STUDIES ON THE MYCOFLORA OF GRAINS OF MAIZE (*ZEA MAYS* L.) AND THE SURVIVAL OF THE CONTAMINANT *ASPERGILLUS* SPECIES

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I, the undersigned, **ROSALIND ELSIE ODATSOO KESSE**, author of this thesis, do hereby declare that the work presented in this thesis

"STUDIES ON THE MYCOFLORA OF GRAINS OF MAIZE (*ZEA MAYS* L.) AND THE SURVIVAL OF CONTAMINANT *ASPERGILLUS* SPECIES"

was done entirely by me in the Department of Botany, University of Ghana, Legon from October, 1993 to September, 1995. This work has never been presented either in whole or in part for any other degree of this University or elsewhere.

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DEDICATION

I dedicate this manuscript to

Dr. Wilfred Kwame Kesse

and to the Glory of God

ABSTRACT

Grains of Zea mays in storage at the Kaneshie and Tema warehouses of the Ghana Food Distribution Corporation with atmospheric humidity fluctuating between 55 and 90% R.H. and those stored at 40°C in the Botany Department were attacked by a large number of fungi. Non-stackburn and stackburn grains at the Kaneshie warehouse contained 26 and 16 fungal species, respectively, and those in Tema warehouse had 32 and 19 species, respectively. Aspergillus was the dominant genus of the mycoflora and Aspergillus flavus was the predominant species occurring at very high levels.

The moisture content of the grains stored in the warehouses showed, an average rise of 6.9 per cent within the six months of storage. During this period the percentage occurrence of A. flavus consistently rose to a peak at the end of the 4th month and declined. Paecilomyces puntonii and Paecilomyces variotii were consistently present in the early months of storage and then disappeared. The rest of the species taking all four batches of grains together, did not show a consistent pattern of occurrence.

The air spora of the Kaneshie and Tema warehouses consisted of 26 and 27 fungal species, respectively, with the same difference in the dominant species. The most abundant species recorded in the Kaneshie warehouses in descending order were Aspergillus flavus, Cladosporium herbarum, Aspergillus flavus-oryzae, Mucor sp., Aspergillus fumigatus, Rhodotorula sp., Penicillium expansum, Aspergillus niger and Penicillium chrysogenum. On the other hand, the most abundant species in the Tema warehouse in descending order were Cladosporium herbarum, Aspergillus flavus, Penicillium expansum, Penicillium chrysogenum, Rhodotorula sp., Rhizoctonia solani, Aspergillus parasiticus and Paecilomyces puntonii.

Grains of Abeleehi, Mixed White, Obatanpa and Yellow maize varieties stored at 40°C had 15, 16, 18 and 18 contaminant fungal species despite marked average loss of 41.4 per cent moisture content over the storage period of 4 months. Aspergillus flavus, under those conditions, was again the predominant species.

Experiments which investigated growth, sporulation, conidial germination capacity, and conidial survival in Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii, showed significant physiological differences among the species. A. clavatus grew best at 38°C and sporulated best at 26°C; it also grew best at 62.4 - 73.4% R.H., and sporulated best at 85.2 - 100% R.H., conidia formed at 62.4 - 100% R.H. showed 90.0 - 99.5 per cent germination in Potato Dextrose Broth and more than 69.6 per cent germination in 1.0 Dextrose, 1.0 Sucrose and 1.0 Peptone solutions, and more than 22.2 per cent in exudates of grains of three maize varieties, but did not germinate in water.

The conidia of the other three species did not also germinate in water. The humidity at which the conidia were formed did not affect their rate of loss of vigour in storage. The conidia survived best at 0,60 and 100% R.H. and lost viability quickest at 20 and 80% R.H.

A. flavus grew and sporulated best at 34°C; grew best at the humidities of 73.4 - 92.8% R.H. and sporulated at 100% R.H. Conidia formed at 62.4 - 100% R.H. showed 80.7 - 94.3 per cent germination in Potato Dextrose Broth and more than 11 per cent germination in maize grain exudates but did not germinate in 1.0% Dextrose, 1.0% Sucrose and 0.1% Peptone solutions. The humidity at which the conidia were formed did not affect their rate of loss of vigour in storage. The conidia survived best at 0, 20, 40, and 100% R.H. and lost viability quickest at 80% R.H.

A. niger grew and sporulated best at 34°C; grew best at the humidities of 92.8 and 100% R.H. and sporulated best at 62.4 - 92.8% R.H. Conidia formed at 62.4 - 100% R.H. showed 83.4 - 98.4 per cent germination in Potato Dextrose Broth but did not germinate in maize grain exudate, and in 1.0% Dextrose, 1.0% Sucrose and 0.1% Peptone solution. Conidia formed at 73.4 - 85.2% R.H. apparently had a greater potential for survival than those formed at the other humidities. A. niger conidia survived longest at 100% R.H. and longevity decreased with decreasing relative humidity.

A. tamarii grew and sporulated best at 30°C; grew best at 62.4 - 73.4% R.H. and sporulated best at 62.4% R.H. Conidia formed at 62.4 - 100% R.H. showed 87.7 - 98.6 per cent germination in Potato Dextrose Broth but did not germinate in maize grain exudate nor in 1.0% Dextrose, 1.0% Sucrose and 0.1% Peptone solutions. Conidia formed at 65.0 - 85.2% R.H. seemingly had a greater potential for survival than those formed at the other humidities. A. tamarii survived longest at 0, 20, 40, and 60% R.H. and lost viability quickest at 100% R.H.

Conidia of all the four species which were swollen prior to germ tube development and were then air-dried died within 1-6 hours.

It was concluded that because of the variation in response of the fungi to temperature and humidity, warehouses held at a set temperature and relative humidity would not be able to control fungal contamination of the grains. More resistant fused pericarp and testa which could emerge from breeding programmes may be one of the more efficient ways by which invasion of the grains could controlled.

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I. INTRODUCTION AND LITERATURE REVIEW

Maize or corn (Zea mays L.) is a major food and livestock feed. It ranks after wheat (Triticum aestivum L.) and rice (Sativa oryzae L.) as the third most important crop in the world (Duncan, 1975). It is the main food for some 100 million people in both tropical and temperate regions (Hoffman, 1965; Inglett, 1970). It may form a meal on its own, or may be an ingredient of various sorts of preparations.

Maize is especially important to many countries in Africa, Asia and Latin America where it serves as a human subsistence crop. It is also an important agricultural "Life line" in the United States of America (U.S.A.) and some developed countries. It is, therefore, important to the economies of producing countries as the major food source on one hand and as a commodity of international trade on the other hand. The world total grain production as estimated 20 years ago stood at 1.2 million tons per year of which wheat, rice and maize accounted for about 75 per cent (Hoff and Janick, 1973). Naturally with the introduction of improved stocks and more intensive cultivation since that time, production would be considerably higher by now.

In Ghana and other countries in the West African sub-region, maize is a major staple diet. This is especially true for inhabitants of the costal regions in Ghana. It is also an important ingredient of infant weaning foods country-wide.

Maize presently is the main source of energy in non-ruminant animal feeds, averaging about 50-60 per cent by weight of the total feed (NARP Report, 1993). It is supplemented by other cereal grains, especially millet (Pennisetum typhoideum) and Sorghum (Sorghum vulgare Pers). Maize grains are an important source of industrial starch and are important for the production of beer and many other beverages.

The cultivation of food crops in Ghana is seasonal, as the country mostly practices rain-fed agriculture, and the abundant food harvested at the end of the raining season should last until the next harvesting time many months away. There is however a lot of wastage during storage due to insect pest damage and fungal contamination. Attempts are being made to improve storage conditions so that this loss could be reduced as much as possible.

In Ghana, most farmers store unshelled dry maize in barns and cribs. Maize is also shelled, dried and put in woven polypropylene or jute sacks which is then sold to market women and wholesalers who store it in warehouses and smaller market stores. The recommended procedure for minimizing insect infestation is to insert the ICI product Actellic into the sacks (Hindmarsh, Tyler and Webley, 1978). Prevention of fungal contamination has been difficult as facilities for storing the grain under low atmospheric humidities and temperatures are not available and the extent of deterioration could be high.

This deterioration is due to a number of interrelated factors, namely biological, physical and technical factors. To begin with, the extent to which these factors will induce deterioration will depend upon the state of the grain. Improper drying provides moisture for fungal growth, broken grains present open wounds which facilitate invasion and fragments of the cob and accumulation of chaff may form a source of inoculum when the contaminant fungi grow on them.

Fungi, however, are able to grow on grains even if they are properly dried and the flora is well documented (Appert, 1987; Christensen and Kauffman, 1969). Christensen and Kauffman (1969), indeed stated that anyone who stores grains and seeds stores fungi as well. Their growth is supported under local conditions by the consistently high temperatures and humidities. In the final analysis the economic value of grains depreciates greatly through changes in colour, texture and odour; loss in nutrients and weight and through contamination by mycotoxins (Broadbent and Oyeniran, 1970, 1973, 1978; Moss, 1977).

Two distinct groups of mycoflora of stored grains and seeds are recognised, namely, field fungi and storage fungi. A large proportion of contaminating fungi of stored grains arrived with the grain from the field. The field fungi of maize grain include species of Alternaria, Cladosporium, Fusarium and Helminthosporium which invade the maturing grains when the moisture content is high and they are metabolically active (Christensen, 1957).

These fungi require a moisture level in equilibrium with a relative humidity of 90 per cent or more. Oyeniran (1981) reported that five per cent of cobs examined in Nigeria had pre-storage mould infection although the infection on the cobs were localised on each cob. Some cobs had about half of the total number of grains affected while others had just two or three rows of grains on the cob infected.

The storage fungi arrive after harvest, and mostly xerophilic as the products, by then, contain very little water. The Aspergillus species are the most important storage fungi of maize grains (Mislivec and Tuite, 1970). They are xerophilic and they grow at low water activity [0.70-0.75 aw] (Magan and Lacey, 1987; Smith and Moss, 1985). The Penicillium species in most instances come next to the Aspergillus species. Some Penicillium species are, however, field fungi while others are storage fungi.

There are a few reports on the flora of stored maize grains in Ghana. Odamtten (1986) identified 26 fungal species belonging to 12 genera. Members of the genus Aspergillus were the most abundant followed by Penicillium species. Aspergillus species identified were A. candidus, A. fumigatus, A. niger, A. ochraceus, A. restrictus, A. tamarii, A. ustus and A. wentii. Species of other genera which were frequently isolated from the grains were Cladosporium herbarum, Neurospora sitophila, Paecilomyces variotii, Penicillium expansum, Penicillium verrucosum, Phoma glomerata, Rhizoctonia solani and Rhizopus oryzae.

Many of these have also been found on stored maize in other countries. Broadbent (1967a and b) and Oyeniran (1972) isolated the Aspergillus species, A. flavus, A. fumigatus, A. niger and A. tamarii and the Penicillium species P. citrinum, P. coryophillium and P. jamthinellium in Nigeria. Species occurring less frequently included Absidia corymbifera, Botryodiplodia theobromae, Fusarium moniliforme, Mucor sp., Rhizopus arrhizus and Syncephalastrum racemosum.

In Iran, Zad and Ale-gha (1985) isolated 22 fungal species from maize, which they claimed to include both endophytic and ectophytic fungi. The list they presented, however, did not indicate the category to which the individual species belonged. The list included Alternaria alternata, Alternaria herbarum, Aspergillus flavus, Aspergillus niger, Cephalosporium maydis, Diplodia zeae, Dreschlera maydis, Fusarium moniliforme, Fusarium roseum, Helminthosporium maydis, Helminthosporium rostratum, Nigrospora oryzae, Physalospora zeae, Penicillium sp., Rhizopus nigricans, Trichothecium roseum and Yeasts.

Singh, Singh and Singh (1987) isolated many fungal species from stored maize belonging to 26 genera in India. Prominent among the genera represented by more than one species were Alternaria (A. sesami and A. tenuis), Aspergillus (A. candidus, A. flavus, A. fumigatus, A. funicolosus, A. luchuensis, A. nidulans, A. niger, A. ochraceus, A. sulphureus, A. sydowi, A. tamarii, A. terreus and A. visicolor), Chaetomium (C. globosum and C. indicum), Cl[illegible]sporium (C. herbarum and C. oxysporum),

Cuvularia (C. lunata and C. pallescens), Dreschlera (D. bicolor, D. halodes, D. hawaiiensis, D. maydis, D. rostrata and D. tetramera), Fusarium (F. equiseti, F. moniliforme and F. semitectum) and Nigrospora (N. oryzae and N. sphaerica).

Tuite and Christensen (1957) indicated that, in addition to field infection and contamination during storage, many fungal species are collected by the products during transit from the field to the warehouse. They reported the presence of fungi growing on all sorts of materials in elevators which were conveying freshly harvested grains. Similar sources could be identified in Ghana. Most common are old sacks, old baskets, trucks and lorries and railway goods' vans.

The physiology of some of the fungi isolated from stored grains in some instances has been studied. However, since the interests of the research workers vary, the available reports in the relevant literature consequently contain results of investigations on only selected aspects of the physiology of these fungi. This investigation was carried out to extend knowledge on the physiology of the fungi of grains in storage.

Contamination of the grains is initiated either by invading germ tubes of germinating spores or by hyphae extending from one grain to another with which it is in contact.

In the case of the former the extent of contamination would depend on the existence of extraneous nutrients as conidia of some species such as Aspergillus species do not germinate in water (Cochrane, 1958) and on environmental factors that influence spore germination and longevity. The survival of conidia of some of the dominant Aspergillus species isolated from samples of stored maize grains in Accra was studied under conditions which were likely to be encountered by the conidia in the warehouses, as studies on the survival of conidia of Aspergillus species seem to be rather limited.

Very great differences have been found in the survival potential of different kinds of fungal spores. The viability of all spores decreases with time and the rate of loss of vigour is dependent on the inherent characteristics of the spore (genetical constitution) and upon the environmental conditions, especially, temperature, humidity and light (Cochrane, 1958, Gottlieb, 1950; Hawker, 1950).

Teitell (1958) found that within the range of atmospheric humidities that was too dry to permit germination, survival of Aspergillus flavus conidia at 29°C was longest at zero and 85% R.H., and the conidia perished quickest at 75% R.H. All spores at 75% R.H. died within 13 days while those at 32% and 85% R.H. retained approximately 50 per cent viability for more than six months. He also found a similar relationship also for Aspergillus terreus.

In this instance also while practically all the conidia held at 81% R.H. died within five days those at 30 and 85% R.H., respectively, were still viable. Teitell (1958) observed that the vulnerable humidity shifted at higher temperatures. At 40-41°C, the conidia of A. flavus died quickest at 81% R.H. instead of 75% R.H.

Under the most suitable environmental conditions conidia of the different Aspergillus species lived for varying lengths of time. A. flavus conidia stored at 25°C and at 100% R.H. survived for five days; A. terreus conidia survived for seven days at 44-45oC and 85% R.H., A. niger conidia retained viability for seven days at 40-41°C and 89% R.H.

Spores of other genera show other patterns of survival in addition to what has been observed in conidia of Aspergillus species. Clerk and Madelin (1965) and Akushie and Clerk (1981) found relative humidity-survival relationship similar to that of A. flavus and A. terreus in conidia of Metarrhizium anisopliae and sporangiospores of Rhizopus oryzae, respectively. A possible third example of this sort of relationship was provided by the ascospores of Endoconidia fagacearum (Merrick and Fergus, 1954). Although they interpreted their results as indicating longest survival at the lowest relative humidity and least at high, they did in fact show that between 12°C and 24°C all the ascospores died in less time at 75% R.H. than at 95, 50, 25 and 10% R.H.

The reports of Clerk and Madelin (1965) and Akushie and Clerk (1981) indicated slight variations in the response of the spores they studied from that of A. flavus and A. terreus in which the unfavourable relative humidity was only a few percent below the minimum required for germination. In the case of M. anisopliae, the unfavourable humidity level, of about 45% R.H., was far below that for spore germination which for this species is between 93 and 97% R.H. There was a percentage viability, for example of 99.2, 99.1, 0.0, 97.8, 97.9 and 98.0 per cent, respectively, after storage for 28 days at 0.0, 12.0, 33.2, 52.8, 75.8, 80.3 and 92.0% R.H.

A striking characteristic of the sporangiospores of R. oryzae which incidentally do not germinate in water, was the existence of three most destructive humidity levels of 20%, 40% and 80% R.H. The sporangiospores, therefore, survived longest at zero and 100% R.H., less so at 60% R.H. and died very rapidly at points 20-40% R.H. and 80% R.H. (Akushie and Clerk, 1981). Citing one of numerous results presented, survival after 30 days storage at 30°C and at the respective humidities of 0, 20, 40, 60, 80 and 100% R.H. was 72.9, 1.0, 2.1, 30.8, 2.4 and 74.3 per cent.

There are three other relative humidity/spore survival relationships which apparently have not been reported in the Aspergillus species. The most common is the relationship in which reduction in humidity serves to increase spore longevity, as found for example in Beauveria bassiana and Paecilomyces farinosus (Clerk and Madelin, 1965), Pircicularia oryzae (Anderson, Henry & Morgan, 1948), Hymenochaete tabacina (Harrison, 1942), Chalara quercina (McLaughlin and True, 1952) and Helminthosporium oryzae (Page, Sherf & Morgan, 1947).

The reverse occurs in spores which cannot withstand desiccation. They are killed more rapidly by low humidities. The conidia of Trachysphaera fructigena were found by Maramba and Clerk (1974) to show the respective percentage viability of 0.5, 1.6, 4.3, 44.1, 92.1 and 94.7 per cent after storage for 30 minutes at 25°C and at 75, 80, 85, 90, 95 and 100% R.H.

After 10 days storage, 90.0 per cent of the conidia at 100% R.H. were still alive while all the conidia at the rest of the storage humidities were dead. Similarly, conidiosporangia of members of the Peronosporales, such as, Phytophthora infestans (Glendenning, Macdonald & Graiger, 1963; Zan, 1962) and Phytophthora meadii (Peries & Fernando, 1966) are readily damaged by desiccation and are conserved for reasonable periods only at very high humidities.

Spores of the last category, lived longest at median humidities and died quickest at extreme humidities. The uredospores of crown rust of oats (Rosen and Weetman, 1940) and Conidia of Monilinia fructicola (Naqvi and Good, 1957) belong to this group.

The effect of light on survival of spores of many species has been reported but no information could be obtained from the pertinent literature on Aspergillus species. Most of the investigations on relative humidity survival relationship which have been mentioned in this Introduction and Literature Review included observations on the effect of light on survival as well. Generally, longevity increased when light was excluded. Thus, for example, under the best conditions of relative humidity (0.0% R.H.) and temperature (8°C) used in the investigation, 90 per cent of the conidia of Beauveria bassiana in dark survived after 635 days storage in contrast to 53 per cent survival in light of intensity of 18lux (Clerk and Madelin, 1965), Rotem and Aust, (1991) found that exposure to sunlight affected the survival of conidia of Alternaria macrospora and Botrytis cinerea in the same way as UV light.

A unique relative humidity/light-spore survival relationship has been discovered in conidia of Cercospora canescens by Teyegaga and Clerk (1972).

They reported that in light the conidia survived best at median humidities and died quickest at the extreme humidities, while in dark the conidia survived longest at the lower humidities and lost viability quickest at the higher humidities. Using typical examples, conidia in light (32.5lux) at 20°C showed the respective viabilities of 33, 77, 100, 62 and 5 percent at 0, 20, 40, 60, and 80% R.H. after 56 days' storage, while in the dark the respective viabilities at the corresponding storage relative humidities were 98, 100, 82, 78 and 69 per cent.

Furthermore, a rare report is provided by the studies of Clerk and Madelin (1965) on the effect of gases on the survival of fungal spores. It would be recalled that they found early death among conidia in normal air at about 45% R.H. and the longest survival of the conidia of Metarrhizium anisopliae at the extreme humidities. Other observations made, during further study of this phenomenon were that (a) conidia washed in Gemex ZII, and Nonidet P.42 both of concentration of 200p.p.m. rendered the conidia more sensitive to the effects of the median humidities and at humidities of 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95%, the respective percentage survival at 25°C after 4 days storage was for unwashed conidia; 100, 100, 100, 99.7, 49.9, 94.3, 98.3, 100, 100, and 100 per cent, respectively; for conidia washed with Gemex ZII; 99.6, 99.8, 99.8, 99.6, 1.5, 1.5, 4.7 5.6 97.9, and 99.6 per cent, respectively, and for conidia washed with Nonidet P.42: 99.9, 99.8, 99.8, 99.8, 56.2, 0.0, 0.0, 5.0, 98.1 and 99.6 per cent, respectively (b) storage of the conidia in air with [illegible] levels of carbon dioxide or without oxygen

prevented early death at the median humidities. Survival of conidia was found to be 30.3, 74.0, 97.0, 98.4 and 100 per cent when they were stored for 7 days, at 25°C at 45% R.H (the most vulnerable humidity level) in normal atmosphere (0.03 per cent carbon dioxide) and in atmospheres with elevated carbon dioxide of 0.52, 7.25 and 18.2 per cent, and in an atmosphere of 100 per cent nitrogen (oxygen free), respectively.

This investigation was carried to study in particular the survival of conidia of Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii. This thesis contains, in the main, results of studies on;

i. the mycoflora of grains stored at warehouses in Accra and Tema.

ii. the effects of relative humidity and temperature on growth of, and conidial formation by, the four Aspergillus species.

iii. the germination capacity of conidia formed at different relative humidities.

iv. the survival capacity of conidia formed at different relative humidities; and

v. the effect of desiccation on the survival of conidia which had already gone through structural and physiological changes that precede germ tube emergence.

II. MATERIALS AND GENERAL METHODS

2.1 MATERIALS

2.1.1 FUNGAL ISOLATES: Isolates of Aspergillus clavatus Desmazieres, Aspergillus flavus Link Fr, Aspergillus niger van Tieghem and Aspergillus tamarii Kita studied were isolated from stored maize grains and maintained on Potato Dextrose Agar (PDA) slants in McCartney tubes at 30°C. They were subcultured fortnightly. Conidia used in the various experiments were removed from 5 day-old cultures raised on PDA Petri plate at 30°C except where otherwise specified.

2.1.2 MAIZE GRAINS: Sample of grains of maize (Zea mays L.) from which fungi were isolated were obtained from Kaneshie and Tema Warehouses of the Food Distribution Corporation, Accra. The grains were stored in polypropylene bags stacked up to 10 bags high in the warehouses, as shown in Plates 1, 2 and 3.

2.1.3 POTATO TUBERS: Tubers of Potato (Solanum tuberosum L.) used in the preparation of Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were bought at the Makola Market, Accra. The tubers were stored in the refrigerator until needed.

PLATE 1. Photograph showing the front view of the warehouse of the Ghana Food Distribution Coporation at Kaneshie, Accra. (x 1/10)

PLATE 2. Photograph showing the front view of the warehouse of Ghana Food Distribution Corporation at Tema. (x 1/16)

PLATE 3. Photograph showing stacked bags of maize in the warehouse of the Ghana Food Distribution Corporation at Kaneshie, Accra (x 1/4)

2.1.4 CULTURE MEDIA: Different media were used for different purposes.

i. Potato Dextrose Agar (PDA) [Ainsworth and Bisby, 1945]

Potato tuber	200g
Dextrose	10g
Agar	15g
Distilled Water	1000ml

Chips of freshly peeled tubers of potato were boiled in 500ml of distilled water. The extract was then strained with muslin and made up to 1,000ml. Ten grams of Dextrose and 15g of Agar were added and the medium was heated in waterbath to melt the Agar before it was autoclaved.

ii. Potato Dextrose Broth (PDB)

Prepared as in the case of PDA, but without Agar.

iii. Maize Meal Agar (Ainsworth and Bisby, 1945)

Maize grains	200g
Dextrose	20g
Agar	15g
Distilled Water	1000ml

An amount of 1,000ml of distilled water was added to 200g ground maize in a round bottom flask and heated for a few minutes without boiling, before adding 20g Dextrose and 15g Agar

iv. Oxytetracycline-Glucose-Yeast extract Agar (OGYE)

(Nout et al, 1987)

Yeast Extract	5.0g
Dextrose	20.0g
Biotin	0.1mg
Agar	12.0g
Distilled water	1000ml

v. Exudate of maize grains

Exudate of maize grains was used for certain tests. Five grams each of Mixed White, Obatanpa and Yellow maize undamaged grains were put in separate lots of 15ml of sterile distilled water in sterile glass vials. For each maize variety the vials were divided into two batches. One batch was allowed to stand for 12 hours and the other for 24 hours. The grains were removed there after and the vials of exudate were stored in a refrigerator at 4°C and the exudate used when needed.

