. The findings of this study would, therefore, guide the development of better postharvest storage practices based on appropriate modification of the microclimatic conditions in Minna, Katsina.

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