2.1.5 CHEMICALS: Chemicals used were purchased from Oxoid Ltd London England; British Drug House (B.D.H.) Chemicals Ltd., Poole, England and Accra Chemist Ltd., Accra.

2.2 GENERAL METHODS

2.2.1 Humidity Chambers

Transparent plastic boxes (24cm long, 12cm wide and 12cm deep) with tightly fitting lids and the edges sealed with cellotape were used as humidity chambers for the growth of the Aspergillus species at different relative humidities. The plastic chambers were sterilised by cleaning the interior with 90 per cent ethanol and three sterilized solid watch glass (3.7 x 3.1 x 1.6cm) also washed with 70 per cent ethanol were placed in each box as stands for the Petri dishes. Distilled water and glycerol solutions which were used to maintain different relative humidities were poured into the different chambers to a depth of 5mm. Distilled water provided 100 per cent R.H. and different concentrations of glycerol solutions shown in Table 1 provided 92.8, 85.2, 73.4, 65.0 and 62.4 per cent R.H.

TABLE 1. Aqueous Glycerol solutions for maintaining Different Relative Humidities (Extracted from data of Johnson, 1940)

% R.H.	Weight of glycerol (g)	Volume of distilled water (ml)
100	0.0	100
92.8	25.0	75.0
85.2	35.0	65.0
73.4	50.0	50.0
65.0	68.0	32.0
62.4	60.0	40.0

Desiccators were used as humidity chambers for the storage of conidia of the four Aspergillus species at different relative humidities in the longevity tests. The interior of the desiccators was sterilised with 90 per cent ethanol. The desired relative humidities of 0.0, 20.0, 40.0, 60.0, 80.0 and 100 per cent were provided by different concentrations of sulphuric acid and distilled water as shown in Table 2.

TABLE 2 Aqueous Sulphuric Acid Solution for Maintaining Different Relative Humidities (Extracted from data of Solomon, 1952).

% R.H.	Weight of Sulphuric Acid (g)	Volume of distilled water (ml)
100	0.00	100
80.0	26.79	73.21
60.0	38.35	61.21
40.0	47.71	52.29
20.0	57.76	42.24
0.0	100	0.00

2.2.2 METHODS OF STERILIZATION

Glass slides, covership, V-shaped glass rods, pipettes, vials and other glassware used were washed thoroughly with detergent, rinsed under running tap and further thoroughly rinsed with distilled water. Glass slides, coverslips and V-shaped glass rods were stored in 90 per cent ethanol.

Culture media, aluminium foil cups, and McCartney tubes and Erlenmeyer flasks with appropriate solutions or media were sterilised by autoclaving for 15 minutes at 121°C at 1.1kg steam pressure. Cotton wool plugs were covered with grease proof paper or aluminium foil to prevent the penetration of condensed water during autoclaving.

Pipettes, measuring cylinders and Petri dishes were sterilized by heating at 160°C for at least 6 hours in an electrically heated oven. Petri dishes and measuring cylinders were air-dried after cleaning before placing them in the oven.

Inoculating needles and loops, forceps and cork borers were sterilised by flaming to red-heat and then cooled by holding them in the air in the sterile inoculation room. The inoculation room was sterilized by spraying with 5 per cent Dettol Solution 15 minutes just before use.

2.2.3 ESTIMATION OF LOAD OF VIABLE FUNGI IN MAIZE GRAINS

Following the procedure described by Lacey, Hill and Edwards (1980) about 10g maize grain were ground in a Warring blender. The chamber was previously washed with 70 per cent ethanol.

Five grams of the resulting powder were suspended in 45ml of aqueous 0.1% agar in a 100ml Erlenmeyer flask and shaken for two minutes. After standing for 30 minutes, a decimal dilution series was prepared in 0.1% agar. Aliquots (1ml) of appropriate dilution were spread on PDA containing 250mg chloramphenicol per ml.

Each aliquot was applied to three pre-poured Petri dishes (2 x 250ul and 1 x 500ul). The Petri plates were incubated at 30°C and colonies which developed were counted after five days. The fungal species were identified on the 7th day using the following books: Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 1972); Manual and Atlas of the Penicillia (Ramirez,1982); Practical Mycology-Manual for Identification of Fungi, (Samson & Hoekstra, 1988); Introduction to Food-Borne Fungi (Funder, 1953); An Introduction To Industrial Mycology (Smith & Raitrick, 1960); A Manual of Aspergilli (Thom & Raper, 1945) and assisted by my supervisors.

2.2.4 DETERMINATION OF MOISTURE CONTENT OF MAIZE GRAINS

Samples of the grain were ground in Warring blender and 10g portions of the flour dried at 108°C in the oven for 24 hours. The flour was placed in a desiccator and allowed to cool and then weighed.

The values obtained were used to calculate the percentage moisture content of the grains.

2.2.5 AIR SPORA STUDIES; PETRI DISH METHOD

Petri dishes containing OGYE were exposed for 5 minutes in the Kaneshie and Tema Warehouses and then closed and incubated at 30°C for 7 days. Colonies were counted on the 5th day of incubation and species were identified on the 7th day using the books listed at section 2.2.3 and assisted by my supervisors.

2.2.6 FUNGUS FLORA OF MAIZE GRAINS KEPT AT MODERATELY HIGH TEMPERATURES

Samples of freshly harvested and bagged grains of Abeleehi, Mixed White, Obatampa and Yellow Maize varieties provided by the Ministry of Food and Agriculture, Accra and the Food Distribution Cooperation, Accra, were kept in the oven at a temperature of 35-40°C and relative humidity of 55-60 per cent for a period of four months. The fungus flora was determined at the beginning and after two and four months of storage.

2.2.7 METHOD OF INOCULATION OF AGAR PLATES

Two diameters at right angles to each other were drawn on the bottom of the Petri dishes with a permanent marker. Agar medium was poured into the Petri dishes and allowed to set. The lid of the Petri dish was removed and the medium was held in an inverted position and inoculated by touching the surface at the intersection of the two diameters with the tip of a flame-sterilised pin carrying spores of the test fungus. The lids were replaced and the plates incubated in the inverted position. Three replicates of Petri plates were used for all growth experiments.

2.2.8. GROWTH MEASUREMENTS OF CULTURES

The diameters of the fungal colonies were measured along the two pre-drawn diameters at desired intervals and the mean calculated.

2.2.9. SPORE GERMINATION TESTS

Slide Method

Conidia of the different Aspergillus species were removed from culture plates by gently touching the culture surface with a sterile inoculating loop and transferring them into McCartney tubes holding appropriate solutions or water. The spore suspension was shaken by hand to give uniform dispersion.

The number of conidia in suspension for every germination test was strictly standardized to 30-40 spores in a high power (x40 objective) microscope field. The conidia in drops of the suspension were then germinated on glass slides.

These were sterile slides (7.5 x 2.5cm), each supported on a V-shaped glass rod in a sterile Petri dish over a small quantity of the same medium as that in which the germination of the spore was tested. This technique obviated both evaporation of the germination test droplet and condensation of moisture. Using a sterile dropping pipette, three separate drops of spore suspension (about 0.1ml in volume) were placed on each thermally equilibrated slide in the Petri dish and incubated. Per cent germination was assessed after the desired period. Each germination count was based on six drops of suspension (ie three separate drops on each of the two slides for each treatment). Petri dishes were always randomized in the incubator to nullify positional effect.

Agar Plate Method

The Agar Method was used in the germination of conidia previously stored dry in longevity tests. Dry conidia were stored, in longevity tests, in aluminium foil cups (3cm in diameter with 4cm high edge).

After the desired storage interval, samples of the conidia were transferred, using a sterile inoculating pin into 10ml of sterile distilled water in McCartney tubes. Potato Dextrose Agar plates were then inoculated with the spore suspension, standardized to give 30-40 conidia in a high power (x40 objective) microscope field. Nine evenly spaced 0.1ml suspension drops were placed on each plate and incubated at 30°C for 12 hours. Two replicate plates (18 test areas) were used for each germination test.

2.2.10 INCUBATION

Cultures on Petri plates, germinating conidia and dry conidia in longevity tests were incubated at 30°C. Light was provided, where continuous illumination was desired, by day-light fluorescent tube placed two metres above the incubated spores.

2.2.11 ASSESSMENT OF CONIDIUM GERMINATION AND GERM TUBE LENGTH

Observations were made and counts of germinating conidia were taken immediately at the end of the desired incubation period. If observations could not be made immediately, a drop of 10 per cent formaldehyde solution was added to each spore suspension on the slides for each inoculated area on the PDA Petri plate to arrest further development of the spores.

The percentage germination was calculated from a minimum of 800 observed conidia randomly selected from all suspension drops for any treatment by the formula:

$$\frac{\text{Number of Germinated Spores} \times 100}{\text{Total Number of Germinated and Ungerminated Spores examined}}$$

The lengths of 20 representative germ tubes were also measured, with an eye piece graticule, and the mean calculated.

A conidium was considered to have germinated if a germ tube was discernible. This assessment of germination was based on morphology. Although Gottlieb and Caltrider (1963) among several workers who have studied both the physiology and ultrastructure of germinating spores have shown that external evidence of spore germination is long preceeded by enzymatic changes within the spore, and that a definition of germination should include this state of physiological change in the spore. Germ tube emergence was adopted in this study to standardize germination assessment as facilities were not available in our laboratories for the measurement of such internal changes.

2.2.12 ESTIMATION OF DEGREE OF SPORULATION OF PETRI PLATE CULTURES AT DIFFERENT RELATIVE HUMIDITIES AND TEMPERATURES

A 3mm diameter disc of culture removed from a standard distance of 2cm from the centre of the culture plate after the desired interval of incubation was put in 10ml of distilled water in the McCartney tubes. A drop of Tween 80 was added and the tube shaken for 10 minutes to dislodge and disperse the conidia. Three replicates were prepared for cultures of each relative humidity. Using a sterile medicine dropper, a drop of the spore suspension was placed onto a hemacytometer slide. The spores in each of ten squares were counted, using a microscope with x10 objective. The mean number of spores per square using values obtained from the three replicate suspensions was used to calculate the number of spores per mililiter of the suspending medium.

2.2.13 ASSESSMENT OF RATE OF SWELLING IN CONIDIAL POPULATIONS

The conidia of the Aspergillus species grew in size before the germ tubes emerged. The rate of swelling in the different spore populations was studied using the Slide Spore Germination Method. Drops of conidial suspension incubated at 30°C were examined under the microscope at regular intervals and the number of swollen conidia and total number of conidia in randomly selected microscope fields recorded, and the percentage of conidia swollen calculated.

2.2.14 EFFECT OF DRYING ON THE SURVIVAL OF SWOLLEN CONIDIA

Using the Slide Method suspensions of conidia of the standard density prepared with Potato Dextrose Broth were incubated at 30°C until 60-80 per cent of the conidia of each species had swollen. Different batches of the suspension drops were then air-dried under a fan for varying lengths of time of 1, 2, 4 and 6 hours. The air-dried conidia were resuspended in PDB on the slides, and incubated at 30°C for 6 hours. Control conidia were not dried. Percentage germination was assessed in all the treatments and the mean germ tube length estimated as described in section 2.2.11.

2.2.15 MEASUREMENT OF pH

The pH of media was measured with Alpha 500 pH meter, EDT Instruments Ltd., Dover, England.

2.2.16 PREPARATION OF SLIDES FOR DRAWINGS

A drop of 10 per cent formaldehyde solution was added to the spore suspension on a glass slide to arrest growth. The spores were then stained with cotton blue in lactophenol. A cover slip was placed on the suspension drop and the excess fluid irrigated with filter paper. Finally a cover slip was placed on each suspension drop. Using camera lucida, drawings of swollen spores were made for the four Aspergillus species.

2.2.17 EXPERIMENTAL PRECAUTIONS

i. Glassware were kept scrupulously clean. Glassware which had already been cleaned with water and detergent were thoroughly rinsed several times with tap water and three times with distilled water and allowed to drain dry before use.

ii In the assessment of percentage germination, spores were counted in all the suspension drops of each treatment.

iii Conidia of the same age were used by obtaining conidia on each occasion from 5-day old cultures.

iv. The edges of the bottom and lid of the desiccators used as humidity chambers were luted with vaseline to make the desiccators air-tight.

v. All plating and inoculation were done in the microflow laminar flow chamber in the inoculating room.

vi. The glycerol-water mixtures and aqueous sulphuric acid solutions were thoroughly shaken on preparation to ensure homogenous mixtures.

vii. When measuring the lengths of germ tubes, those at the edge of the suspension drops were avoided as these were under more aerated condition, and tended to grow much faster than the submerged germ tubes.

2.2.18 STATISTICAL ANALYSIS

Experimental results, where necessary, were analyzed statistically using calculated confidence limits quoted at 95 per cent. Figures bearing the same letters are not significantly different.

III. EXPERIMENTAL DETAILS

A. MYCOFLORA OF MAIZE GRAINS STORED INITIALLY AT THE KANESHIE AND TEMA WAREHOUSES AND SUBSEQUENTLY AT LEGON

The original design to study the mycoflora of maize grains being stored at the Kaneshie and Tema Warehouses over a period of six months had to be modified when the Food Distribution Management decided to sell the stock only two months after the beginning of sampling. Samples from the two warehouses were, therefore, transferred to the laboratory. They were kept in woven polypropylene bags at 30°C and 75-80% R.H.. The humidities in the two warehouses recorded with thermohydrographs fluctuated between 55 and 90% RH.

The fungal species present at the time of transfer were studied and subsequent studies were made at two month intervals over a period of six months. At each fungal population assessment time, the moisture content of samples of the grains was also determined. Eighty grams of grains were ground on each occasion, and five samples of the flour of 10g each were used in the determination of the moisture content. The species isolated under various occasions and their frequencies are shown in Tables 3 and 4, and the moisture content of the grains at the corresponding times are shown in Table 5.

B. MYCOFLORA OF MAIZE GRAINS STORED AT SUB-LETHAL TEMPERATURE 40°C FOR FOUR MONTHS

Sample of maize grain of different varieties were next kept at 40°C and at 55-60 percent R.H. in woven polypropylene bags inside an incubator to study the changes that might occur in the fungus flora of the grain. Grains of four varieties namely, Abeleehi, Obatanpa, Mixed White and Yellow were used. They had all been previously kept for approximately two months after harvest at atmospheric temperature of 30-32°C. Abeleehi and Obatanpa varieties were obtained from stores of Ministry of Food and Agriculture, Mixed White variety from the Kaneshie Warehouse and Yellow variety from the Tema Warehouse. The samples of Mixed White and Yellow did not, however, come from the same stock as those of the preceding study.

The fungi were isolated at the beginning and after two and four months storage in the incubator. The moisture content of samples of the grains was also determined at monthly intervals. The species isolated on each occasion and the frequency and occurrence of each one presented in Tables 6, 7, 8 and 9. The monthly percentage moisture content of the grain obtained appears in Table 10.

C. STUDIES ON THE AIR SPORA OF THE WAREHOUSES AT KANESHIE AND TEMA

Studies on the air spora of the warehouses at Kaneshie and Tema were started at the same time with studies on the contaminating fungi of the grains in storage. When it became necessary to transfer some of the grains to the laboratory at Legon to continue the investigation on the fungal species in the grains, it was decided, nonetheless, to continue with the studies of the air spora.

Agar Petri plates were exposed at different locations in the warehouse on each occasion to obtain as much information as possible. To standardize sampling, plates were exposed on the north, south, east and west between the stack and the wall at a height of 2 metres. Four plates were exposed at each location. The mean colonies per plate for each location have been presented in Tables 11 and 12 to provide a clear picture of not only the identity of the fungi but also their distribution inside the warehouse.

D. EFFECT OF EXUDATES OF GRAINS OF MAIZE VARIETIES, MIXED WHITE, OBATANPA AND YELLOW, ON THE GERMINATION OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

Compounds of different sort from the grains were likely to enter drops of condensed water on grains in storage. If the nutrients occur in greater proportions the resultant mixture is likely to stimulate spore germination. Spore may be inhibited, on the other hand, in the case of greater concentrations of toxic compounds such as organic acids.

What may happen to the fungus spores when water condenses on the stored grain was investigated.

The conidia of Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii were germinated using the Slide Method, in exudates of maize prepared as described under Materials and General Methods. The Percentage Germination and Mean Germ Tube Lengths of germ tubes produced by germinating conidia incubated at 30°C were assessed after 6, 9, and 12 hours. Degree of stimulation, where it occurred, was compared with germination of conidia in 0.1% Peptone, 1.0% Dextrose and 1.0% Sucrose. The results are presented in Tables 13 to 16.

E. EFFECT OF RELATIVE HUMIDITY ON GROWTH AND SPORULATION OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

The influence of relative humidity prevailing in the warehouses on the growth of the fungi and the degree of sporulation will greatly influence the persistence of the fungi in storage. The response of Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii to humidities of 100, 92.8, 85.2, 73.4, 65.0 and 62.4% R.H. was, therefore, investigated. There were three Petri plates at each humidity.

The inoculated plates at the different humidities were examined daily and the diameters of the colonies measured. At the end of the incubation period the degree of sporulation was determined by estimating the density of conidia on the standard sized culture disc removed from spots of identical distances from the culture centre for each treatment as described under Materials and General Methods. The results are presented in Tables 17 to 21.

F. GERMINATION CAPACITY OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

Germination of spores of fungi in stored products is one of the important factors contributing to the persistence of the fungi. Conidia formed at different relative humidities may differ in their ability to germinate. The germination of conidia formed at 100, 92.8, 85.2, 73.4, 65.0 and 62.4% R.H. in the previous experiment was studied using the Slide Method, and Potato Dextrose Broth as the germinating medium. Drops of the spore suspension prepared with the broth were incubated at 30°C and the percentage germination for each treatment was assessed at 3-hour intervals for a total incubation period of 12 hours. The results are presented in Tables 22 to 25.

G. EFFECT OF TEMPERATURE ON GROWTH AND SPORULATION OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

The fungi sporulated to different degrees in atmospheres of different relative humidities. The slight fluctuations in temperature which occur locally are also likely to influence the growth and sporulation of fungi of the warehouses. Different batches of three inoculated plates of Maize Meal Agar each were incubated at 22, 26, 30, 34 and 38°C. The diameters of the growing colonies were measured daily and the degree of sporulation in each treatment was estimated in the same way as was done with cultures growing at the different relative humidities. The results are shown in Tables 26 to 30.

H. SURVIVAL OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII* FORMED AT DIFFERENT RELATIVE HUMIDITIES

The conditions under which spores are formed may influence their longevity. Subsequent tests on longevity of the conidia formed at different relative humidities were carried out using conidia of all the four Aspergillus species. In this experiment dry conidia were removed from cultures growing at 100, 92.8, 85.2, 73.4, 65.0 and 62.4% R.H. and stored for 28 days.

Different samples of conidia formed at each humidity were kept in light at 100, 80.0, 60.0, 40.0, 20.0 and 0.0% R.H. at 30°C. Some of the conidia of each treatment were removed at regular intervals and germinated on Potato Dextrose Agar plates at 30°C. The percentage of conidia which germinated represented the percentage viability of the sample. Germ tubes of the germinated conidia were also measured as an indication of the vigour of the germinating conidia.

i. Survival of Conidia of Aspergillus clavatus.
The results obtained are shown in Tables 31 and 32.

ii. Survival of Conidia of Aspergillus flavus.
The results obtained are presented in Tables 33 and 34.

iii. Survival of Conidia of Aspergillus niger.
The results obtained are given in Tables 35 and 36.

iv. Survival of Conidia of Aspergillus tamarii.
The results obtained appear in Tables 37 and 38.

I. ABILITY OF CONIDIA SWOLLEN IN NUTRIENT BROTH TO WITHSTAND SUBSEQUENT DESICCATION

Physiological and structural changes take place in germinating spores long before the emergence of the germ tubes. The evidence of this was clear in conidia of the Aspergillus species studied in the germination tests conducted. The conidia enlarged ominously prior to germ tube emergence. Since in nature small condensation droplets holding swollen conidia may dry up, the processes leading to final germination may be interrupted. It was, considered necessary to find out the fate of such desiccated swollen conidia.

Conidia swollen in drops of Potato Dextrose Broth and then dried were kept for varying periods up to 6 hours in the dried condition and then resuspended in Potato Dextrose Broth and incubated at 30°C for 6 hours. Percentage germination of these conidia was assessed at the end of the incubation period. Control conidia were not dried. The results are presented in Tables 39 to 43.

IV RESULTS

A. MYCOFLORA OF MAIZE GRAINS STORED INITIALLY AT THE KANESHIE AND TEMA WAREHOUSES AND SUBSEQUENTLY AT LEGON

Using the appropriate fungal taxonomic texts (Barnett and Hunter, 1972; Funder, 1953; Malone and Muskett, 1964; Ramirez, 1982; Smith and Raistrick, 1960; Thom and Raper, 1945), several fungal species were identified from stored grains of both Mixed White and Yellow varieties as shown in Tables 3 and 4. The fungi were identified by the culture characteristics and pigmentation and by the reproductive structures, and unique structures such as sclerotia. During storage, the moisture content of the grains remained between 13.7 and 15.8 per cent during the first two months and at the end of storage period as shown in Table 5. The statistical analysis of the percentage moisture content of the grains is indicated in the table).

Tables 3 and 4 contain the fungus species isolated from normal and brown-coloured (stackburn) kernels from Kaneshie and Tema warehouses, respectively. The genus Aspergillus was represented by the largest number of species in the four batches of grains. The Aspergillus made up 68.8 per cent of the number of species encountered in the normal grains from Kaneshie, 34.0 per cent of the species from the stackburn grains from Kaneshie and 60.3 and 47.95 per cent, respectively, from the normal and stackburn grains from the Tema warehouse.

Comparing the normal and brown-coloured grains from each warehouse, the later contained fewer Aspergillus species. Therefore, the normal grain from Kaneshie contained Aspergillus candidus, A. clavatus and A. fumigatus which were absent in the stackburn kernels. The normal grains of Tema also contained Aspergillus candidus and A. parasiticus which were not found in stackburn grains. Comparing the normal grains from the two warehouses, Aspergillus effusus and A. wentii which were absent in the Kaneshie grains were obtained from the Tema grains.

The normal grains also contained more of the other species than the stackburn grains. Thus the Kaneshie stock showed the following species only in the normal grains: Neurospora sitophila, Paecilomyces puntonii, P. variotii, Phoma glomerata and Rhizoctonia solani. The Tema stock also showed the following species in the normal Yellow Grains only: Curvularia lunata, Dreschlera maydis, Fusarium moniliforme, Fusarium sp. and Gliocladium sp.

Similarly, some other species occurred in grains from one warehouse but not in grains from the other warehouse. Thus species of the Kaneshie grains only were Chaetomium sp. and Rhodotorula sp. and those of the Tema grains only were Curvularia lunata, Dreschlera maydis, Gliocladium sp., Paecilomyces carneus, Scopulariopsis and Stemphylium lanugolosum.

For species occurring in both the normal and stackburn grains in a warehouse, the patterns of percentage frequency were similar in the case of the majority. The patterns of percentage frequency divided the species into five categories. Using examples in which the same pattern occurred in normal and stackburn grains from both warehouses, the five categories are:

i. Percentage frequency decreased with time e.g. Mucor sp., Paecilomyces puntonii sp., P. variotii and Rhizoctonia solani.

ii. Percentage frequency increased with time e.g. Aspergillus candidus, A. ustus, Chaetomium sp. and Verticillium lecanii.

iii. Percentage frequency increased and later declined e.g. Aspergillus flavus, Fusarium sp., Paecilomyces carneus and Penicillium sp.

iv. Percentage frequency decreased and later rose e.g. Cladosporium herbarum.

v. Percentage frequency was irregular e.g. Aspergillus niger, A. ochraceus, A. sulphureus, Penicillium chrysogenum, P. expansum, Phoma glomerata and Rhodotorula sp.

Table 3

Percentage frequency of Fungal Species isolated from white maize variety obtained from Kaneshie Warehouse and subsequently stored in the laboratory at 30°C for six months

Fungal Species	Normal coloured				Brown discoloration (Stackburn)			
	Storage period in months				Storage period in months			
	0	2	4	6	0	2	4	6
Aspergillus candidus	0.0	0.0	0.0	12.8	0.0	0.0	0.0	0.0
Aspergillus clavatus	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus	12.6	15.9	33.1	12.8	22.4	28.4	37.9	15.6
Aspergillus flavus-oryzae	0.0	0.0	0.0	8.2	0.0	0.0	0.0	18.4
Aspergillus fumigatus	0.0	0.0	0.0	16.4	0.0	0.0	0.0	0.0
Aspergillus niger	15.4	13.6	12.6	1.5	18.0	8.0	1.1	1.7
Aspergillus ochraceus	0.0	5.0	1.4	1.4	0.0	0.0	0.0	2.8
Aspergillus parasiticus	3.7	0.0	0.0	0.0	15.7	9.6	17.9	1.7
Aspergillus sulphureus	9.7	0.0	0.0	0.7	8.9	1.9	0.0	0.0
Aspergillus tamarii	0.0	2.1	2.0	0.3	0.0	0.0	0.0	0.8
Aspergillus ustus	0.0	10.1	0.7	15.0	0.0	0.0	0.0	3.8
Chaetomium sp.	0.0	0.0	0.0	1.6	0.0	0.0	0.0	2.8
Cladosporuim herbarum	9.1	9.2	0.0	4.7	4.1	13.5	0.0	3.8
Mucor sp.	4.3	4.6	0.0	0.0	4.4	9.5	0.0	0.0
Fusarium moniliforme	11.5	2.6	0.7	0.7	0.0	16.9	9.5	0.0
Neurospora sitophila	0.0	1.4	4.0	4.0	0.0	0.0	0.0	0.0
Paecilomyces puntonii	5.3	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Paecilomyces variotii	2.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium chrysgenum	11.9	4.7	0.0	0.0	22.4	8.4	0.0	12.0

Table 3 Cont'd

Percentage frequency of Fungal Species isolated from white maize variety obtained from Kaneshie Warehouse and subsequently stored in the laboratory at 30°C for six months

Fungal Species	Normal coloured				Brown discoloration (Stackburn)			
	Storage period in months				Storage period in months			
	0	2	4	6	0	2	4	6
Penicillium expansum	5.1	6.9	23.1	0.0	4.1	4.2	1.2	16.6
Penicillium sp.	7	8.5	22.4	11.2	0.0	0.0	0.0	0.0
Phoma glomerata	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Rhizoctonia solani	5.4	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhodotorula sp.	[illegible]	1.4	0.0	0.0	0.0	0.0	0.6	3.8
Verticillium lecanii	[illegible]	0.0	0.0	4.8	0.0	0.0	0.0	4.2
Unidentified spec. 1	[illegible]	6.8	0.0	6.1	0.0	0.0	0.0	22.8

Table 4

Percentage frequency of Fungal Species isolated from yellow maize variety obtained from Tema Warehouse and subsequently stored in the laboratory at 30°C for six months

Fungal Species	Normal coloured				Brown discoloration(Stackburn)			
	Storage period in months				Storage period in months			
	0	2	4	6	0	2	4	6
Aspergillus candidus	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0
Aspergillus clavatus	0.0	2.3	0.0	0.0	0.0	12.7	0.0	0.0
Aspergillus effusus	0.0	0.0	0.0	4.8	0.0	0.0	0.0	0.8
Aspergillus flavus	10.3	13.5	45.9	21.5	22.2	26.8	100	4.0
Aspergillus flavus-oryzae	0.0	0.0	0.0	6.4	0.0	0.0	0.0	18.1
Aspergillus fumigatus	0.0	0.0	0.0	2.6	0.0	8.0	0.0	7.1
Aspergillus glaucus	0.0	0.0	0.0	9.6	0.0	0.0	0.0	14.3
Aspergillus niger	9.3	14.3	0.0	2.6	68.3	20.7	0.0	0.7
Aspergillus parasiticus	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus sulphureus	0.0	2.5	0.0	3.6	0.0	0.0	0.0	1.0
Aspergillus tamarii	0.0	0.0	0.0	2.5	0.0	6.4	0.0	0.7
Aspergillus ustus	0.0	0.0	0.0	0.5	0.0	0.0	0.0	2.0
Aspergillus wentii	0.0	0.0	0.0	3.6	5.2	2.9	0.0	0.0
Cladosporuim herbarium	0.0	0.0	0.0	15.2	0.0	0.0	0.0	16.0
Curvularia lunata	0.0	8.3	0.0	2.4	0.0	9.5	0.0	6.6
Dreschlera maydis	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0
Fusarium moniliforme	24.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0
Fusarium sp.	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0
Gliocladium sp.	0.0	20.8	0.0	0.0	0.0	0.0	0.0	0.0
Mucor sp.	0.0	3.0	0.0	0.0	0.0	6.6	0.0	0.7

Table 4. Cont'd

Percentage frequency of Fungal Species isolated from yellow maize variety obtained from Tema Warehouse and subsequently stored in the laboratory at 30°C for six months

Fungal Species	Normal coloured				Brown discoloration (Stackburn)			
	Storage period in months				Storage period in months			
	0	2	4	6	0	2	4	6
Neurospora sitophila	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paecilomyces variotii	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Paecilomyces puntonii	28.8	2.8	0.0	0.0	0.0	0.0	0.0	0.0
Paecilomyces variotii	15.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium citrinum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.4
Penicillium chryosgenum	0.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium cyclopium	0.0	0.0	0.0	4.3	0.0	0.0	0.0	1.0
Penicillium expansum	0.0	7.1	0.0	11.9	33.3	15.9	0.0	1.8
Penicillium sp.	0.0	7.8	54.1	0.0	0.0	0.0	0.0	0.8
Phoma glomerata	0.0	1.3	0.0	2.4	0.0	0.0	0.0	0.0
Scopulariopsis sp.	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0
Stemphylium lanugulosum	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Verticillium lecanii	0.0	0.0	0.0	3.9	0.0	0.0	0.0	3.6

Table 5

Moisture content of maize grains stored for 6 months at 30°C and at 75-80% R.H.

Date and period of storage in (months)		Mean percentage moisture content (± SE) of grains of			
		Mixed white variety		Yellow variety	
		Normal coloured	Brown (Stackburn)	Normal coloured	Brown (Stackburn)
15th November, 1993	0	13.7 ± 0.2 A	13.8 ± 0.2 A	15.0 ± 0.2 A	15.6 ± 0.1 A
14th January, 1994	2	14.2 ± 0.2 A	14.5 ± 0.1 B	15.4 ± 0.1 B	15.8 ± 0.0 B
12th May, 1994	6	15.1 ± 0.1 B	15.4 ± 0.2 C	15.6 ± 0.4 AB	15.8 ± 0.0 B

* *Calculated confidence limits at 95%, figures bearing the same letters are not significantly different. (Vertical direction)*

B. MYCOFLORA OF MAIZE GRAINS STORED AT SUB-LETHAL TEMPERATURE OF 40°C FOR FOUR MONTHS

This experiment which was carried out as a sequel to experiment A studied other aspects of contamination of the grains. Only normal grains were used to study:

(a) Infection of grains of four varieties: Abeleehi, Mixed White, Obatanpa and Yellow Maize varieties.

(b) Infection and persistence of causal fungi at sub-lethal temperatures.

Since the samples were kept in an enclosed incubator it is reasonable to assume that changes in the flora over the period of four months would involve more particularly the fungi already present before incubation.

During incubation samples were withdrawn at monthly intervals for the determination of the moisture content of the grains. Table 10 shows that the grains of the varieties had different initial percentage moisture content. It was highest in the Mixed White and the Obatanpa grains and the lowest in the Abeleehi grains. There was gradual decrease in moisture content with time at different rates. For example, whereas, only a third of the moisture content of Abeleehi variety was lost over four months decreasing from 12.1 to 8.0 per cent, Obatanpa grains lost almost 50 per cent of its water content which fell from 14.0 to

The number of fungal species isolated varies from variety to variety. Abeleehi variety contained 15 fungal species while Mixed White variety contained 16 fungal species. Obatanpa and Yellow Maize varieties yielded 18 fungal species each.

The genus Aspergillus was represented by the largest number of species in the four varieties, than any other. Comparing the four maize varieties, Abeleehi, Mixed White and Obatanpa each contained seven Aspergillus species while the Yellow Maize variety contained nine Aspergillus species (Tables 6, 8 and 9).

Other common features were the fewest number of species at the initial stage and then the number increased with increase in storage time. Thus the respective number of species at zero, two and four months for the different varieties were;

Abeleehi	-	2, 6 and 15
Mixed White	-	2, 7 and 12
Obatanpa	-	4,6 and 17
Yellow	-	2,3 and 16

It was noteworthy that the percentage occurrence of all species present initially decreased with increase in storage time. For each maize variety Aspergillus flavus was the predominant species.

Stackburn was not observed in any of the samples.

Table 6

Frequency of Fungal Species isolated from grains of Abeleehi maize variety stored at 40°C

Fungal Species	Storage after indicated months		
	0	2	4
Aspergillus flavus	53.9	56.9	5.9
Aspergillus fumigatus	0.0	14.6	3.0
Aspergillus giganteus	46.1	11.1	9.9
Aspergillus niger	0.0	0.0	4.9
Aspergillus sulphureus	0.0	0.0	2.9
Aspergillus tamarii	0.0	0.0	3.0
Aspergillus ustus	0.0	0.0	3.0
Chaetomium sp.	0.0	0.0	14.8
Neurospora sitophila	0.0	1.4	7.4
Paecilomyces variotii	0.0	14.6	4.9
Penicillium citrinum	0.0	0.0	9.9
Penicillium sp.	0.0	1.4	5.9
Phoma glomerata	0.0	0.0	7.4
Scopulariopsis sp.	0.0	0.0	7.4
Verticillium lecanii	0.0	0.0	4.9
Unidentified species	0.0	0.0	4.9

Table 8

Frequency of Fungal Species isolated from grains of Obatanpa maize variety stored at 40°C

Fungal Species	Storage after indicated months		
	0	2	4
Aspergillus candidus	0.0	0.0	5.8
Aspergillus flavus	64.8	49.9	17.0
Aspergillus flavus-oryzae	0.0	0.0	14.3
Aspergillus fumigatus	0.0	4.5	1.2
Aspergillus niger	0.0	21.9	4.7
Aspergillus sulphureus	0.0	0.0	1.1
Aspergillus tamarii	0.0	0.0	1.0
Cladosporium herbarum	0.0	0.0	12.1
Fusarium sp.	0.0	0.0	15.1
Paecilomyces variotii	0.0	4.2	0.0
Penicillium brevicompactum	3.7	8.6	4.3
Penicillium citrinum	0.0	0.0	8.7
Penicillium sp.	3.8	0.0	3.4
Phoma glomerata	0.0	0.0	1.0
Rhizoctonia solani	27.7	10.9	1.1
Rhodotorula sp.	0.0	0.0	3.4
Scopulariopsis	0.0	0.0	2.4
Verticillium lecanii	0.0	0.0	3.4

Table 9

Frequency of Fungal Species isolated from grains of yellow maize variety stored at 40°C

Fungal Species	Storage after indicated months		
	0	2	4
Aspergillus effusus	0.0	0.0	12.7
Aspergillus flavus	62.7	28.6	6.3
Aspergillus flavus-oryzae	0.0	0.0	4.8
Aspergillus fumigatus	0.0	0.0	4.8
Aspergillus niger	0.0	0.0	1.7
Aspergillus ochraceus	0.0	0.0	1.7
Aspergillus sulphureus	0.0	0.0	2.6
Aspergillus tamarii	0.0	0.0	1.7
Aspergillus ustus	0.0	0.0	3.5
Cladosporium herbarum	0.0	0.0	11.4
Cladosporium sp.	0.0	0.0	12.7
Curvularia lunata	0.0	0.0	9.5
Neurospora sitophila	37.3	14.3	0.0
Penicillium brevicompactum	0.0	0.0	9.5
Penicillium citrinum	0.0	0.0	9.5
Penicillium sp.	0.0	57.1	0.0
Penicillium sp.	0.0	0.0	0.0
Rhodotorula sp.	0.0	0.0	3.8
Verticillium lecanii	0.0	0.0	6.8

Table 10

Moisture content of maize grains stored for 4 months at 3(°C and at 75-80% R.H.

Date and period of storage in (months)	Mean percentage moisture content (± SE) of grains of			
	Mixed white variety		Yellow variety	
	Abeleehi	Mixed White	Obatanpa	Yellow
0	12.1 ± 0.1 A	14.4 ± 0.2 A	14.0 ± 0.1 A	13.4 ± 0.7 A
1	12.0 ± 0.4 A	12.0 ± 0.0 B	12.1 ± 0.1 B	12.2 ± 0.1 A
2	9.3 ± 0.4 B	9.2 ± 0.1 C	9.5 ± 0.2 C	9.3 ± 0.0 B
3	8.2 ± 0.2 C	8.9 ± 0.1 D	8.3 ± 0.3 D	8.5 ± 0.3 C
4	8.0 ± 0.1 C	8.3 ± 0.2 E	7.9 ± 0.1 D	7.5 ± 0.4 C

By Calculated Confidence limits at 95%, values bearing the same letters (vertical rows) are not significantly different.

C. STUDIES ON THE AIR SPORA OF THE WAREHOUSES AT KANESHIE AND TEMA

The air spora of the warehouses at Kaneshie and Tema were studied as described in Materials and General Methods by exposing Oxytetracycline-Glucose-Yeast Extract Agar Petri plates on 19th November, 1993; 18th January, 1994, 17th March, 1994 and 16th May, 1994. Very interesting features of the air spora of the warehouse were observed. The information contained in Tables 11 and 12 could be summarized as follows;

(a) There were many species in the atmosphere of each warehouse. A total of 26 and 28 fungal species were isolated from the Kaneshie and Tema warehouses respectively.

(b) Only a few species, namely, Aspergillus flavus A. fumigatus, Cladosporium herbarum, Mucor sp. and Rhodotorula sp. grew on the plates exposed in the Kaneshie warehouse on all four sampling times. For the Tema warehouse more species were isolated from the atmosphere. These were Aspergillus flavus, A. flavus-oryzae, A. niger, Cladosporium herharum, Penicillium cyclopium, P. expansum, Rhizoctonia solani and Scopulariopsis sp.

(c) On any occasion and with any of the species isolated, different numbers of colonies were isolated from the four sampling locations.

(d) From the total of the four values (N,S, E and W) for each species, the patterns of percentage frequency divided the species into five categories. Using examples from the totals for the Kaneshie warehouse, the five categories were:

i. percentage frequency increased with time e.g. Aspergillus effusus, A. flavus and Verticillium lecanii.

ii. percentage frequency decreased with time e.g. Dreschlera maydis and Rhizoctonia solani.

iii. percentage frequency increased and later decreased with time e.g. Aspergillus flavus-oryzae, A. niger, A. ochraceus, A. parasiticus, A. tamarii, Cladosporium sp., Mucor sp. Paecilomyces puntonii, Penicillium cyclopium, Penicillium sp., Phoma glomerata, Scopulariopsis and Stemphylium lanugolosum.

iv. percentage frequency decreased and later rose e.g. Aspergillus sulphureus, Cladosporium herbarum, Fusarium moniliforme, Paecilomyces variotii and Penicillium chrysogenum.

v. Percentage frequency was random e.g. Aspergillus fumigatus and Rhodotorula sp.

Using examples from totals for the Tema warehouse, the five categories were;

i. percentage frequency increased with time e.g. Aspergillus flavus, A. flavus-oryzae, A. fumigatus, A. sulphureus, A. tamarii and Cladosporium sp.

ii. Percentage frequency decreased with time e.g. Aspergillus parasiticus, Penicillium Chrysogenum.

iii. Percentage frequency increased and later declined e.g. Aspergillus ochraceus, A. ustus, Curvularia lunata, Helminthosporium, Neurospora sitophila, Paecilomyces variotii, Penicillium expansum and Verticillium lecanii.

iv. percentage frequency decreased and later rose e.g. Mucor sp., Paecilomyces puntonii, Penicillium cyclopium and Scopulariopsis sp.

v. Percentage frequency was random e.g. Aspergillus niger, Penicillium sp. and Phoma glomerata.

(e) In both cases the dominant species were Aspergillus flavus and Cladosporium herbarum.

(f) It is noteworthy that the Penicillium species occurred in greater numbers in the atmosphere of Tema warehouse than that of the Kaneshie Warehouse. The total number of colonies of Penicillium chrysogenum, P. cyclopium, P. expansum and other Penicillium sp. were 55.8, 18.9, 76.7 and 41.0. The respective corresponding total number of colonies of the Tema warehouse were, 118.4, 34.7, 157.4 and 69.9.

Table 11

Percentage frequencies of Fungi isolated from Air in the Kaneshie Warehouse over a 6-month period (November,1993 to May,1994) Using the Agar Plate Method

Fungal Species	0				2				4		
	19/11/93				18/1/94				17/3/944		
	N	S	E	W	N	S	E	W	N	S	E
Aspergillus effusus	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
Aspergillus flavus	32.1	0.0	0.0	3.5	48.2	17.1	10.7	58.4	49.6	22.0	15.3
Aspergillus flavus-oryzae	0.0	0.0	0.0	0.0	0.0	0.0	11.4	0.0	0.0	0.0	0.7
Aspergillus fumigatus	0.0	0.0	0.0	12.6	3.0	0.0	6.1	0.0	3.7	0.0	7.1
Aspergillus niger	.0	5.9	0.0	5.0	0.0	18.5	0.0	20.0	0.0	0.0	0.0
Aspergillus ochraceus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.2	0.0	12.0	0.0
Aspergillus parasiticus	0.0	0.0	0.0	0.0	0.0	17.1	0.0	0.0	0.0	10.0	0.0
	0	19.0	5.4	0.0	0.0	0.0	6.3	0.0	0.0	0.0	7.0

Table 11

Percentage frequencies of Fungi isolated from Air in to May,1994) Using the Agar Plate Method

Fungal Species	0				2	
	19/11/93				18/1	
	N	S	E	W	N	S
Penicillium chrysogenum	9.9	9.1	11.6	0.0	0.0	0.0
Penicillium cyclopium	0.0	0.0	0.0	0.0	6.2	0.0
Penicillium expansum	0.0	9.5	12.3	0.0	0.0	0.0
Penicillium sp.	0.0	0.0	0.0	0.0	0.0	0.0
Phoma glomerata	0.0	0.0	0.0	0.0	0.0	0.0
Rhizoctonia solani	15.4	10.6	0.0	7.0	0.0	13.8
Rhodotorula sp.	22.8	13.5	9.7	4.0	6.0	0.0
Scopulariopsis sp.	0.0	0.0	0.0	0.0	0.0	19.7
Stemphylium lanugulosum	0.0	0.0	0.0	0.0	0.0	0.0
Verticillium lecanii	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified species	0.0	0.0	0.0	0.0	0.0	0.0

Tab

Percentage frequencies of fungi isolated from air i
May,1994) Using the Agar Plate Method

Fungal Species	0				2		
	19/11/93				18/1/94		
	N	S	E	W	N	S	
Aspergillus flavus	7.6	7.2	6.0	7.7	7.9	8.1	8.
Aspergillus flavus-oryzae	4.0	0.0	0.0	0.0	6.3	0.0	0.
Aspergillus fumigatus	0.0	0.0	0.0	0.0	0.0	0.0	0.
Aspergillus glaucus	0.0	0.0	0.0	0.0	0.0	0.0	0.
Aspergillus niger	4.0	0.0	4.0	0.0	4.4	0.0	2.
Aspergillus ochraceus	0.0	0.0	0.0	0.0	0.0	0.0	0.
Aspergillus parasiticus	7.4	25.7	0.0	0.0	6.3	11.7	0.
Aspergillus sulphureus	0.0	0.0	0.0	0.0	0.0	0.0	0.
Aspergillus tamarii	0.0	0.0	0.0	0.0	0.0	0.0	0.
Aspergillus ustus	0.0	0.0	0.0	0.0	0.0	0.0	0.
Cladosporium herbarum	33.6	24.9	33.6	54.0	45.4	28.8	36.
Cladosporium sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.
Curvularia lunata	0.0	0.0	0.0	5.0	0.0	5.2	0.
Helminthosoporium sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.

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Percentage frequencies of fungi isolated from Air i
May,1994) using the Agar Plate Method

Fungal Species	0					
	19/11/93				18	
	N	S	E	W	N	S
Mucor sp.	7.4	0.0	0.0	0.0	6.0	0.0
Neurospora sitophila	0.0	0.0	0.0	0.0	0.0	0.0
Paecilomyces puntonii	10.6	0.0	0.0	2.7	9.4	0.0
Paecilomyces variotii	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium chrysogenum	9.4	15.0	23.6	0.0	6.3	13.7
Penicillium cyclopium	0.0	0.0	0.0	6.4	0.0	0.0
Penicillium expansum	6.4	0.0	22.2	9.3	5.6	16.1
Penicillium sp.	9.6	14.7	0.0	7.7	2.4	0.0
Phoma glomerata	0.0	4.4	0.0	0.0	0.0	2.4
Rhizoctonia solani	0.0	0.0	10.6	0.0	0.0	5.2
Rhodotorula sp.	0.0	8.1	0.0	0.0	6.0	8.8
Scopulariopsis sp.	0.0	0.0	0.0	7.2	0.0	0.0
Verticillium lecanii	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified species	0.0	0.0	0.0	0.0	0.0	0.0

D. EFFECT OF EXUDATES OF GRAINS OF MAIZE VARIETIES, MIXED WHITE, OBATANPA AND YELLOW, ON THE GERMINATION OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

Four Aspergillus species from the earlier isolations from grains used were Aspergillus clavatus, A. flavus, A. niger and A. tamarii. It is clear from the data in the Table of results, Table 3, shows that Aspergillus flavus, A. niger colonies were abundant while the colonies of A. clavatus and A. tamarii were very sparse. Aspergillus clavatus and A. tamarii were deliberately selected to find out from the germination, growth and survival experiments whether they were present in the warehouses but did not survive long or were really of low occurrence.

The conidia were germinated in exudates of grains of Mixed White, Obatanpa and Yellow Maize varieties. The results are presented in Tables 13, 14, 15 and 16.

Conidia of the different species responded differently. Aspergillus niger conidia did not swell in the Peptone, Dextrose and Sucrose solutions and did not germinate after 12 hours. Conidia of Aspergillus flavus and A. tamarii enlarged to different proportions in these three media but did not also germinate. Only conidia of Aspergillus clavatus germinated after swelling in Peptone, Dextrose and Sucrose solution.

In Aspergillus clavatus which germinated in Peptone, Dextrose and Sucrose, germination of the conidia was slower than germination in 24-hour exudates of the three varieties of maize. Conidia of Aspergillus flavus did germinate in the exudates of the grains while conidia of both Aspergillus niger and A. tamarii were swollen in the exudates but never germinated. In the two instances response to the exudates of maize varieties varied and the different percentage germination are recorded in Tables of results. However, the exudates of Obatanpa was inferior to exudates of the other two varieties.

Germ tube growth seemed to be responding differently to medium from conidial germination. A clear example was shown by conidial of Aspergillus flavus (Table 14) where percentage germination in exudate of Mixed White, Obatanpa and Yellow were 57.8, 13.2 and 11.2 per cent respectively, while the mean germ tube lengths were 29.0, 76.8 and 10.9µm respectively.

Table 13

Germination of conidia of *Aspergillus clavatus* in exudates of grains of three maize varieties and solutions of organic compounds at 30°C

Germination Medium	Germination after indicated hours								
	6			9			12		
	% of spores swollen	% Germination	*MGTL in µm	% of spores swollen	% Germination	MGTL in µm	% of spores swollen	% Germination	MGTL in µm
12h- Exudate of Maize Variety									
Mixed White	81.7	0.0	-	97.5	20.8	18.2	91.3	40.0	27.5
Obatanpa	94.4	1.0	5.0	93.6	11.3	10.2	91.6	22.2	24.4
Yellow	98.5	1.8	5.2	92.1	75.8	14.2	97.6	96.8	23.4
24h- Exudate of Maize Variety									
Mixed White	87.2	0.0	-	88.2	18.7	13.5	92.5	73.4	28.8
Obatanpa	95.1	15.8	7.9	94.3	32.3	17.5	93.5	57.7	31.7
Yellow	92.2	0.0	-	96.9	36.7	6.6	92.3	96.6	16.2
Peptone 0.1%	70.2	0.0	-	73.3	0.0	-	72.0	27.8	7.9
Dextrose 1.0%	84.4	6.7	6.3	82.7	15.5	22.2	83.7	51.5	32.3
Sucrose 1.0%	63.2	0.0	-	67.0	0.0	-	69.6	26.8	5.9

*Mean Germs Tube Length.

Table 14

Germination of conidia of *Aspergillus flavus* in exudates of grains of three maize varieties and solutions of organic compounds at 30°C

Germination Medium	Germination after indicated hours								
	6			9			12		
	% of spores swollen	% Germination	*MGTL in µm	% of spores swollen	% Germination	MGTL in µm	% of spores swollen	% Germination	MGTL in µm
12h- Exudate of Maize Variety									
Mixed White	81.6	0.0	-	85.8	57.2	10.2	94.8	57.8	29.0
Obatanpa	87.4	0.0	-	91.0	8.5	5.6	97.0	13.2	26 8
Yellow	83.9	0.0	-	84.3	0.0	-	96.2	11.2	10 9
24h- Exudate of Maize Variety									
Mixed White	91.1	0.0	-	96.3	51.5	13.2	98.2	55.5	24.1
Obatanpa	95.1	0.0	-	83.2	0.0	-	90.6	16.3	17 0
Yellow	92.2	0.0	-	90.5	26.8	11.9	88.5	30.8	14 2
Peptone 0.1%	60.0			67.8	0.0	-	74.3	0.0	-
Dextrose 1.0%	49.9			49.5	0.0	-	52.0	0.0	
Sucrose 1.0%	41.9	0.0		46.0	0.0	-	53.7	0.0	

*Mean Germ Tube Length.

Table 15

Germination of conidia of *Aspergillus niger* in exudates of grains of three maize varieties and solutions of organic compounds at 30°C

Germination Medium	Germination after indicated hours								
	6			9			12		
	% of spores swollen	% Germination	*MGTL in µm	% of spores swollen	% Germination	MGTL in µm	% of spores swollen	% Germination	MGTL in µm
12h- Exudate of Maize Variety									
Mixed White	29.2	0.0	-	28.7	0.0	-	2.8	0.0	-
Obatanpa	49.8	0.0	-	69.0	0.0	-	4.7	0.0	-
Yellow	18.2	0.0	-	22.0	0.0	-	6.7	0.0	-
24h- Exudate of Maize Variety									
Mixed White	65.7	0.0	-	68.8	0.0	-	93.3	0.0	-
Obatanpa	52.3	0.0	-	55.0	0.0	-	69.3	0.0	-
Yellow	42.2	0.0	-	80.5	0.0	-	91.2	0.0	-
Peptone 0.1%	11.3	0.0	-	36.0	0.0	-	46.2	0.0	-
Dextrose 1.0%	67.0	0.0	-	80.2	0.0	-	89.3	0.0	-
Sucrose 1.0%	57.0	0.0	-	63.0	0.0	-	89.3	0.0	-

*Mean Germ Tube Length.

Table 16

Germination of conidia of *Aspergillus tamarii* in exudates of grains of three maize varieties and solutions of organic compounds at 30°C

Germination Medium	Germination after indicated hours								
	6			9			12		
	% of spores swollen	% Germination	*MGTL in µm	% of spores swollen	% Germination	MGTL in µm	% of spores swollen	% Germination	MGTL in µm
12h- Exudate of Maize Variety									
Mixed White	40.0	0.0	0.0	48.3	0.0	0.0	60.0	0.0	0.0
Obatanpa	12.5	0.0	0.0	25.5	0.0	0.0	47.5	0.0	0.0
Yellow	0.0	0.0	0.0	10.7	0.0	0.0	10.0	0.0	0.0
24h- Exudate of Maize Variety									
Mixed White	0.0	0.0	0.0	24.3	0.0	0.0	41.8	0.0	0.0
Obatanpa	0.0	0.0	0.0	15.2	0.0	0.0	34.7	0.0	0.0
Yellow	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Peptone 0.1%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dextrose 1.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sucrose 1.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Mean Germ Tube Length.

E. EFFECT OF RELATIVE HUMIDITY ON GROWTH AND SPORULATION OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

All the four Aspergillus species; A. clavatus, A. flavus, A. niger and A. tamarii were able to grow on Maize Meal Agar. Different sets of the inoculated plates were incubated at relative humidities ranging from 62.4 to 100% RH. The mean diameters of each treatment recorded at 2, 3, 4, 5, 6, and 7 days are given in Tables 17, 18, 19 and 20. Fig. 1 compares the final diameters of the various treatments obtained on the 7th day. Every fungus grew steadily at the different relative humidities and the results showed that maximum growth had not been achieved on the 7th day because the mean diameter for each treatment was the highest on that day.

Since they are different species with different genetical constituent they will be expected to grow at different rates on the media. A. tamarii grew best on the medium. Its rate of growth was only slightly better than those of A. flavus and A. niger while A. clavatus was distinctly the slowest as clearly illustrated in Fig. 1

The four species in addition responded differently to the atmospheric relative humidities. Thus, the mean diameters of colonies of A. clavatus on the 7th day at 100, 92.8. 85.2, 73.4. 65.0 and 62.4% Rh were 36.5, 41.7, 47.0. 47.8 and 51.5mm respectively (Table 17) and those of A. tamarii at the corresponding relative humidities were 61.8, 66.7, 67.3. 67.5 and 68.8mm respectively

Growth of A. flavus on the other hand was better at the median humidities of 73.4, 85.2 and 92.8% than at the extreme humidities of 62.4, 65.0 and 100% (Table 18). Only A. niger grew best at the highest humidities of 92.8 and 100% and slowest at the lowest humidities of 62.4, 65.0 and 73.4% R.H. (Table 19).

The statistical analysis of growth of each species at the various relative humidities calculated at 95 per cent, indicate which difference among the diameters of the colonies at the different relative humidities on each occasion is statistically significant.

The various degrees of sporulation of the fungi at different relative humidities assessed according to the method described under Material and General Methods are presented in Table 21. The species again exhibited differences in habit with regards to conidiation. The data showed that Aspergillus clavatus and A. niger sporulated better on the Maize Meal Agar t an A. flavus and A. tamarii. The humidities that supported best sporulation varied from species to species. A. clavatus sporulated best at 85.2 to 100% R.H. producing 180-200 x 100 conidia per ml of the standard suspension. A. flavus also sporulated best at the highest relative humidity of 100% R.H. and the spore density of 180 x 100 per ml of standard suspension was more than double the value obtained at 62.4, 65.0 and 85.2%.

In contrast, A. tamarii sporulated best at the lowest humidity of 62.4% Rh. The spore density of 168 x 100 per ml of standard suspension decreased steadily with increasing relative humidities to 60 x 100 per ml of standard suspension at 100% R.H.

A. niger did not show any recognisable pattern. There were wide fluctuations with the peaks and troughs occurring randomly over the relative humidities used. High densities of 208 and 213 x 100 per ml of standard suspension were recorded at 62.4 and 92.8% R.H., respectively, and low densities of 119 and 128 x 100 per ml of standard suspension were obtained at 100 and 65.0%, respectively.

Table 17

Growth of *Aspergillus clavatus* incubated at 27°C in atmospheres of different relative humidities

Relative Humidity (%)	Mean culture diameters (mm) after following days of incubation					
	2	3	4	5	6	7
100	10.8 ± 1.6	18.3 ± 0.7	27.6 ± 0.3	31.5 ± 1.0	34.7 ± 3.0	36.5 ± 0.6 A
92.8	10.3 ± 0.2	17.3 ± 0.3	27.0 ± 0.0	31.8 ± 0.7	36.3 ± 1.8	41.7 ± 1.2 B
85.2	10.7 ± 1.1	18.8 ± 0.8	27.8 ± 0.9	32.0 ± 1.0	41.8 ± 1.6	46.7 ± 1.6 C
73.4	10.0 ± 0.4	17.5 ± 0.6	26.3 ± 0.9	33.3 ± 0.5	42.8 ± 0.8	47.0 ± 0.8 C
65.0	9.7 ± 0.5	19.0 ± 1.1	27.3 ± 0.8	34.3 ± 0.8	42.3 ± 1.1	48.0 ± 1.4 CD
62.4	9.0 ± 0.5	18.5 ± 0.8	27.0 ± 0.4	34.6 ± 2.0	44.5 ± 0.5	51.5 ± 1.0 D

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 18

Growth of *Aspergillus flavus* incubated at 27°C in atmospheres of different relative humidities

Relative Humidity (%)	Mean culture diameters (mm) after following days of incubation					
	2	3	4	5	6	7
100	[illegible] ± 0.6	35.7 ± 1.1	48.0 ± 2.0	55.5 ± 0.3	57.0 ± 0.9	59.0 ± 2.1 A
[illegible]	[illegible] ± [illegible]	37.0 ± 1.0	52.5 ± 1.1	59.0 ± 0.7	59.5 ± 0.7	65.0 ± 1.1 B
[illegible]	21.7 ± 0.7	[illegible] ± [illegible]	51.0 ± 1.2	62.7 ± 0.7	65.0 ± 1.1	67.8 ± 1.4 C
73.4	20.7 ± 0.3	36.4 ± 0.6	45.7 ± 1.7	49.0 ± 0.4	52.0 ± 0.6	65.5 ± 1.5 BC
65.0	21.8 ± 0.5	36.7 ± 0.5	51.3 ± 0.5	59.4 ± 1.6	61.8 ± 0.6	62.5 ± 2.4 AB
62.4	20.5 ± 0.4	36.5 ± 0.3	49.5 ± 0.9	57.8 ± 3.6	60.3 ± 2.7	57.5 ± 2.4 A

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 19

Growth of *Aspergillus niger* incubated at 27°C in atmospheres of different relative humidities

Relative Humidity (%)	Mean culture diameters (mm) after following days of incubation					
	2	3	4	5	6	7
100	12.3 ± 1.0	32.0 ± 0.0	43.0 ± 1.4	56.5 ± 0.9	61.0 ± 0.8	63.3 ± 0.2 A
97.6	12.5 ± 0.2	32.3 ± 0.9	38.8 ± 1.1	52.0 ± 1.0	56.0 ± 0.8	63.0 ± 0.9 A
85.2	12.6 ± 0.5	26.3 ± 0.5	38.5 ± 1.0	52.3 ± 1.0	55.8 ± 1.4	61.0 ± 0.8 B
73.4	12.8 ± 0.3	26.5 ± 0.3	41.7 ± 0.6	55.7 ± 0.5	58.2 ± 0.4	62.0 ± 0.3 AB
65.0	12.3 ± 0.6	26.2 ± 1.1	41.0 ± 0.9	54.5 ± 0.6	57.3 ± 0.3	60.7 ± 0.4 B
52.4	10.0 ± 0.5	25.2 ± 1.3	38.5 ± 1.7	49.5 ± 1.1	54.2 ± 0.8	58.0 ± 1.3 C

By calculated Confidence Limits, at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 20

Growth of *Aspergillus tamarii* incubated at 27°C in atmospheres of different relative humidities

Relative Humidity (%)	Mean culture diameters (mm) after following days of incubation					
	2	3	4	5	6	7
100	25.5 ± 1.2	36.8 ± 2.1	46.8 ± 1.8	55.5 ± 2.2	59.5 ± 3.0	61.8 ± 1.8 A
92.8	26.7 ± 0.9	42.3 ± 0.5	54.7 ± 0.5	60.5 ± 1.0	65.1 ± 1.7	66.1 ± 1.2 B
85.2	24.8 ± 1.5	37.0 ± 1.3	45.3 ± 2.1	54.0 ± 2.8	64.1 ± 1.6	66.7 ± 0.9 B
73.4	28.0 ± 0.7	45.3 ± 0.4	53.3 ± 1.5	60.7 ± 2.5	61.2 ± 2.5	67.3 ± 0.2 B
65.0	25.2 ± 2.2	45.5 ± 1.0	57.0 ± 2.0	61.5 ± 1.0	66.5 ± 1.2	67.5 ± 0.7 B
52.4	24.7 ± 0.5	48.0 ± 0.7	57.1 ± 1.1	60.8 ± 0.7	67.2 ± 0.9	68.8 ± 0.9 C

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 21

Degree of sporulation of *Aspergillus* species on Maize Meal Agar, at different relative humidities

Relative Humidity (%)	Number of spores per ml (x 100) of standard suspension prepared with 8-day old cultures			
	Aspergillus clavatus	Aspergillus flavus	Aspergillus niger	Aspergillus tamarii
100	196	188	119	60
[illegible]	180	136	213	68
[illegible]	[illegible]	88	176	128
[illegible]	[illegible]	124	144	144
[illegible]	[illegible]	84	128	148
[illegible]	[illegible]	[illegible]	208	168

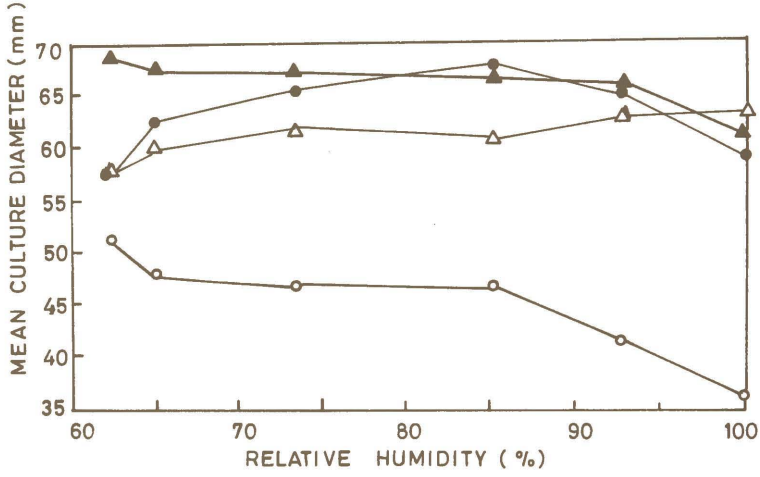

FIG. 1 MEAN CULTURE DIAMETER OF COLONIES OF A. CLAVATUS (o—o A. FLAVUS (●—●), A. NIGER (△—△), A. TAMARII (▲—▲), GROWN AT DIFFERENT RALATIVE HUMIDITIES AT 30 °C FOR 7 DAYS.

F. GERMINATION CAPACITY OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII* FORMED AT DIFFERENT RELATIVE HUMIDITIES

The germination capacity of the conidia of four Aspergillus species was tested in Peptone Dextrose Broth and in distilled water at 30°C. Slides with suspension drops were withdrawn after 3, 6, 9 and 12 hours and the percentage germination determined. The period of incubation was long enough to enable the rich Potato Dextrose Broth to support vigorous germ tube growth which made the measurement of the germ tube lengths difficult. The results appear in Table 22, 23, 24 and 25.

For all four Aspergillus species there was no conidium germination in distilled water. In all cases a period of three hours was too short and no germination was observed in all the treatments. All germinated after 6 hours and germination after that interval was best in A. clavatus. It was followed in descending order by A. flavus, A. tamarii and A. niger.

After incubation for 12 hours very high percentage germination of 90.0 to 99.5 per cent was recorded for A. clavatus (see Table 22), 80.7 to 94.3 per cent for A. flavus (see Table 23), 83.4 to 98.4 per cent for A. niger (see Table 24), and 87.7 to 98.6 per cent for A. tamarii (see Table 25).

Table 22

Germination capacity of conidia of *Aspergillus clavatus* formed at different relative humidities. (Percentage germination based on 500-800 observed conidia in Potato Dextrose Broth after incubation for different periods at 30°C)

% R.H. of humidity chamber of source of conidia	Percentage germination after following hours of incubation			
	3	6	9	12
100	0.0	78.5	80.5	90.0
92.8	0.0	85.4	89.6	92.9
85.2	0.0	97.9	99.5	99.5
73.4	0.0	91.0	93.7	96.3
65.0	0.0	97.0	99.0	99.5
62.4	0.0	93.0	94.0	97.4

Table 23

Germination capacity of conidia of *Aspergillus flavus* formed at different relative humidities. (Percentage germination based on 500-800 observed conidia in Potato Dextrose Broth after incubation for different periods at 30°C)

% R.H. of humidity chamber of source of conidia	Percentage germination after following hours of incubation			
	3	6	9	12
100	0.0	72.7	79.2	80.7
92.8	0.0	74.3	74.9	86.5
85.2	0.0	89.0	89.9	92.1
73.4	0.0	71.3	72.5	81.1
65.0	0.0	68.7	84.7	94.3
62.5	0.0	73.5	79.5	85.1

Table 24

Germination capacity of conidia of *Aspergillus niger* formed at different relative humidities. (Percentage germination based on 500-800 observed conidia in Potato Dextrose Broth after incubation for different periods at 30°C)

% R.H. of Humidity chamber of source of conidia	Percentage germination after following hours of incubation			
	3	6	9	12
100	0.0	8.9	73.4	83.4
92.8	0.0	37.1	44.1	84.4
85.2	0.0	56.0	96.1	96.8
73.4	0.0	41.8	60.3.	89.5
65.0	0.0	54.8	85.1	95.8
62.4	0.0	67.9	92.4	98.4

Table 25

Germination capacity of conidia of *Aspergillus tamarii* formed at different relative humidities. (Percentage germination based on 500-800 observed conidia in Potato Dextrose Broth after incubation for different periods at 30°C)

% R.H. of humidity chamber of source of conidia	Percentage germination after following hours of incubation			
	3	6	9	12
100	0.0	80.6	88.0	90.0
92.8	0.0	95.9	98.0	98.6
85.2	0.0	93.9	96.9	97.5
73.4	0.0	78.5	95.2	96.6
65.0	0.0	93.4	94.0	94.7
62.4	0.0	70.0	77.9	87.7

G. EFFECT OF TEMPERATURE ON GROWTH AND SPORULATION OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII*

The effect of temperature on biochemical activities and therefore, growth and sporulation is predictable. For any fungus there is an optimum temperature and activities decrease with temperatures above and below the optimum. Every fungus has its own optimum temperature and this experiment used only a fairly narrow range of 22 to 30°C to find out whether an optimum could be found for any of the fungi.

Several Maize Meal agar plates were inoculated and batches were incubated at 22, 26, 30, 34 and 38°C. Diameters of the growing colonies were measured after 2, 3, 4, 5, 6 and 7 days. On the 8th day the degree of sporulation was also assessed. The results are presented in Tables 26, 27, 28, 29 and 30. Fig. 2 is a graph of the mean diameters of the colonies after 7 days growth.

The optimum temperature for growth of A. clavatus, A. flavus, A. niger and A. tamarii were found to be 38, 34, 34, and 30°C respectively. The mean diameters at these optimal temperatures varied with the species. It was 81.5mm for A. clavatus, 72.0mm for A. flavus, 70.7mm for A. niger and 76.8mm for A. tamarii. The optimum temperature was identifiable at each sampling time in A. flavus and A. niger (Tables 27 and 28). But in A. clavatus and A. tamarii the optimum temperature became evident only after 5 days (Tables 26 and 29).

Growth at 22°C was poor in all species (see Fig. 2) and the mean colony diameters were 22.8, 18.2, 23.2 and 18.7mm after 7 days for A. clavatus, A. flavus, A. niger and A. tamarii respectively. Plate 4 shows the colonies of A. flavus and A. niger at the different temperatures after 7 days. The results were analysed statistically and the significant differences between means are shown in table of results.

The data in Table 30 showed that A. clavatus sporulated best at 26°C, A. flavus and A. niger sporulated best at 34°C and A. tamarii at 30°C. From the values recorded, abundant conidia were formed by A. niger followed by A. flavus, A. clavatus and A. tamarii. The statistical analysis of the data is presented in Table 30 to show significant differences between means.

Table 26

Growth of *Aspergillus clavatus* incubated at different temperatures

Temperature (°C)	Mean culture diameters (mm) after following days of incubation						
	2	3	4	5	6	7	
[illegible]	[illegible] ± [illegible]	5.7 ± 0.2	10.8 ± 0.3	13.0 ± 0.8	19.0 ± 1.5	22.8 ± 0.3	A
[illegible]	[illegible] ± 0.4	13.8 ± 0.5	19.5 ± 0.8	26.7 ± 0.8	32.0 ± 0.3	36.8 ± 0.8	B
[illegible]	11.5 ± 0.5	13.9 ± 0.6	21.2 ± 1.0	28.7 ± 1.0	34.0 ± 1.1	40.3 ± 0.5	C
[illegible]	[illegible] 8 ± 0.9	20.0 ± 0.6	30.5 ± 1.2	37.3 ± 1.2	45.3 ± 0.6	55.0 ± 0.6	D
[illegible]	[illegible] ± [illegible]	14.0 ± 0.6	26.5 ± 0.5	49.5 ± 0.5	63.5 ± 0.5	81.5 ± 0.3	E

[illegible] Confidence Limits at 95%, mean culture diameters bearing the [illegible] are significantly different.

Table 27

Growth of *Aspergillus Flavus* incubated at different temperatures

Temperature (°C)	Mean culture diameters (mm) after following days of incubation						
	2	3	4	5	6	7	
22	2.7 ± 0.5	3.0 ± 0.6	7.8 ± 0.5	9.3 ± 0.6	15.8 ± 1.1	18.2 ± 0.4	A
26	2.1 ± 0.3	11.2 ± 0.9	36.3 ± 0.6	39.7 ± 0.6	52.0 ± 0.2	58.5 ± 0.5	B
30	3.5 ± 0.4	11.7 ± 0.7	37.3 ± 1.1	44.5 ± 0.1	55.0 ± 0.8	61.8 ± 1.2	C
34	8.7 ± 0.6	24.2 ± 0.7	41.7 ± 0.9	49.8 ± 1.2	61.3 ± 0.5	72.0 ± 0.0	D
38	5.7 ± 0.7	14.5 ± 1.1	23.0 ± 0.8	29.4 ± 1.1	32.7 ± 0.1	42.3 ± 0.6	E

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 28

Growth of *Aspergillus niger* incubated at different temperatures

Temperature (°C)	Mean culture diameters (mm) after following days of incubation						
	2	3	4	5	6	7	
22	3.0 ± 0.3	3.3 ± 0.4	9.5 ± 0.6	11.2 ± 0.6	16.2 ± 1.2	23.2 ± 0.5	A
26	3.7 ± 0.5	17.3 ± 0.6	30.2 ± 0.6	43.0 ± 0.7	50.8 ± 0.6	56.0 ± 1.1	B
30	3.5 ± 0.7	14.0 ± 0.6	30.8 ± 1.2	46.8 ± 1.1	53.5 ± 1.1	61.5 ± 0.3	C
34	11.6 ± 0.3	26.2 ± 0.6	47.7 ± 0.6	58.3 ± 0.8	63.8 ± 0.5	70.7 ± 0.7	D
38	8.3 ± 1.1	20.7 ± 1.1	31.8 ± 0.1	40.2 ± 0.7	47.3 ± 1.1	55.2 ± 1.1	B

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 29

Growth of *Aspergillus tamarii* incubated at different temperatures

Temperature (°C)	Mean culture diameters (mm) after following days of incubation						
	2	3	4	5	6	7	
22	3.5 ± 0.6	4.9 ± 0.7	8.3 ± 0.4	10.0 ± 0.4	15.2 ± 0.8	18.7 ± 0.8	A
26	14.8 ± 0.5	28.2 ± 0.6	38.0 ± 0.5	52.0 ± 1.0	63.5 ± 0.8	67.8 ± 0.7	B
30	5.5 ± 0.4	13.0 ± 0.5	38.7 ± 0.8	54.7 ± 0.7	67.2 ± 1.4	76.8 ± 1.3	C
34	10.3 ± 0.9	21.5 ± 0.6	38.7 ± 0.3	54.4 ± 0.3	64.8 ± 0.3	71.2 ± 0.2	D
38	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	35.5 ± 0.4	3.5 ± 0.4	E

By calculated Confidence Limits at 95%, mean culture diameters bearing the same letters are not significantly different.

Table 30

Degree of sporulation of *Aspergillus* species on Maize Meal Agar, at different temperatures

Temperature (°C)	Number of spores per ml (x 100) of standard suspension prepared with 8-day old cultures			
	Aspergillus clavatus	Aspergillus flavus	Aspergillus niger	Aspergillus tamarii
22	84	120	128	108
[illegible]	160	128	176	102
[illegible]	[illegible]	112	144	112
[illegible]	[illegible]	208	[illegible]	[illegible]
[illegible]	[illegible]	124	[illegible]	[illegible]

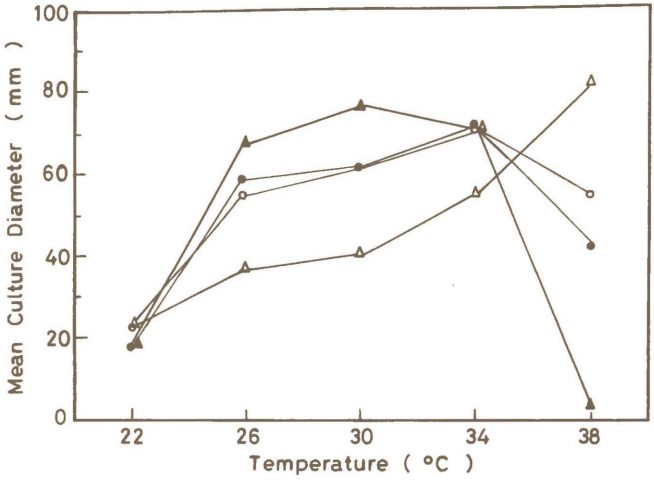

FIG. 2. Mean culture diameter of colonies of A. clavatus (△—△), A. flavus (●—●), A. niger (○—○) and A. tamarii (▲—▲) grown at different temperatures for 7 days.

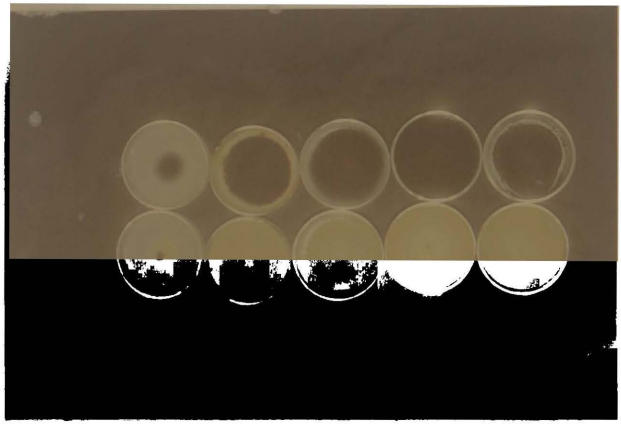

PLATE 4 Photograph of cultures of A. flavus and A. niger incubated at different temperatures showing growth after 7 days (x 2/9)

Top. A. niger : from left: 22, 26, 30, 34 and 38°C.

Bottom. A. flavus: from left: 22, 26, 30, 34 and 38°C.

H. SURVIVAL OF CONIDIA OF *ASPERGILLUS CLAVATUS*, *ASPERGILLUS FLAVUS*, *ASPERGILLUS NIGER* AND *ASPERGILLUS TAMARII* FORMED AT DIFFERENT RELATIVE HUMIDITIES

i. Survival of conidia of Aspergillus clavatus.

The percentage survival of conidia of A. clavatus which had been formed at different relative humidities ranging from 100 to 62.4% R.H. and then stored at relative humidities of 100 to 0.0% R.H. for 28 days is shown in Tables 31, 32 and in Fig. 3. The aim of the series of experiments in this chapter was not to determine how long the conidia will remain viable at the different relative humidities, but to find out whether the humidity of formation of the conidia would affect their response to storage humidity. The storage period of 28 days was adequate to identify such responses.

The results of the test with A. clavatus could be summarised as follows:

(a) There was no obvious indication that the humidities at which the conidia were formed influenced their subsequent survival at the different relative humidities of storage. There was no particular humidity of formation which produced consistently the best surviving conidia or the poorest surviving conidia. This can be observed clearly in Table 32.

(b) The conidia survived longest at relative humidities of 100 and 0.0% R.H. The shortest longevity was obtained at 20.0 and 80.0% R.H. Conidia at 40.0 and 60.0% R.H. were intermediate.

(c) As summarized in Table 32 and in Fig. 3, mean germ tube lengths were generally directly related to percentage survival.

The details commented in (a), (b) and (c) will be followed for the remaining three Aspergillus species.

Table 31a

Percentage of conidia of *Aspergillus clavatus* formed at different % R.H. able to survive at zero % R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	0.5	51.9	-	5.6 ± 0.2	23.4 ± 0.3
	92.8	0.0	0.0	67.8	-	-	19.8 ± 0.3
	85.2	0.0	0.0	54.4	-	-	22.1 ± 0.5
	73.4	0.0	0.5	62.2	-	5.0 ± 0.2	11.9 ± 0.3
	65.0	0.0	0.0	50.2	-	-	18.0 ± 0.3
	62.4	0.0	0.0	56.0	-	-	19.0 ± 0.3
28	100	0.0	0.0	39.2	-	-	15.8 ± 0.2
	92.8	0.0	0.0	40.0	-	-	10.6 ± 0.2
	85.2	0.0	0.0	42.2	-	-	7.9 ± 0.1
	73.4	0.0	0.0	54.4	-	-	7.4 ± 0.1
	65.0	0.0	0.0	40.0	-	-	8.1 ± 0.2
	62.4	0.0	0.0	42.8	-	-	8.4 ± 0.1
56	100	0.0	0.0	20.3	-	-	11.2 ± 0.2
	92.8	0.0	0.0	23.1	-	-	8.1 ± 0.2
	85.2	0.0	0.0	22.8	-	-	5.9 ± 0.1
	73.4	0.0	0.0	27.2	-	-	6.6 ± 0.0
	65.0	0.0	0.0	0.0	-	-	0.0
	62.4	0.0	0.0	0.0	-	-	0.0

Table 31b

Percentage of conidia of *Aspergillus clavatus* formed at different % R.H. able to survive at 20% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	0.0	11.7	-	-	10.6 ± 0.3
	92.8	0.0	1.9	8.1	-	7.6 ± 0.2	11.1 ± 0.2
	85.2	0.0	0.8	4.7	-	5.5 ± 0.2	19.1 ± 0.3
	73.4	0.0	0.0	4.4	-	-	11.9 ± 0.3
	65.0	0.0	0.0	6.3	-	-	8.9 ± 0.2
	62.4	0.0	0.0	4.2	-	-	6.3 ± 0.2
28	100	0.0	0.0	9.7	-	-	6.6 ± 0.2
	92.8	0.0	0.0	6.1	-	-	5.0 ± 0.2
	85.2	0.0	0.0	4.7	-	-	8.8 ± 0.2
	73.4	0.0	0.0	4.2	-	-	5.3 ± 0.2
	65.0	0.0	0.0	5.8	-	-	6.3 ± 0.2
	62.4	0.0	0.0	3.1	-	-	4.3 ± 0.2
56	100	0.0	0.0	4.4	-	-	5.3 ± 0.2
	92.8	0.0	0.0	1.9	-	-	4.0 ± 0.2
	85.2	0.0	0.0	2.7	-	-	6.3 ± 0.1
	73.4	0.0	0.0	0.6	-	-	3.3 ± 0.0
	65.0	0.0	0.0	1.1	-	-	5.9 ± 0.1
	62.4	0.0	0.0	1.4	-	-	4.2 ± 0.2

Table 31c

Percentage of conidia of *Aspergillus clavatus* formed at different % R.H. able to survive at 40% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	Storage in Light					
		% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (µm)		
		6	9	12	6	9	12
14	100	0.0	0.0	34.4	-	-	25.1 ± 0.5
	92.8	0.0	0.0	30.3	-	-	29.0 ± 0.4
	85.2	0.0	0.0	25.6	-	-	41.3± 0.3
	73.4	0.0	0.0	33.6	-	-	20.8 ± 0.3
	65.0	0.0	0.0	32.8	-	-	12.5 ± 0.3
	62.4	0.0	0.0	32.2	-	-	16.8 ± 0.3
28	100	0.0	0.0	20.0	-	-	8.9 ± 0.2
	92.8	0.0	0.0	20.8	-	-	11.2 ± 0.2
	85.2	0.0	0.0	16.3	-	-	20.7± 0.3
	73.4	0.0	0.0	22.8	-	-	11.2± 0.2
	65.0	0.0	0.0	17.5	-	-	9.6 ± 0.2
	62.4	0.0	0.0	16.1	-	-	15.8± 0.2
56	100	0.0	0.0	2.5	-	-	6.9 ± 0.1
	92.8	0.0	0.0	10.3	-	-	7.9 ± 0.2
	85.2	0.0	0.0	3.1	-	-	14.9 ± 0.2
	73.4	0.0	0.0	3.3	-	-	10.6 ± 0.1
	65.0	0.0	0.0	3.1	-	-	6.6 ± 0.0
	62.4	0.0	0.0	2.2	-	-	10.9 ± 0.2

Table 31f

Percentage of conidia of *Aspergillus clavatus* formed at different % R.H. able to survive at 100% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	Storage in Light					
		% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	8.1	36.1	-	3.3 ± 0.0	7.6 ± 0.2
	92.8	0.0	4.4	47.8	-	3.6 ± 0.1	8.6 ± 0.2
	85.2	0.0	47.8	61.1	-	3.3 ± 0.0	7.3 ± 0.1
	73.4	0.0	1.1	51.4	-	5.3 ± 0.2	7.6 ± 0.1
	65.0	0.0	0.8	62.5	-	4.0 ± 0.1	7.2 ± 0.1
	62.4	0.0	1.9	51.9	-	4.3 ± 0.2	6.0 ± 0.0
28	100	0.0	0.0	39.2	-	-	15.8 ± 0.2
	92.8	0.0	0.0	40.0	-	-	10.6 ± 0.2
	85.2	0.0	0.0	42.2	-	-	7.9 ± 0.1
	73.4	0.0	0.0	54.4	-	-	7.4 ± 0.1
	65.0	0.0	0.0	40.0	-	-	8.1 ± 0.2
	62.4	0.0	0.0	42.8	-	-	8.4 ± 0.1
56	100	0.0	0.0	18.9	-	-	6.6 ± 0.0
	92.8	0.0	0.0	23.3	-	-	7.6 ± 0.2
	85.2	0.0	0.0	44.7	-	-	6.9 ± 0.1
	73.4	0.0	0.0	35.6	-	-	6.3 ± 0.1
	65.0	0.0	0.0	48.9	-	-	6.6 ± 0.2
	62.4	0.0	0.0	36.4	-	-	5.6 ± 0.2

Table 32

Percentage survival (to the nearest whole number) of conidia of *Aspergillus clavatus* formed at different relative humidities and later stored at different relative humidities in light at 30°C for 56 days. (Data extracted from tables 31a, b,c,d, e and f) (Viability assessed by germinating the conidia on PDA for 12 hours)

Period of Storage (Days)	% R.H. of which conidia were formed	Percentage survival of conidia stored at indicated % R.H.					
		100	80	60	40	20	0
14	100	56	11	64	34	12	52
	92.8	66	13	67	30	8	68
	85.2	67	8	48	26	5	54
	73.4	76	19	28	34	4	62
	65.0	74	11	24	33	6	50
	62.4	91	10	30	32	4	56
28	100	36	11	49	20	10	39
	92.8	48	10	42	21	6	40
	85.2	61	5	48	16	5	42
	73.4	51	9	23	23	4	54
	65.0	63	9	18	18	6	40
	62.4	52	8	21	16	3	43
56	100	19	1	33	3	4	20
	92.8	23	2	23	10	2	23
	85.2	45	1	44	3	3	23
	73.4	36	2	8	3	1	27
	65.0	49	2	5	3	1	00
	62.4	36	1	12	2	1	00

ii. Survival of conidia of Aspergillus flavus.

The results of the test with A. flavus conidia are presented in Tables 33 and 34 and Fig 3.

(a) There was again no obvious indication that the humidities at which the conidia were formed influenced their subsequent survival at the different relative humidities of storage.

(b) The conidia survived shortest at 80.0% R.H. This is shown clearly in the summary Table of Table 34 and in Fig 3.

(c) The mean lengths of the germ tubes were generally directly related to percentage survival.

Table 33a

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at zero % R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	29.7	77.1	-	9.2 ± 0.3	23.4 ± 0.2
	92.8	0.0	53.7	78.1	-	13.9 ± 0.2	36.3 ± 0.2
	85.2	1.9	49.3	72.5	4.2 ± 0.2	12.3 ± 0.0	20.1 ± 0.4
	73.4	5.3	65.0	73.9	5.3 ± 0.3	34.3 ± 0.3	49.5 ± 0.3
	65.0	0.0	64.4	71.9	-	18.2 ± 0.2	41.9 ± 0.4
	62.4	0.0	17.1	93.8	-	13.2 ± 0.2	17.5 ± 0.3
28	100	0.0	0.0	40.6	-	-	6.6 ± 0.0
	92.8	0.0	0.0	48.0	-	-	6.9 ± 0.2
	85.2	0.0	0.3	40.6	-	5.0 ± 0.5	11.8 ± 0.3
	73.4	0.0	0.8	45.8	-	5.5 ± 0.2	7.9 ± 0.2
	65.0	0.0	0.0	51.1	-	-	4.7 ± 0.2
	62.4	0.0	0.8	43.6	-	4.4 ± 0.2	8.6 ± 0.4
56	100	0.0	0.0	27.8	-	-	6.3 ± 0.2
	92.8	0.0	0.0	33.1	-	-	5.3 ± 0.2
	85.2	0.0	0.3	18.6	-	4.7 ± 0.1	7.9 ± 0.2
	73.4	0.0	0.0	31.9	-	-	4.8 ± 0.2
	65.0	0.0	0.0	40.8	-	-	3.7 ± 0.1
	62.4	0.0	0.0	30.3	-	-	7.3 ± 0.1

Table 33b

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at 20% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	9.1	75.6	80.8	4.0 ± 0.0	22.8 ± 0.2	42.6 ± 0.2
	92.8	10.6	76.7	78.6	3.3 ± 0.3	27.7 ± 0.2	47.5 ± 0.2
	85.2	9.0	58.6	80.5	4.3 ± 0.2	3.5 ± 0.0	64.7 ± 0.2
	73.4	16.4	71.7	85.6	9.6 ± 0.2	27.4 ± 0.3	80.9 ± 0.2
	65.0	8.3	43.3	66.9	4.3 ± 0.2	9.9 ± 0.2	31.0 ± 0.3
	62.4	20.6	67.2	82.8	8.6 ± 0.2	20.8 ± 0.2	42.9 ± 0.3
28	100	0.0	0.0	54.7	-	-	25.3 ± 0.2
	92.8	0.0	0.6	58.3	-	8.3 ± 0.2	17.6 ± 0.2
	85.2	0.0	1.4	50.8	-	4.6 ± 0.5	18.6 ± 0.3
	73.4	0.0	4.4	59.7	-	40.0 ± 0.2	19.6 ± 0.2
	65.0	0.0	0.6	65.6	-	6.6 ± 0.0	16.9 ± 0.2
	62.4	0.0	0.8	53.6	-	6.0 ± 0.2	17.9 ± 0.2
56	100	0.0	2.2	46.9	-	5.0 ± 0.2	11.9 ± 0.2
	92.8	0.0	0.3	56.1	-	4.0 ± 0.1	9.6 ± 0.2
	85.2	0.0	0.5	43.9	-	4.0 ± 0.1	9.3 ± 0.3
	73.4	0.0	0.0	24.2	-	-	8.6 ± 0.2
	65.0	0.0	0.3	44.4	-	3.7 ± 0.1	8.3 ± 0.2
	62.4	0.0	0.5	43.5	-	4.1 ± 0.2	5.3 ± 0.2

Table 33c

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at 40% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	17.7	22.5	38.8	4.3 ± 0.2	12.2 ± 0.2	22.4 ± 0.3
	92.8	10.0	20.0	27.5	11.6 ± 1.0	11.9 ± 0.4	21.8 ± 0.3
	85.2	0.0	0.0	38.6	-	-	24.4 ± 0.4
	73.4	13.6	18.1	61.4	8.3 ± 0.2	13.5 ± 0.2	63.4 ± 0.4
	65.0	0.3	8.9	70.6	3.3 ± 0.0	6.6 ± 0.2	11.6 ± 0.3
	62.4	1.7	2.5	63.0	3.9 ± 0.2	6.9 ± 0.3	11.2 ± 0.2
28	100	0.0	0.0	11.1	-	-	5.6 ± 0.2
	92.8	0.0	1.1	25.8	-	7.4 ± 0.2	9.6 ± 0.2
	85.2	0.0	0.0	33.6	-	-	15.8 ± 0.4
	73.4	0.0	7.4	58.8	-	9.9 ± 0.2	13.2 ± 0.3
	65.0	0.0	0.0	51.4	-	-	6.6 ± 0.6
	62.4	0.0	0.0	59.1	-	-	9.2 ± 0.3
56	100	0.0	0.0	5.3	-	-	5.0 ± 0.2
	92.8	0.0	0.0	16.9	-	-	7.4 ± 0.2
	85.2	0.0	0.0	30.8	-	-	12.9 ± 0.2
	73.4	0.0	4.2	42.8	-	6.3 ± 0.2	9.2 ± 0.2
	65.0	0.0	0.0	46.7	-	-	5.3 ± 0.2
	62.4	0.0	0.0	55.8	-	-	8.3 ± 0.2

Table 33d

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at 60% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	8.6	10.8	23.3	7.9 ± 0.2	9.6 ± 0.2	22.1 ± 0.2
	92.8	15.3	18.3	19.7	5.9 ± 0.3	13.5 ± 0.3	17.5 ± 0.1
	85.2	14.7	17.5	29.4	3.6 ± 0.3	6.2 ± 0.2	36.3 ± 0.2
	73.4	11.9	15.3	27.2	10.8 ± 0.2	16.5 ± 0.5	55.8 ± 0.3
	65.0	0.0	6.4	14.7	-	7.6 ± 0.2	27.4 ± 0.2
	62.4	0.0	9.4	18.9	-	5.3 ± 0.2	19.8 ± 0.6
28	100	0.0	0.0	12.5	-	-	5.1 ± 0.2
	92.8	0.0	1.9	13.1	-	6.6 ± 0.2	6.1 ± 0.2
	85.2	0.0	6.1	18.3	-	16.5 ± 0.4	27.4 ± 0.3
	73.4	0.0	2.2	7.2	-	15.3 ± 0.2	36.3 ± 0.2
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	0.0	-	-	-
56	100	0.0	0.0	6.9	-	-	4.6 ± 0.2
	92.8	0.0	1.7	10.6	-	3.9 ± 0.3	7.9 ± 0.2
	85.2	0.0	4.4	12.5	-	7.3 ± 0.2	12.9 ± 0.2
	73.4	0.0	2.2	12.2	-	10.6 ± 0.2	13.9 ± 0.3
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	0.0	-	-	-

Table 33e

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at 80% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	6.7	36.1	-	7.9 ± 0.2	16.5 ± 0.1
	92.8	3.9	10.6	28.6	4.0 ± 1.0	5.0 ± 0.2	20.1 ± 0.4
	85.2	0.0	1.7	51.7	-	8.9 ± 0.3	34.1 ± 0.3
	73.4	4.4	10.0	20.3	4.6 ± 0.3	11.2 ± 0.2	15.2 ± 0.2
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	8.1	-	-	6.6 ± 0.3
28	100	0.0	0.0	10.8	-	-	5.5 ± 0.2
	92.8	0.0	0.0	16.7	-	-	5.0 ± 0.5
	85.2	0.0	1.1	24.9	-	7.4 ± 0.2	15.3 ± 0.2
	73.4	0.0	1.1	14.2	-	9.1 ± 0.3	13.2 ± 0.3
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	0.8	-	-	-
56	100	0.0	0.0	0.0	-	-	-
	92.8	0.0	0.0	0.0	-	-	-
	85.2	0.0	0.0	0.0	-	-	-
	73.4	0.0	0.0	0.0	-	-	-
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	0.0	-	-	-

Table 33f

Percentage of conidia of *Aspergillus flavus* formed at different % R.H. able to survive storage at 100% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (µm)		
		6	9	12	6	9	12
14	100	0.0	13.1	23.9	-	10.2 ± 0.2	23.4 ± 0.3
	92.8	1.9	6.4	52.8	3.8 ± 0.3	4.0 ± 0.3	16.8 ± 0.2
	85.2	2.2	16.1	18.1	4.6 ± 0.2	6.3 ± 0.3	9.6 ± 0.3
	73.4	11.1	18.6	53.6	8.3 ± 0.3	21.5 ± 0.1	37.0 ± 0.3
	65.0	20.0	42.2	76.7	8.5 ± 0.4	13.2 ± 0.3	28.1 ± 0.2
	62.4	1.1	8.9	35.0	4.1 ± 0.2	4.0 ± 0.3	14.5 ± 0.2
28	100	0.0	6.6	18.3	-	5.1 ± 0.2	12.2 ± 0.2
	92.8	0.0	2.8	16.4	-	4.0 ± 0.1	9.6 ± 0.3
	85.2	0.0	2.2	15.0	-	5.3 ± 0.2	6.6 ± 0.0
	73.4	0.6	1.4	22.8	3.3 ± 0.0	7.9 ± 0.2	15.3 ± 0.3
	65.0	0.3	1.7	21.9	3.3 ± 0.0	5.5 ± 0.2	16.5 ± 0.4
	62.4	0.0	0.6	14.4	-	5.0 ± 0.5	6.6 ± 0.0
56	100	0.0	4.4	15.8	-	4.7 ± 0.2	10.9 ± 0.2
	92.8	0.0	2.2	11.7	-	3.3 ± 0.0	7.9 ± 0.2
	85.2	0.0	1.1	13.1	-	5.0 ± 0.2	6.3 ± 0.2
	73.4	0.0	6.4	20.8	-	7.6 ± 0.2	13.9 ± 0.2
	65.0	0.0	3.6	18.9	-	4.6 ± 0.2	11.2 ± 0.3
	62.4	0.0	0.8	13.3	-	3.9 ± 0.2	5.3 ± 0.2

Table 34

Percentage survival (to the nearest whole number) of conidia of *Aspergillus flavus* formed at different relative humidities and later stored at different relative humidities in light at 30°C for 56 days. (Data extracted from tables 33a, b,c,d, e and f) Viability assessed by germinating the conidia on PDA for 12 hours)

Period of Storage (Days)	% R.H. at which conidia were formed	Percentage Survival of Conidia stored at indicated % R.H.					
		100	80	60	40	20	0
14	100	24	0	23	39	39	77
	92.8	53	0	20	28	28	78
	85.2	18	0	29	39	39	73
	73.4	54	0	27	61	86	74
	65.0	77	0	15	71	67	72
	62.4	35	0	19	63	83	94
28	100	18	0	13	11	55	41
	92.8	16	0	13	26	58	48
	85.2	15	0	18	34	51	41
	73.4	23	0	7	59	60	46
	65.0	22	0	0	51	66	51
	62.4	14	0	0	59	54	44
56	100	16	0	7	5	47	28
	92.8	12	0	10.6	17	56	33
	85.2	13	0	12.5	31	44	19
	73.4	21	0	12.2	43	24	32
	65.0	19	0	0	47	44	41
	62.4	13	0	0	56	44	30

iii. Survival of conidia of Aspergillus niger

The results of the test with A. niger conidia are shown in Tables 35, 36 and in Fig 3. The response of the conidia appear quite different from that of A. clavatus and A. flavus.

(a) Survival over 28 days was fairly high for most treatments.

(b) Conidia which were formed at the median humidities of 73.4 and 85.2% R.H. and stored at the different humidities on most occasions survived better than those formed at the humidities of 65.0, 62.4 and 100% R.H.

(c) The conidia survived best at 100% storage R.H. and percentage survival decreased with decrease in relative humidity.

(d) With a few exceptions, the higher the percentage survival, the longer the mean germ tube length.

Table 35a

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at zero % R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	0.0	58.8	-	-	21.1 ± 0.4
	92.8	0.0	14.6	58.6	-	10.9 ± 0.3	18.8 ± 0.3
	85.2	0.0	36.7	73.6	-	10.1 ± 0.2	30.4 ± 0.3
	73.4	0.0	23.6	71.4	-	11.7 ± 0.2	23.4 ± 0.2
	65.0	0.0	0.0	53.1	-	-	18.5 ± 0.3
	62.4	0.0	0.0	64.7	-	-	19.1 ± 0.5
28	100	0.0	0.0	19.7	-	-	13.2 ± 0.3
	92.8	0.0	5.6	53.6	-	4.6 ± 0.2	15.1 ± 0.3
	85.2	0.0	31.4	36.7	-	7.9 ± 0.2	12.2 ± 0.2
	73.4	0.0	20.0	30.0	-	4.0 ± 0.1	8.9 ± 0.3
	65.0	0.0	0.0	12.8	-	-	6.9 ± 0.1
	62.4	0.0	0.0	9.4	-	-	6.2 ± 0.3
56	100	0.0	0.0	6.9	-	-	3.3 ± 0.0
	92.8	0.0	0.0	37.2	-	-	8.6 ± 0.2
	85.2	0.0	5.8	30.6	-	6.9 ± 0.1	10.5 ± 0.2
	73.4	0.0	0.0	9.4	-	-	4.6 ± 0.2
	65.0	0.0	0.0	12.2	-	-	3.6 ± 0.2
	62.4	0.0	0.0	6.1	-	-	3.3 ± 0.0

Table 35b

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at 20% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	0.0	50.5	-	-	23.4 ± 0.2
	92.8	0.0	0.0	53.1	-	-	14.9 ± 0.3
	85.2	0.0	0.0	72.5	-	-	25.4 ± 0.3
	73.4	0.0	0.0	69.2	-	-	22.8 ± 0.4
	65.0	0.0	0.0	46.1	-	-	21.8 ± 0.3
	62.4	0.0	0.0	30.3	-	-	13.2 ± 0.2
28	100	0.0	0.0	47.8	-	-	14.2 ± 0.2
	92.8	0.0	0.0	42.7	-	-	17.5 ± 0.2
	85.2	0.0	0.0	55.8	-	-	22.8 ± 0.2
	73.4	0.0	0.0	59.7	-	-	17.5 ± 0.2
	65.0	0.0	0.0	35.2	-	-	7.9 ± 0.2
	62.4	0.0	0.0	19.2	-	-	15.1 ± 0.3
56	100	0.0	0.0	9.4	-	-	3.6 ± 0.1
	92.8	0.0	0.0	35.3	-	-	8.9 ± 0.2
	85.2	0.0	0.0	52.8	-	-	20.1 ± 0.2
	73.4	0.0	0.0	57.5	-	-	14.9 ± 0.2
	65.0	0.0	0.0	11.1	-	-	5.0 ± 0.2
	62.4	0.0	0.0	11.4	-	-	4.0 ± 0.1

Table 35c

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at 40% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E.(μm)		
		6	9	12	6	9	12
14	100	0.0	0.0	33.8	-	-	21.8 ± 0.3
	92.8	0.0	0.0	48.3	-	-	19.1 ± 0.5
	85.2	0.0	0.0	76.7	-	-	9.6 ± 0.2
	73.4	0.0	0.0	75.6	-	-	18.8 ± 0.3
	65.0	0.0	0.0	52.8	-	-	17.4 ± 0.4
	62.4	0.0	0.0	40.3	-	-	21.1 ± 0.4
28	100	0.0	0.0	23.6	-	-	8.6 ± 0.2
	92.8	0.0	0.0	33.6	-	-	15.4 ± 0.2
	85.2	0.0	0.0	74.7	-	-	16.9 ± 0.1
	73.4	0.0	0.0	68.9	-	-	11.2 ± 0.2
	65.0	0.0	0.0	45.0	-	-	9.2 ± 0.3
	62.4	0.0	0.0	34.4	-	-	17.6 ± 0.2
56	100	0.0	0.0	15.6	-	-	3.6 ± 0.1
	92.8	0.0	0.0	12.8	-	-	4.4 ± 0.2
	85.2	0.0	0.0	67.7	-	-	14.9 ± 0.2
	73.4	0.0	0.0	47.2	-	-	15.8 ± 0.1
	65.0	0.0	0.0	31.1	-	-	9.3 ± 0.1
	62.4	0.0	0.0	15.6	-	-	5.0 ± 0.2

Table 35d

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at 60% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	20.8	45.0	-	5.4 ± 0.2	7.3 ± 0.2
	92.8	1.4	32.2	77.4	4.0 ± 0.1	13.2 ± 02	30.7 ± 0.4
	85.2	1.9	47.5	73.4	3.3 ± 0.0	9.6 ± 0.3	25.4 ± 0.3
	73.4	1.7	23.9	80.0	4.2 ± 0.2	14.2 ± 0.2	29.4 ± 0.2
	65.0	0.0	19.4	51.4	-	4.6 ± 0.2	6.9 ± 0.2
	62.4	0.0	15.0	53.6	-	3.6 ± 0.1	5.9 ± 0.3
28	100	0.0	15.8	25.8	-	9.9 ± 0.3	9.9 ± 0.2
	92.8	0.0	26.9	33.3	-	5.3 ± 0.2	13.3 ± 0.3
	85.2	0.0	32.2	59.2	-	3.6 ± 0.1	9.9 ± 0.3
	73.4	0.0	23.1	73.1	-	7.2 ± 0.1	14.2 ± 0.3
	65.0	0.0	1.1	40.0	-	5.6 ± 0.2	11.9 ± 0.3
	62.4	0.0	1.7	34.0	-	5.4 ± 0.2	16.5 ± 0.4
56	100	0.0	1.7	11.4	-	3.3 ± 0.0	4.0 ± 0.1
	92.8	0.0	15.0	24.4	-	5.3 ± 0.2	6.9 ± 0.1
	85.2	0.0	16.7	51.7	-	6.9 ± 0.1	10.6 ± 0.1
	73.4	0.0	17.5	61.7	-	6.9 ± 0.1	13.2 ± 0.2
	65.0	0.0	0.0	31.4	-	-	10.9 ± 0.2
	62.4	0.0	0.0	19.4	-	-	7.3 ± 0.1

Table 35e

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at 80% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	1.1	60.3	75.3	4.1 ± 0.2	11.2 ± 0.2	23.8 ± 0.3
	92.8	2.2	51.4	83.9	3.7 ± 0.1	8.6 ± 0.2	19.8 ± 0.2
	85.2	4.2	50.6	78.3	4.0 ± 0.1	9.9 ± 0.2	28.7 ± 0.3
	73.4	3.0	60.0	94.7	3.3 ± 0.0	8.9 ± 0.2	22.8 ± 0.3
	65.0	2.2	41.4	85.0	4.7 ± 0.1	17.5 ± 0.2	29.7 ± 0.4
	62.4	0.5	42.8	83.3	3.3 ± 0.0	7.5 ± 0.2	18.8 ± 0.3
28	100	0.0	19.4	57.9	-	4.6 ± 0.2	15.8 ± 0.3
	92.8	0.0	32.2	46.4	-	6.6 ± 0.2	12.5 ± 0.2
	85.2	0.0	45.8	63.9	-	11.2 ± 0.2	20.5 ± 0.2
	73.4	0.0	50.6	75.6	-	9.6 ± 0.2	14.9 ± 0.2
	65.0	0.0	18.3	43.3	-	5.3 ± 0.2	10.6 ± 0.2
	62.4	0.0	20.8	36.7	-	5.0 ± 0.2	14.9 ± 0.2
56	100	0.0	9.7	30.6	-	3.3 ± 0.0	3.6 ± 0.1
	92.8	0.0	16.7	41.1	-	5.6 ± 0.2	8.6 ± 0.2
	85.2	0.0	28.9	51.9	-	7.3 ± 0.1	12.5 ± 0.2
	73.4	0.0	33.3	70.3	-	4.0 ± 0.1	7.6 ± 0.2
	65.0	0.0	8.6	30.6	-	5.3 ± 0.2	7.9 ± 0.2
	62.4	0.0	11.1	30.8	-	4.0 ± 0.1	5.3 ± 0.2

Table 35f

Percentage of conidia of *Aspergillus niger* formed at different % R.H. able to survive storage at 100% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	49.7	68.3	-	4.6 ± 0.2	11.7 ± 0.2
	92.8	0.0	30.3	60.0	-	3.3 ± 0.0	9.6 ± 0.2
	85.2	0.0	56.4	74.7	-	9.6 ± 0.3	24.1 ± 0.2
	73.4	0.0	37.8	77.8	-	5.0 ± 0.2	12.9 ± 0.2
	65.0	0.0	35.8	56.4	-	5.6 ± 0.2	10.2 ± 0.2
	62.4	0.0	22.2	69.4	-	8.6 ± 0.2	17.5 ± 0.2
28	100	0.0	46.9	58.9	-	9.6 ± 0.3	12.8 ± 0.3
	92.8	0.0	28.6	58.1	-	5.6 ± 0.2	13.9 ± 0.3
	85.2	0.0	48.6	60.0	-	8.6 ± 0.2	13.6 ± 0.2
	73.4	0.0	30.3	62.8	-	5.7 ± 0.2	9.9 ± 0.2
	65.0	0.0	31.4	49.7	-	3.3 ± 0.0	8.6 ± 0.2
	62.4	0.0	17.2	51.4	-	3.3 ± 0.0	9.9 ± 0.2
56	100	0.0	35.8	56.9	-	3.3 ± 0.0	4.0 ± 0.1
	92.8	0.0	14.2	53.1	-	4.0 ± 0.1	4.8 ± 0.2
	85.2	0.0	28.6	45.0	-	4.4 ± 0.2	7.6 ± 0.2
	73.4	0.0	17.8	53.1	-	3.3 ± 0.0	3.6 ± 0.1
	65.0	0.0	18.3	45.0	-	3.6 ± 0.1	7.9 ± 0.2
	62.4	0.0	11.9	44.2	-	5.6 ± 0.2	8.9 ± 0.2

Table 36

Percentage survival (to the nearest whole number) of conidia of *Aspergillus niger* formed at different relative humidities and later stored at different relative humidities in light at 30°C for 56 days. (Data extracted from tables 35a, b,c,d, e and f) (Viability assessed by germinating the conidia on PDA for 12 hours)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	Percentage Survival of Conidia stored at indicated % R.H.					
		100	80	60	40	20	0
14	100	68	84	45	34	51	50
	92.8	60	78	77	48	53	59
	85.2	75	95	73	77	73	74
	73.4	78	85	80	76	69	71
	65.0	69	83	51	53	46	53
	62.4	97	94	54	40	30	64
28	100	59	58	26	24	48	20
	92.8	58	46	33	34	42	54
	85.2	60	64	59	75	56	37
	73.4	63	76	73	69	60	30
	65.0	50	43	40	45	35	13
	62.4	51	37	34	34	19	9
56	100	57	31	11	16	9	7
	92.8	53	41	24	13	35	37
	85.2	45	52	52	68	53	31
	73.4	53	70	62	47	58	9
	65.0	45	31	31	31	11	12
	62.4	44	31	19	16	11	6

iv. Survival of conidia of A. tamarii

The results of the test with A. tamarii conidia are presented in Tables 37, 38 and in Fig 3.

(a) Conidia which were formed at 65.0 to 85.2% R.H. and subsequently stored at 0 to 100% R.H. seemingly survived better than those formed at the remaining humidities of incubation of the cultures.

(b) The conidia survived better at the lowest humidities and lost viability more rapidly at highest humidities of 80.0 and 100% R.H.

(c) Again the lengths of the germ tubes reflected the percentage survival. The graph in Fig 3 which was drawn with data obtained after 28 days of storage, illustrates the survival-relative humidity relationship showed by the four species.

Table 37a

Percentage of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at zero % R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	0.0	100	100	-	58.4 ± 1.7	63.4 ± 0.2
	92.8	0.0	91.6	96.8	-	39.9 ± 0.2	53.1 ± 0.2
	85.2	0.0	100	100	-	63.4 ± 0.3	75.6 ± 0.3
	73.4	0.0	100	100	-	82.1 ± 0.2	91.7 ± 0.3
	65.0	0.0	100	100	-	98.7 ± 0.3	10.6 ± 0.3
	62.4	0.0	100	100	-	10.4 ± 0.2	11.6 ± 0.3
28	100	0.0	43.3	79.7	-	20.1 ± 0.2	40.6 ± 0.3
	92.8	0.0	32.5	64.2	-	13.9 ± 0.3	28.4 ± 0.3
	85.2	0.0	79.4	90.0	-	12.2 ± 0.2	24.0 ± 0.3
	73.4	0.0	41.7	82.0	-	17.2 ± 0.4	24.1 ± 0.4
	65.0	0.0	40.6	83.4	-	11.9 ± 0.2	41.3 ± 0.3
	62.4	0.0	48.3	61.1	-	24.1 ± 0.4	47.2 ± 0.4
56	100	0.0	9.4	66.1	-	8.9 ± 0.2	25.7 ± 0.3
	92.8	0.0	5.3	59.2	-	13.2 ± 0.3	22.1 ± 0.2
	85.2	0.0	22.8	75.8	-	5.0 ± 0.2	12.2 ± 0.2
	73.4	0.0	21.9	75.6	-	5.9 ± 0.1	20.1 ± 0.2
	65.0	0.0	16.7	64.7	-	8.3 ± 0.2	25.4 ± 0.2
	62.4	0.0	9.2	47.2	-	7.8 ± 0.2	35.0 ± 0.4

Table 37b

Longevity of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at 20% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (mm)		
		6	9	12	6	9	12
14	100	12.3	86.0	90.3	7.8 ± 0.2	25.6 ± 0.2	28.5 ± 0.2
	92.8	1.4	58.2	66.7	8.6 ± 0.2	14.5 ± 0.2	26.4 ± 0.3
	85.2	15.3	94.2	97.8	6.3 ± 0.2	37.9 ± 0.2	58.4 ± 0.3
	73.4	26.4	91.4	94.6	11.2 ± 0.2	34.7 ± 0.3	46.6 ± 0.2
	65.0	20.3	94.6	97.9	11.9 ± 0.2	23.1 ± 0.2	31.0 ± 0.3
	62.4	22.8	99.0	99.0	8.9 ± 0.1	28.7 ± 0.2	40.3 ± 0.3
28	100	0.8	26.4	60.3	6.6 ± 0.3	22.1 ± 0.2	26.5 ± 0.3
	92.8	0.3	20.6	42.5	6.8 ± 0.2	14.6 ± 0.2	27.5 ± 0.2
	85.2	4.0	22.5	91.0	5.6 ± 0.3	23.7 ± 0.3	40.4 ± 0.3
	73.4	0.6	20.0	60.0	5.0 ± 0.2	6.3 ± 0.2	20.8 ± 0.2
	65.0	0.2	28.9	76.7	6.0 ± 0.1	9.3 ± 0.2	30.0 ± 0.3
	62.4	1.1	37.2	92.2	6.6 ± 0.4	9.2 ± 0.2	37.0 ± 0.3
56	100	0.3	7.5	21.1	4.6 ± 0.2	14.9 ± 0.2	24.8 ± 0.2
	92.8	0.0	0.0	18.6	-	-	-
	85.2	0.8	22.2	61.4	5.0 ± 0.2	8.3 ± 0.2	8.3 ± 0.2
	73.4	0.3	5.8	35.5	3.3 ± 0.0	5.9 ± 0.2	5.9 ± 0.2
	65.0	0.0	7.2	29.7	5.0 ± 0.2	7.3 ± 0.2	7.3 ± 0.2
	62.4	0.6	12.5	74.4	5.9 ± 0.2	7.9 ± 0.1	21.5 ± 0.1

Table 37c

Percentage of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at 40% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% of R.H. of Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	78.6	83.9	92.6	5.9 ± 0.2	12.2 ± 1.3	21.1 ± 0.3
	92.8	16.3	91.5	95.7	8.9 ± 0.2	20.8 ± 0.3	30.1 ± 0.3
	85.2	89.8	96.8	98.9	14.9 ± 0.2	48.8 ± 0.3	65.3 ± 0.3
	73.4	72.0	91.4	96.9	20.5 ± 0.1	34.7 ± 0.3	53.5 ± 0.3
	65.0	77.4	98.9	100	19.5 ± 0.3	99.7 ± 0.4	10.5 ± 0.4
	62.4	46.2	98.8	100	45.9 ± 0.3	45.9 ± 0.3	57.1 ± 0.2
28	100	3.9	62.5	87.9	5.7 ± 0.2	6.5 ± 0.2	18.8 ± 0.3
	92.8	0.8	23.5	48.1	3.3 ± 0.0	20.7 ± 0.2	41.3 ± 0.4
	85.2	5.6	45.6	55.6	8.6 ± 0.2	19.1 ± 0.4	29.0 ± 0.4
	73.4	2.5	93.1	93.3	9.9 ± 0.2	31.7 ± 0.3	47.4 ± 0.3
	65.0	11.7	68.1	88.6	7.6 ± 0.2	47.9 ± 0.3	62.3 ± 0.4
	62.4	23.9	92.3	97.2	5.9 ± 0.2	31.0 ± 0.3	50.2 ± 0.3
56	100	1.9	46.7	74.7	3.3 ± 0.0	9.9 ± 0.2	14.9 ± 0.2
	92.8	0.0	8.3	31.4	17.2± 0.2	16.8 ± 0.3	28.1 ± 0.3
	85.2	1.4	20.3	46.0	4.6 ± 0.2	11.2 ± 0.3	4.9 ± 0.2
	73.4	1.9	44.7	70.5	4.2 ± 0.2	12.5 ± 0.3	19.8 ± 0.4
	65.0	28.6	41.7	84.7	6.6 ± 0.2	23.1 ± 0.3	38.6 ± 0.4
	62.4	29.4	55.6	82.8	5.0 ± 0.2	17.2 ± 0.2	28.7 ± 0.3

Table 37d

Percentage of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at 60% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	Storage in Light					
		% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (μm)		
		6	9	12	6	9	12
14	100	83.5	96.8	98.9	7.3 ± 0.1	39.9 ± 0.3	49.2 ± 0.3
	92.8	18.3	55.4	80.4	6.9 ± 0.1	15.1 ± 0.2	28.7 ± 0.3
	85.2	88.5	97.9	98.0	17.8 ± 0.2	34.0 ± 0.3	48.5 ± 0.3
	73.4	85.1	97.7	98.7	18.2 ± 0.2	47.5 ± 0.3	59.5 ± 0.3
	65.0	93.7	100	100	34.3 ± 0.2	67.2 ± 0.2	81.5 ± 0.5
	62.4	97.9	100	100	26.4 ± 0.2	10.7 ± 0.3	12.4 ± 0.4
28	100	0.3	40.0	63.6	5.0 ± 0.2	15.2 ± 0.2	22.8 ± 0.4
	92.8	0.3	4.2	39.7	3.3 ± 0.2	18.5 ± 0.2	28.4 ± 0.5
	85.2	1.1	75.6	81.1	5.5 ± 0.2	32.7 ± 0.3	47.5 ± 0.3
	73.4	0.3	56.7	63.3	3.3 ± 0.0	22.8 ± 0.2	38.6 ± 0.3
	65.0	0.3	83.3	88.9	3.3 ± 0.0	68.0 ± 0.3	86.1 ± 0.2
	62.4	0.3	49.7	59.4	3.3 ± 0.0	31.7 ± 0.2	38.9 ± 0.3
56	100	0.0	52.2	52.5	-	7.3 ± 0.2	10.6 ± 0.2
	92.8	0.0	10.8	46.9	-	8.6 ± 0.2	17.2 ± 0.3
	85.2	0.0	25.3	53.1	-	8.4 ± 0.2	25.4 ± 0.3
	73.4	0.0	20.0	47.8	-	10.9 ± 0.2	28.1 ± 0.3
	65.0	0.0	30.8	68.3	-	20.1 ± 0.2	40.4 ± 0.4
	62.4	0.0	22.2	42.2	-	8.5 ± 0.2	24.8 ± 0.3

Table 37e

Percentage of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at 80% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. of Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E (µm)		
		6	9	12	6	9	12
14	100	19.1	38.7	85.6	5.3 ± 0.2	13.9 ± 0.2	35.3 ± 0.2
	92.8	0.0	66.6	79.7	-	14.0 ± 0.2	29.9 ± 0.2
	85.2	24.7	79.6	94.7	11.9 ± 0.2	21.5 ± 0.2	39.6 ± 0.2
	73.4	46.9	64.9	83.9	13.5 ± 0.2	19.1 ± 0.2	30.7 ± 0.2
	65.0	84.6	86.0	86.6	7.6 ± 0.2	8.9 ± 0.2	18.2 ± 0.2
	62.4	46.2	76.6	93.7	4.3 ± 0.2	18.5 ± 0.2	30.1 ± 0.2
28	100	0.0	8.1	8.1	-	6.9 ± 0.2	14.2 ± 0.2
	92.8	0.0	0.3	4.2	-	9.9 ± 0.2	14.9 ± 0.2
	85.2	0.0	16.7	61.7	-	11.6 ± 0.2	16.2 ± 0.2
	73.4	0.0	16.0	69.1	-	17.5 ± 0.3	24.2 ± 0.3
	65.0	0.0	26.7	76.4	-	20.8 ± 0.3	44.9 ± 0.3
	62.4	0.0	24.2	50.8	-	20.1 ± 0.3	24.3 ± 0.3
56	100	0.0	1.7	2.8	-	3.6 ± 0.2	10.9 ± 0.3
	92.8	0.0	0.0	0.0	-	-	-
	85.2	0.0	2.2	19.2	-	5.4 ± 0.2	11.2 ± 0.2
	73.4	0.0	1.7	24.7	-	6.6 ± 0.2	10.2 ± 0.3
	65.0	0.0	2.5	11.4	-	10.6 ± 0.2	26.9 ± 0.3
	62.4	0.0	0.6	11.1	-	5.0 ± 0.2	21.1 ± 0.3

Table 37f

Percentage of conidia of *Aspergillus tamarii* formed at different % R.H. able to survive at 100% R.H. at 30°C in light. (Viability assessed by germinating the conidia on PDA)

Period of Storage (Days)	Source of conidia (% R.H. Culture)	% Germination after indicated hours of Incubation			Mean Germ Tube Length after indicated hours of Incubation ± S.E. (µm)		
		6	9	12	6	9	12
14	100	0.0	4.3	84.3	-	24.3 ± 0.3	57.6 ± 0.3
	92.8	0.0	7.4	89.7	-	17.9 ± 0.3	42.2 ± 0.3
	85.2	55.9	83.9	97.8	7.6 ± 0.2	27.5 ± 0.3	59.9 ± 0.3
	73.4	80.6	90.1	93.7	4.0 ± 0.1	10.6 ± 0.3	63.4 ± 0.3
	65.0	68.8	84.9	98.9	18.2 ± 0.2	15.1 ± 0.2	60.4 ± 0.2
	62.4	83.3	89.0	97.3	5.0 ± 0.2	8.9 ± 0.3	37.6 ± 0.3
28	100	0.0	0.0	12.5	-	-	55.4 ± 0.5
	92.8	0.0	0.0	1.0	-	-	4.0 ± 0.1
	85.2	0.0	27.8	10.2	-	14.9 ± 0.2	35.6 ± 0.4
	73.4	0.0	0.0	6.3	-	-	56.1 ± 0.0
	65.0	0.0	0.0	1.7	-	-	51.4 ± 0.3
	62.4	0.0	0.0	0.8	-	-	26.4 ± 0.3
56	100	0.0	0.0	1.1	-	-	15.8 ± 0.3
	92.8	0.0	0.0	0.0	-	-	-
	85.2	0.0	0.0	5.6	-	-	7.6 ± 0.2
	73.4	0.0	0.0	0.0	-	-	-
	65.0	0.0	0.0	0.0	-	-	-
	62.4	0.0	0.0	0.8	-		-

Table 38

Percentage survival (to the nearest whole number) of conidia of *Aspergillus tamarii* formed at different relative humidities and later stored at different relative humidities in light at 30°C in light at 56 days. (Data extracted from Tables 37a,b,c,d,e and f) (Viability assessed by germinating the conidia on PDA for 12 hours)

Period of Storage (Days)	% R.H. at which conidia were formed	Percentage survival of conidia stored at indicated % R.H.					
		100	80	60	40	20	0
14	100	84	86	99	93	90	100
	92.8	90	80	80	96	67	97
	85.2	98	95	98	99	98	100
	73.4	94	84	99	97	95	100
	65.0	99	87	100	100	98	100
	62.4	97	94	100	100	99	100
28	100	13	8	64	88	60	80
	92.8	1	4	40	48	43	64
	85.2	10	62	81	56	91	90
	73.4	6	69	63	93	60	82
	65.0	2	76	89	89	77	83
	62.4	1	51	97	89	92	61
56	100	1	3	53	75	21	66
	92.8	0	0	47	31	19	59
	85.2	6	19	53	46	61	76
	73.4	0	25	48	71	36	76
	65.0	0	11	68	85	30	65
	62.4	0	11	42	83	74	47

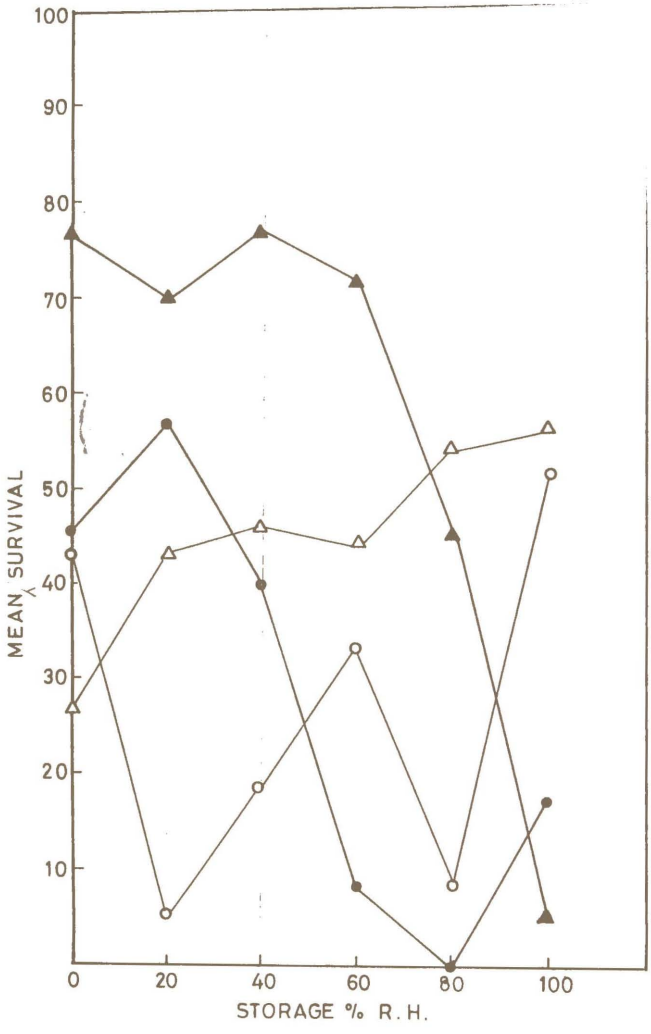

FIG. 3 SURVIVAL PATTERN OF CONIDIA OF A. CLAVATUS (o——o), A. FLAVUS (●——●), A. NIGER (△——△) AND A. TAMARII (▲——▲) STORED AT DIFFERENT RELATIVE HUMIDITIES AT 30 °C FOR 28 DAYS.

I. ABILITY OF CONIDIA SWOLLEN IN NUTRIENT BROTH TO WITHSTAND SUBSEQUENT DESICCATION

Germination of fungal spores is characterised by the absorption of water (Strobel, 1965), and by increases in respiration and the biosynthesis of many cell components (Gottlieb, 1960). Morphological changes also occur at this time; the spore generally swells (Mandels and Derby 1953) and forms a germ tube and eventually a mature thallus.

The individual conidia of the Aspergillus species were observed to swell ominously in the germination tests already described. In this experiment the rates of swelling of the conidia of the different species were closely followed. The conidia were germinated at 30°C in PDB and percentage of spores which were swollen were recorded at one hour intervals.

The observation was carried out over a total of six hours and estimation of percentage of spores swollen was discontinued in any sample which started to produce germ tubes within this period. The rate of spores swelling of the conidia of A. clavatus, A. flavus, A. niger and A. tamarii are shown in Table 39.

The conidia of A. clavatus, and A. flavus started to show germ tubes on the fifth hour of incubation. Germ tubes had not emerged from the conidia of A. niger and A. tamarii at the time the observation was stopped, that is, after six hours of incubation.

Conidia of A. clavatus, A. flavus and A. tamarii showed noticeable swelling after only three hours of incubation. Swelling proceeded faster in A. clavatus and A. tamarii after that than in A. flavus. At the fourth hour the percentage of spores swollen in A. clavatus, A. flavus and A. tamarii was 88.6, 77.4 and 95.8 per cent respectively.

A. niger showed a slower rate of germination. The first swollen spores were observed after two hours compared to the other three but at four hours only 32.2 per cent of spores were swollen.

Having then established the possible maximum time of incubation of the conidia of the four species in Potato Dextrose Broth to induce the possible maximum percentage of the conidia that would swell, the subsequent test was carried out to find the ability of the swollen spores to stand desiccation.

Conidia of A. clavatus and A. flavus were immersed in Potato Dextrose Broth for four hours. A. niger and A. tamarii were suspended in Potato Dextrose Broth for six hours. Samples of the spore suspensions of each species were dried for 1, 2, 4 and 6 hours and then resuspended in Potato Dextrose Broth. The conidia were incubated at 30°C and percentage of conidia germinated after six hours was determined. The mean germ tube lengths were also calculated. The results are presented in Tables 39, 40, 41, 42 and 43.

The conidia showed different degrees of ability to withstand desiccation. The results could be summarised as follows:

(a) Conidia of A. niger which had swollen were killed by desiccation of only one hour (see Table 42).

(b) Swollen conidia of A. clavatus, A. flavus and A. tamarii were able to stand desiccation for four hours.

(c) The percentage germination after six hours could be used to compare the ability of the different species to withstand desiccation. A. flavus conidia survived best and swollen conidia desiccated for four hours showed 22.2 per cent germination (See Table 41) and under the same conditions, conidia of A. clavatus and A. tamarii showed only 8.0 and 4.8 per cent viability, respectively (see Tables 40 and 43).

Figures 4 to 7 are camara lucida drawings of conidia of A. clavatus, A. flavus, A. niger and A. tamarii showing extend of swelling of spores after different hours of incubation in Potato Dextrose Broth.

Table 39

Rate of swelling of conidia of four *Aspergillus* species of placed in Potato Dextrose Broth at 30°C (Each value of percentage of conidia swollen based on 600-800 conidia)

Time of incubation (hours)	Aspergillus species			
	A. flavatus	A. flavus	A. niger	A. tamarii
1	29.8	25.9	0.0	49.3
2	71.1	50.6	2.8	69.5
3	79.4	53.6	10.7	84.4
4	88.6	77.4	32.2	95.8
5	-*	-*	58.2	98.1
6	-	-	68.7	98.4

*Observation discontinued, because of emergence of germ tubes.

Table 40

Percentage of conidia of *Aspergillus Clavatus* able to germinate in Potato Dextrose Broth at 30°C after they had been made to swell over 4 hours in Potato Dextrose Broth and then air-dried. (Percentage germination of each treatment based on 500-700 observed conidia)

Time of drying conidial sample containing 79.7% swollen spores (hours)	Time of germination in PDB (hours)			
	3		6	
	% Germination	Mean germ tube length (μm)	% Germination	Mean germ tube length (μm)
0	45.0	4.6 ± 0.2	92.2	24.8 ± 0.3
1	0.2	50.6	41.8	15.2 ± 0.2
2	0.0	53.6	22.5	12.2 ± 0.2
4	0.0	77.4	8.0	4.3 ± 0.2
6	0.0		0.0	-

Table 41

Percentage of conidia of *Aspergillus flavus* able to germinate in Potato Dextrose Broth at 30°C after they had been made to swell over 4 hours in Potato Dextrose Broth and then air-dried. (Percentage germination of each treatment based on 500-700 observed conidia)

Time of drying conidial sample containing 76.5% swollen spores (hours)	Time of germination in PDB (hours)			
	3		6	
	% Germination	Mean germ tube length (μm)	% Germination	Mean germ tube length (μm)
0	63.0	25.4 ± 0.2	89.4	51.5 ± 0.2
1	58.0	24.1 ± 0.2	83.0	47.4 ± 0.2
2	10.3	13.2 ± 0.2	54.0	36.3 ± 0.3
4	4.0	73 ± 0.1	22.2	27.1 ± 0.2
6	0.0	-	0.0	-

Table 42

Percentage of conidia of *Aspergillus niger* able to germinate in potato Dextrose Broth at 30°C after they had been made to swell over 6 hours in Potato Dextrose Broth and then air-dried. (Percentage germination of each treatment based on 500-700 observed conidia)

Time of drying conidial sample containing 69.3% swollen spores (hours)	Time of germination in PDB (hours)			
	3		6	
	% Germination	Mean germ tube length (μm)	% Germination	Mean germ tube length (μm)
0	0.0	-	70.8	5.0 ± 0.2
1	0.0	-	0.0	-
2	0.0	-	0.0	-
4	0.0	-	0.0	-
6	0.0	-	0.0	-

Table 43

Percentage of Conidia of *Aspergillus tamarii* able to germinate in Potato Dextrose Broth at 30°C after they had been made to swell over 6 hours in Potato Dextrose Broth and then air-dried. (Percentage germination of each treatment based on 500-700 observed conidia)

Time of drying conidial sample containing 69.3% swollen spores (hours)	Time of germination in PDB (hours)			
	3		6	
	% Germination	Mean germ tube length (μm)	% Germination	Mean germ tube length (μm)
0	0.0	-	96.2	28.7 ± 0.2
1	0.0	-	29.8	12.2 ± 0.2
2	0.0	-	14.0	8.6 ± 0.2
4	0.0	-	4.8	3.3 ± 0.0
6	0.0	-	0.0	-

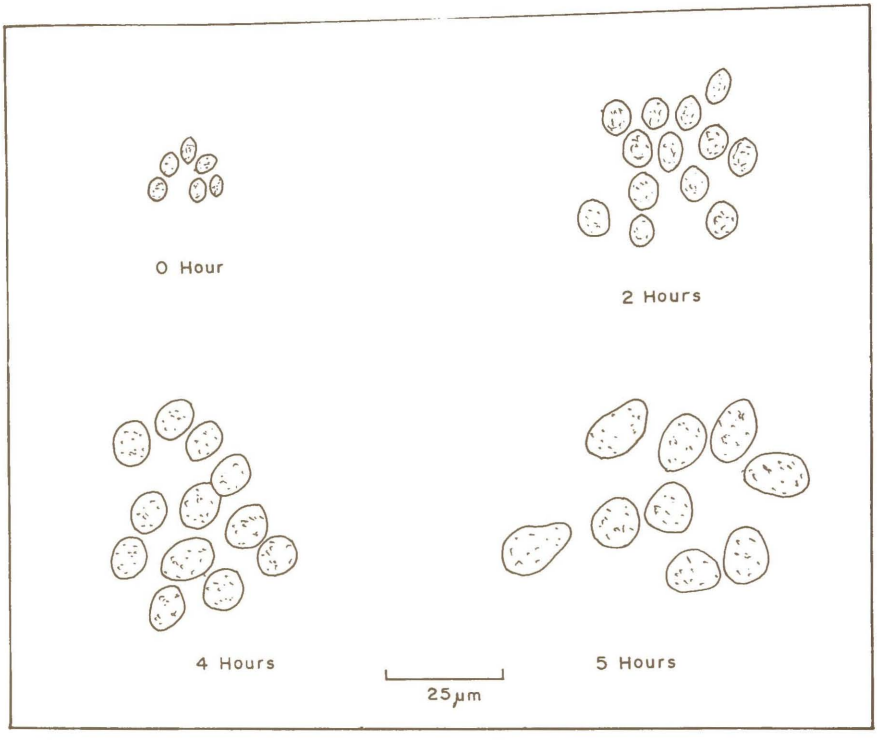

Fig. 4 Camera Lucida drawings of Conidia of Aspergillus clavatus incubated in Potato Dextrose Broth at 30°C for the indicated hours.

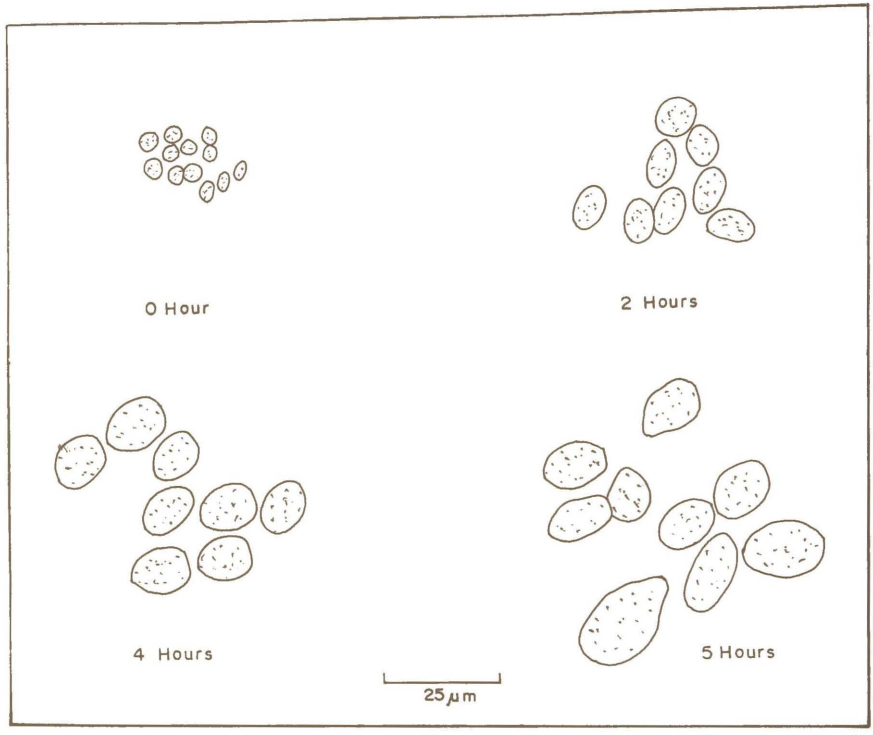

Fig. 5 Camera Lucida Drawings of Conidia of Aspergillus Flavus incubated in Potato Dextrose Broth at 30°C for the indicated hours.

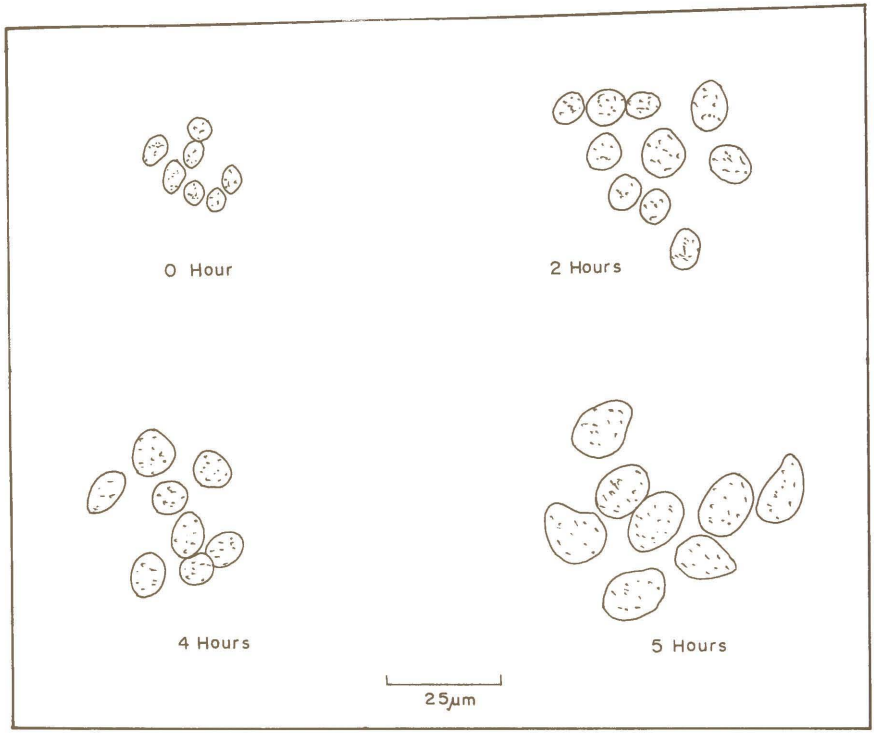

Fig. 6 Camera Lucida drawings of Conidia of Aspergillus niger incubated in Potato Dextrose Broth at 30°C for the indicated hours.

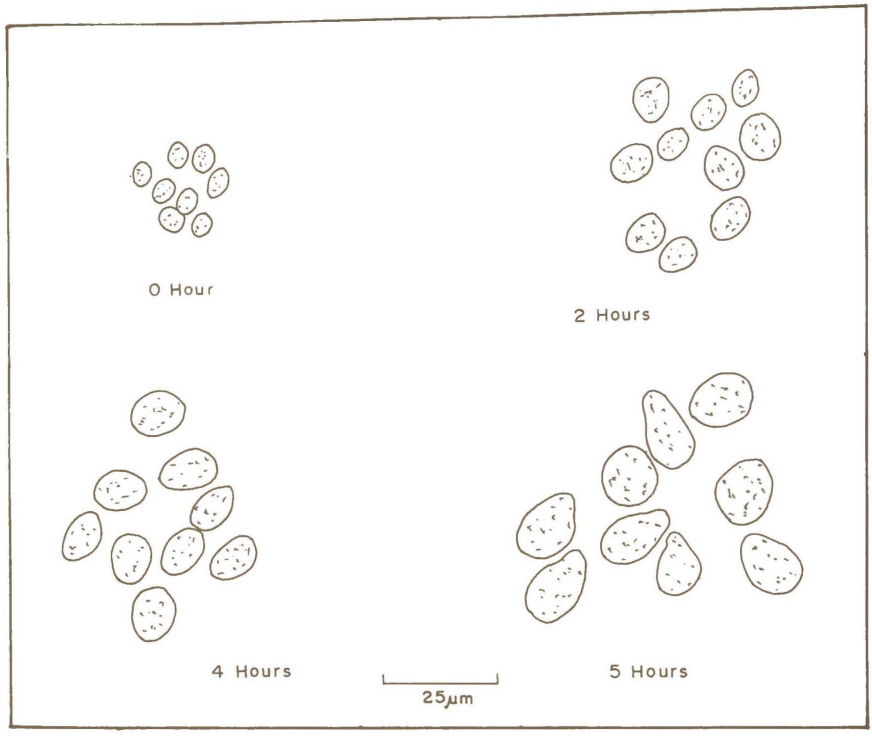

Fig. 7 Camera Lucida drawings of Conidia of Aspergillus tamarii incubated in Potato Dextrose Broth at 30°C for the indicated hours.

V. DISCUSSION

Maize, as a major staple food in Ghana, is grown extensively in the country. Cultivation in recent times has even been extended to the drier Sudan savanna regions in the north with the introduction of irrigation to those areas. The annual yield has consequently increased substantially in recent years. The excess that remains after the cropping season is partly stored traditionally on-the-cob in cribs and partly as shelled grains packed in propylene or jute bags and stacked in warehouses and market stores.

Storage of the grains and seeds is, however, beset with many problems. The most important are predation by rodents and insect pests and decay by fungi. Fungal contamination is, infact, manifested in many ways apart from grain decay. The texture of the grains changes, the grains are discoloured, the grains develop unpleasant odours, preparations made with the grains have an off-taste and they may be contaminated by toxins synthesized by the fungi. Where the contaminant fungus does not destroy the seed it would later destroy the seedling through seedling blight.

Respiration of large bulks of grains in storage inevitably raise the ambient temperature and humidity which encourage the development of the contaminant fungi (Christensen, 1957). The multiple effects of the contaminant fungi of maize grains and seeds in storage justify the intensive attention they have received in numerous studies.

Many of the studies on fungal contaminants of maize grains involved initially a study of the components of the mycoflora (e.g. Broadbent, 1967a and b; Odamtten, 1986; Oyeniran, 1973). Some of the mycofloral studies have been related to such environmental factor as, moisture content of the seeds and grain (Christensen, and Kaufmann, 1969), atmospheric humidity of store houses (FAO Report, 1970) and temperature of store houses (Christensen, 1970).

A new factor concerning stored maize grains which has emerged recently is what has been termed "stackburn". It is the discoloration of maize grains packed in propylene bags and stacked in the warehouses to various shades of brown. The first case of stackburn was reported from Zimbabwe (Anon, 1995), followed by a report in 1995 (Anon, 1995) of the incidence of stackburn in Ghana. Studies in progress in Zimbabwe (Sarah Phillips, personal communication) and the studies made during this investigation provide the first information on the mycoflora of stackburn maize grains.

This thesis contains in addition results of studies carried out on the survival of some of the species of Aspergillus which were isolated from the grains to provide further knowledge on survival of fungi, in general. The investigation sought, in particular, the relationship between the humidity at which conidia of Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii were formed and the longevity of these conidia stored later at different relative humidities. Very little attention has been given to that aspect of physiology of fungi.

In the isolation of the contaminant fungi of the normal-looking and stackburn maize grains, the serial dilution method according to Lacey, Hill and Edwards (1980), and two varieties of maize - white and yellow grains were used. From the normal looking white maize grains 26 fungal species belonging to 12 genera were isolated. At the same time, 16 fungal species belonging to 9 genera were isolated from the stackburn white variety grains. On the other hand, 32 fungal species belonging to 14 genera were encountered in the normal-looking yellow grains and 19 species belonging to 6 genera were isolated from the stackburn yellow grains (see Tables 3 to 4). In each instance a lesser number of fungal species occurred in the stackburn grains which were stored under the same conditions as the normal grains.

This could be the consequence of some changes that might have taken place in the stackburn grains. Studies on the chemical composition of the two types of grains which would be carried out later during this project would provide an explanation for the differences in the levels of contamination recorded here.

Differences were also observed when the percentage frequency of the dominant species were compared. The Aspergillus species which were the predominant contaminants were more abundant in the normal grains. Thus, they constituted 68.8 and 60.3 per cent, respectively, of the flora of the normal grains from the Kaneshie and Tema Warehouses of the Food Distribution Corporation, while the corresponding percentage frequency in the stackburn grains were 34.0 and 47.9 per cent, respectively. The loss suffered by the incidence of stackburn is somewhat compensated for by the inability of stackburn grains to support as much infection as the normal grains and, therefore, becoming less suitable as a reservoir of fungal inoculum for further spread of the contaminating fungi. This advantage will obviously be lost if stackburn incidence is extensive. A reasonable conclusion from these results is that since stackburn grains harboured fewer fungi, fungi are not likely to have caused the stackburn phenomenon.

Although the grains used in this study and those used by Odamtten (1986) were stored under different conditions the results of the two studies were quite similar. The grains of the present study were packed in propylene bags while those studied by Odamtten (1986) were stored in jute bags. Since the jute material is more porous than propylene, aeration would be different in the two types of bags. This would, consequently, affect both the temperature and humidity levels in the jute and propylene bags. Nonetheless, Odamtten (1986) recorded 26 fungal species belonging to 12 genera, white maize grains without stackburn - the same figures obtained in this work. The lists of fungal species of the two were, however, not identical. Another significant difference was that whereas count of Aspergillus flavus was consistently highest over the period of six months of storage in the work of Odamtten, it was also the predominant species initially in the present work but declined by the end of the six-month storage period. The results of Danquah (1973) are in rather sharp contrast. First, he isolated only one Aspergillus species and, secondly, the percentage occurrence of Fusarium and Penicillium species was higher than the Aspergillus species. It is not unusual for such differences to occur since conditions of storage of grains and seeds vary and different methods of isolation and media are used by different investigators.

The 'Blotter test' method used by Danquah (1973) would evidently favour the growth of fungi in the outer tissues of the maize grains and the absence of a nutrient medium was to the disadvantage of some species. There were other significant differences. The list of Danquah (1973) alone contained Alternaria zinniae, Botryodiopliodia theobromae, Dreschlera sorokiniana, Macrophomina sp., Melanospora sp., Nigrospora sp. and Phomopsis sp., while Chaetomium sp., Mucor, Paecilomyces puntonii, Penicillium chrysogenum and Verticillium lecanii were isolated only in the present investigation.

Depending on the species, the percentage occurrence followed five different patterns reflecting most probably the physiology of the species, the response of the species to changing internal factors of the grains and also the competitive ability of the species. The percentage frequency of some (e.g. Mucor sp., Paecilomyces variotii decreased with storage time and it increased in others (e.g. Aspergillus candidus, chaetomium sp). The percentage frequency of some species such as Aspergillus flavus and Fusarium increased and then declined getting to the end etc., while the reverse occurred in Cladosporium herbarum. Lastly, percentage occurrence was irregular in some species such as Aspergillus ochraceus, Aspergilllus niger, Aspergillus sulphurens etc. (see Tables 3 to 4). It will not be possible to explain these patterns at present on the basis of the available information. This could be examined further in subsequent investigation.

For any contaminant, there is a minimum moisture content in grains below which the fungus cannot grow. The minimum moisture content have been determined for most of the storage fungi growing on cereal grains (Christensen and Kaufmann, 1969). Davey and Elcoate (1965) and Lutey and Christensen (1963) put the safe moisture content at 12-14 per cent at 20-30°C. Noubasher et. al (1972), indeed, recorded a very high fungal population from grains of a moisture of 15.2 per cent and stored at 30°C for four months. The data in Table 5 showed that the moisture content of the grains of the four types increased gradually from a range of 13.7 to 15.6 initially to a range of 15.1 to 15.8 per cent at the end of the 6th month of storage. This could be due to water absorbed from the atmosphere of the warehouse and to metabolic water produced by the activities of the contaminant. This increase in moisture content was accompanied by an increase in the incidence of the contaminant fungi (see Tables 3 to 4).

In order to provide further information on stackburn, an experiment was set up at 40°C to find whether fungi, were directly involved in stackburn development. Batches of freshly harvested and dried maize grains of Abelechi, Mixed White, Obatampa and Yellow varieties packed in mini propylene bags were stored at 40°C for a total period of four months. Similar observations as in the previous experiments were made. The number of contaminant fungal species increased with time (see Tables 6 to 10) even though the high temperature of 40°C caused a marked decrease in the moisture content of the grains.

Stackburn, again, did not develop in any of the samples, confirming, the earlier conclusion made that the fungi might not directly be the cause of stackburn.

Comparing the data in Tables 3, 4, 11 and 12 containing lists of fungi in the atmosphere of the warehouses and in the maize grains, it would be observed that most of the fungi were common to both. This indicated that the atmosphere of the warehouses was a major source of the contaminants. The warehouses were not airtight and there was free exchange between the warehouse atmosphere and the external atmosphere. The warehouse air spora was, therefore, directly related to the general air spora of the area. There could also be two other sources of the contaminants. First, mouldy materials, such as old jute bags and old stocks of other products such as wheat present in the warehouses contributed some of the inoculum. Secondly, the presence of species like Cladosporium and Mucor, which are well-known field fungi, also suggested that part of the infection could have come from the field.

Whatever the source of the invading fungi, successful establishment would depend greatly on the moisture available to the fungi and to the temperature to which the grains are exposed. The effects of humidity and temperature on the growth and sporulation of Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergilllus tamarii selected from among the many contaminant fungi were studied to give an indication of the activities of these species within the stored maize grains.

In those experiments, using temperatures of 22, 26, 30, 34 and 38°C, A. clavatus, A. flavus, A. niger and A. tamarii grew best at 38, 34, 34 and 30°C, respectively (see Tables 27, 28, 29 and 30). The relevant literature contains rather scanty information on the temperature growth relationships of the Aspergillus species. Thom and Raper (1945) observed an optimum temperature of 45-50°C for Aspergillus fumigatus, which is much higher than what was obtained for the species of this study. Because the Aspergillus species form a considerable portion of the mycoflora population of stored seeds and grains a greater attention should be given to the physiology of these species. Future studies should also use a greater temperature range than was adopted here so that the minimum and maximum temperatures could be identified for all the species. Infact, the highest temperature of 38°C used in the present study turned out to be the temperature which supported the greatest growth in A. clavatus. It is not certain whether that was, indeed, the maximum temperature.

Growth of A. flavus, A. niger and A. tamarii was poor at the extreme temperatures of 22 and 38°C, while growth of A. tamarii was moderate at 22°C and poor only at 38°C. It could be suggested that these Aspergillus species were sensitive to moderately high temperatures. Since temperatures could be high among stored grains the activities of these four Aspergillus species are more likely to be limited in the grains with increasing storage period.

Fungi often do not sporulate best at temperatures which support the greatest growth. For example, Gnomonia valgaris grows and sporulates best at 17 and 15°C respectively (Wolf and Wolf, 1947). Furthermore, the temperature ranges supporting growth and sporulation mostly do not coincide. Penicillium digitatum, for example, is able to grow at 30°C but no conidia are formed at this temperature, and sporulation by Peronospora tabacina occurs within a range of temperature from 5 to 8°C and is most abundant at 7°C. Mycelial growth, however, occurs at temperatures below and above this range (Wolf and Wolf, 1947). A. clavatus was found to belong to this category. It grew best at 38°C but sporulated best at 26°C (see Table 26). The three other species, namely, A. flavus, A. niger and A. tamarii differed from A. clavatus in this respect. In their case, optimum growth and optimum sporulation temperatures coincided.

It is remarkable that different fungi also respond differently to humidity. Many fungi grow most rapidly at 100% R.H. where abundant moisture is available. The best growth in some species, however, occurs at lower humidities. For example, Chona (1932) grew cultures of Aspergillus glaucus inoculated onto both Richard's and Czapek's agar media at 55, 75, 80, 90 and 100% R.H. and found that A. glaucus grew best in atmospheres of 80 and 90% R.H. Above and below this humidity range there was a decline in growth. Also, the most abundant conidia and perithecia were formed at 80 and 90% R.H.

The four Aspergillus species used in this investigation provided one more evidence of varying responses to humidity. They all grew over the humidities of 62.4 to 100% R.H. at the incubation temperature of 30°C. The maximum growth of A. niger occurred at 100% R.H. at 30°C. A. clavatus, A. flavus and A. tamarii, however, attained maximum growth at other relative humidities. A. clavatus and A. tamarii grew best at 62.4% R.H., while the best growth of A. flavus was recorded at 85.2% R.H. The respective mean colony diameters at 100, 92.8, 85.2, 73.4 65.0 and 62.4% R.H. on the 7th day for A. clavatus, A. flavus, A. niger and A. tamarii in that order were 36.5, 41.7, 46.7, 47.0, 48.0 and 51.5mm; 59.0, 65.0, 67.8, 65.5, 62.5 and 57.5mm; 63.3, 63.0, 61.0, 62.0, 60.7 and 58.0mm; and 61.8, 66.1, 66.7, 67.3, 67.8 and 68.8mm (see Tables 17-20). It is likely that neither the maize grains tissues nor the atmosphere of warehouses would be saturated, and so only A. niger will not be at its best in the grains.

An aspect of fungal contamination of grains which should receive attention is the survival of the mycelium in the grains. Both temperature and relative humidity would certainly affect longevity of the mycelium. This is the subject of a study in progress in this Department by Clerk (personal communication) as part of the stackburn project. Kaiser (1973) reported a relevant study on Ascochyta rabiei, a pathogen of chickpea, Cicer arietinum. At 30 and 35°C, A. rabiei could no longer be recovered from infected tissue after 115 weeks.

The fungus was still viable at 136 weeks at 10, 15 and 20°C. At lower humidities of 0-30% R.H. the fungus was viable at 128 weeks. However, this relationship between temperature and moisture requirements of species of fungi and their occurrence in maize kernels in storage, does not exclude other factors which probably would be involved: ability to penetrate and utilize host tissue, growth rates, and production and/or tolerance to antibiotic substances.

As had been shown by the report of Chona (1932), sporulation will not also always be greatest at 100% R.H. where moisture would be readily available. A. clavatus, A. niger and A. tamarii appeared to belong to this group. These fungi sporulated best at 85.2, 92.8 and 62.4% R.H. (See Table 21). An atmospheric humidity of 100% R.H. was, on the other hand, most suitable for A. flavus (see Table 21). It is not possible to find a common reason for the preference of humidities lower than 100% R.H. by some fungi. In certain species the low moisture level may retard growth processes thereby triggering onset of sporulation.

Since vigorous vegetative growth, does not usually permit abundant sporulation (Hawker, 1950) it was not, too surprising that whereas A. clavatus A. flavus and A. niger grew best at 62.4, 85.2 and 100% R.H., respectively (see Table 21), they sporulated best at 85.2, 100 and 92.8% R.H., (See Table 21), respectively.

From the point of view of survival, A. tamarii which required the same humidity, 62.4% R.H. (See Tables 20 and 21), has a slight advantage over the other three species which required different optimum humidities for growth and sporulation. Humidities over the range of 62.4 to 100% R.H. however, permitted adequate sporulation of all the four species and the survival of none would be overly affected by humidities of warehouses which fluctuated between 55 and 95% recorded in this study.

Survival of these fungi involves longevity of the hyphae in the grain tissues, longevity of the conidia, and, the germination capacity of the conidia. Whatever the humidity in the warehouse is immaterial to the conidia directly because they would not germinate in distilled water. Humidity of 100% R.H. would, however, play an indirect role. Droplets of condensed water would form on the surfaces of the grains at that humidity which would become enriched by exudates of the grains turning them into suitable germination media. The nutrient level would become even greater if the water droplet forms on open wound created during shelling whereby it is an extract that would be derived rather than an exudate. The conidia of the four Aspergillus species responded differently to exudates of the maize grains.

The conidia of Aspergillus clavatus and A. flavus germinated to varying degrees in exudates of Mixed White, Obatanpa and Yellow Maize (See Tables 13 and 14). The variation in germination should be expected as the grains of the different varieties would be somewhat different in composition. Using exudate prepared by submerging the grains in sterile distilled water for 12 hours (12-hour exudate) and 24 hours (24-hour exudate), respectively, there was 96.8 and 96.6 per cent germination of conidia of A. clavatus in exudates of yellow maize grains, 40.9 and 73.4 per cent, respectively, in Mixed White exudates and 22.2 and 57.7 per cent, respectively, in Obatanpa exudates.

Conidia of A. flavus showed lower percentage germination of 57.8 and 55.5 per cent, respectively in the 12-hour and 24-hour exudates of Mixed White maize grains; 13.2 and 16.3 per cent, respectively, in Obatanpa exudates and 11.2 and 30.8 per cent, respectively, in Yellow maize exudates (see Tables 13 and 14).

A very significant finding was the inability of the, conidia of A niger and A tamarii to germinate in these exudates (see Tables 15 and 16). Apparently, they did not contain some essential compounds required for the germination of conidia of these two species. What was missing would probably play a critical role connected with the later stages of germination leading to germ tube emergence, because the early mechanisms of germination were initiated.

The conidia became swollen in the solutions - a process that involves metabolism and synthesis of cell materials (Sussman, 1960). The inability of maize grain exudates to support conidial germination in nature would depend on both the maize variety and fungus species.

The suggestion that these conidia may require special extraneous compounds to induce germination was supported by the results in Tables 14, 15 and 16. Conidia of A. clavatus gave 26.8 to 51.5 per cent germination in solutions of 0.1% Peptone, 1.0% Dextrose and 1.0% Sucrose while conidia of A. flavus, A. niger and A. tamarii in stored grains would be relatively curtailed by the inability of conidia to germinate in media low in nutrients, such as condensed water drops on the grains. Their germination required rich nutrients, such as Potato Dextrose Broth in which more than 94 per cent of the conidia of all four Aspergillus species germinated (See Tables 23, 24 and 25) but which are unlikely to be available to them.

Since the atmospheric humidity of the warehouses fluctuates, the fate of swollen conidia of A. niger and A. tamarii in the exudate of maize grains is predictable. When the humidity falls, the droplets of exudate will dry up. The swollen conidia will, consequently, become desiccated. Swollen conidia of A. clavatus and A. flavus whose germ tubes did not emerge before the humidity falls would be similarly desiccated. It was found in this investigation that the conidia of the different species will suffer varying degrees of damage.

Desiccated swollen conidia of A. niger died within one hour. Greater longevity was shown by swollen conidia of A. clavatus, A. flavus and A. tamarii. After four hours of desiccation, 22.2, 8.0 and 4.8 per cent, respectively, of the swollen conidia were still viable (See Table 39). The survival of the desiccated swollen conidia of A. tamarii for some hours is not likely to be of any particular importance in the warehouse, since re-hydration in exudates when the humidity rises will not lead to any eventual germination. Only A. clavatus and A. flavus have an advantage. Ordinary conidia placed at 0% R.H. far outlived the desiccated swollen conidia. The treatment might have affected the stability of either membrane systems or enzyme systems or both in swollen spores which had changed from a quiescent to an active state.

It is this knowledge of fluctuating humidities in the warehouses which determined the experimental design to investigate the third form of survival of the Aspergillus species. Viz., longevity of the normal conidia. Conidia formed at a particular humidity would be most likely exposed later to quite a different atmospheric humidity. Therefore, to study the longevity of the conidia, conidia formed at different relative humidities were stored over a range of relative humidities.

Very great differences have been found in the survival potential of different kinds of fungal spores. The viability of all spores decreases with time and the rate of loss of vigour is dependent on the inherent characteristics of the spore and upon environmental conditions, especially, temperature, humidity and light (Cochrane, 1958).

Temperature greatly influences the longevity of spores, and, long survival is correlated with low temperature while high temperatures shorten this period. The effect of particularly high temperature on viability is probably due to the gradual denaturing of the proteins of the cell's protoplasm. It is also likely that moderately high temperatures increase the respiration rate of the quiescent spore, with both consequential early depletion of essential reserve materials or greater accumulation of toxic metabolites resulting in spore death. The effect of temperature was not included in this study due to lack of refrigerated incubators that would provide a reasonable range of temperatures. When these facilities become available the exact response to temperature should be determined for each species.

Radiation shortens the period of viability of some spores and blue light appears to be the most active portion of the visible spectrum (Dillon-Weston and Halnan, 1932). Maize grains in the warehouses and stores are hardly ever in continuous darkness. The conidia were stored in light and, infact, also formed in light, in order to obtain results that would be relevant to the house environment.

Varying proportions of the conidia were viable after storage for 56 days at 0, 20, 40, 60, 80 and 100% R.H. The highest percentage viability recorded for conidia of A. clavatus, A. flavus, A. niger and A. tamarii after 56 days' storage was 48.9 percent of conidia formed at 65.0% R.H. and stored at 100% R.H. (see Table 31f), 56.1 per cent of conidia formed at 92.8% and stored at 20.0% R.H. (see Table 33b), 70.3 per cent of conidia formed at 73.4% R.H. and stored at 80.0% R.H. (see Table 35c), and 84.7 per cent of conidia formed at 65.0% R.H. and stored at 40.0% R.H. (see Table 37c), respectively. This shows that the conidia have fairly long life and a portion would be viable for most part of the period that the grains would be kept in the warehouse. Furthermore, there would naturally, be a relay of re-infections of the grains and reproduction of crops of conidia throughout the storage period.

Survival of the conidia was apparently not related to the humidity at which the conidia of A. clavatus and A. flavus were formed. This is observable in the summary tables of Tables 32 and 34. There was no occasion when conidia of the two species formed at a particular humidity proved to be either the longest-living or shortest-living spores. What was certainly important was the humidity at which these conidia were stored, conidia of A. niger and A. tamarii showed a phenomenon that had rarely, if ever, been reported.

Conidia of A. niger formed at 73.4 - 85.2% R.H. and A. tamarii conidia formed at 65.0 - 85.2% R.H. showed a greater potential for survival than those formed at the other humidities (see Tables 35 - 38). The absence of the appropriate facilities did not permit an investigations into the physiological basis for this striking phenomenon, which could be related to membrane stability or store of enzyme systems or nature of organelles or nature of metabolites or a combination of these.

The four Aspergillus species exhibited four different humidity-survival relationships as illustrated in Fig 1. In the first case, the conidia were damaged by desiccation. Conidia of A. niger died quickest at 0% R.H. and lived longest at the highest humidities (see Tables 35 and 36). Other species whose spores have been found to be killed more rapidly by low humidities include Phytophthora infestans (Glendenning, McDonald and Graiger, 1963; Zan 1962), Phytophora meadi (Pereis and Fernado, 1966) and Trachysphaera fructigena (Maramba and Clerk, 1974). A. tamarii conidia, in contrast, survived better at low humidities than at high humidities (see Tables 37 and 38). And so are conidia of Beauveria bassiana and Paecilomyces farinosus (Clerk and Madelin, 1965), Chalara quercina (McLaunghlin and True, 1952) and Pyricularia oryzae (Anderson, Henry and Morgan, 1948). In this group of fungi, there was, most likely, greater metabolism and accumulation of toxic metabolites in conidia at the high humidities.

A. flavus conidia died quickest at 80% R.H. and survived longer at 0, 20, 40, and 100% R.H. (see Tables 33 and 34). This confirmed the similar observation made by Teitell (1958), in his studies on A. flavus. He found a similar relationship also for an isolate of Aspergillus terreus. Clerk and Madelin (1965) reported the same phenomenon in conidia of Metarrihizium anisopliae. Teitell (1958) suggested that at the vulnerable humidity, the conidia were not as functional as those at the higher humidities while they lacked the protection of full desiccation of those at lower humidities. Clerk and Madelin (1965), on the other hand, suggested that permeability of cell membranes was impaired at the vulnerable humidity. Future studies should re-examine these two different views.

Lastly, A. clavatus conidia lost viability quicker at 20 and 80% R.H. than at the other humidities (see Tables 31 and 32). The only other report of this type of response by fungus spores to storage relative humidity was that of Akushie and Clerk (1981) on sporangiospores of Rhizopus oryzae. It is difficult, with the evidence available, to explain why 20 and 80% R.H. should be particularly lethal to conidia of A. clavatus, while 40 and 60% R.H. lying in between should prolong longevity of the conidia.

By coincidence, the four species selected for studies have shown how varied the response of the conidia of the contaminating fungi to relative humidity could be. Since there is so much variation it will be difficult to minimize contamination with one particular relative humidity. Mislivec and Tuite (1970) also recognised that low temperatures could not be used in controlling infection of yellow dent corn kernels by Penicillium species. While P. citrinum, P. funiculosum, P. oxalicum, P. purpurogenum and P. variable had an optimum temperature of 30°C for growth, nine other Penicillium contaminants, namely P. brevicompactum, P. chrysogenum, P. cyclopium, P. expansum, P. frequentans, P. palitans, P. puberulum, P. urticae and P. viridicatum had an optimum temperature as low as 23°C.

No direct relationship has been established between fungal activity and stackburn. The large number of contaminants encountered over six months suggested that substantial economic loss would be caused by fungus damage in the storage of maize grains from harvest to final processing. The mould genus that would be most responsible is Aspergillus Link. The study of the mycoflora in itself is important and should be sustained. The knowledge of species composition may aid in determining the quality and the history of a given lot of maize. With extensive breeding programmes on maize in progress in many maize-growing countries, it is possible that varieties with more robust testa and pericarp not easily penetrable by fungi will emerge. This could be achieved and we look forward to combating contamination both in the field and storage in this way, with hope.

VI SUMMARY

1. Twenty-six fungal species belonging to 12 genera were isolated from Mixed White maize grains, while 16 fungal species were isolated from the stackburn Mixed White grains.

2. Normal Yellow maize grains and stackburn Yellow maize grains contained 32 fungal species belonging to 14 genera, and 19 species belonging to 6 genera respectively.

3. The humidity of the atmosphere of the warehouses fluctuated between 55 and 90% R.H.

4. The mycoflora of each of the four batches of maize grains was dominated by the members of the genus Aspergillus.

5. The percentage occurrence of Aspergillus species in:
   (a) the non-stackburn White Maize from the Kaneshie Warehouse was 68.8 per cent

   (b) the stackburn White Maize from the Kaneshie Warehouse was 34.0 per cent.

   (c) the non-stackburn Yellow Maize from the Tema Warehouse was 60.3 per cent.

(d) the stackburn Yellow Maize from the Tema Warehouse was 47.9 per cent.

6. Aspergillus flavus was the most important species, after storage for 4 months, with the highest percentage of:

(a) 33.1 per cent in non-stackburn White Maize.

(b) 37.9 per cent in stackburn White Maize.

(c) 45.9 per cent in non-stackburn Yellow Maize.

(d) 100 per cent for stackburn Yellow Maize.

7. Genera recorded on the grains were:

(a) Aspergillus, Chaetomium, Cladosporium, Fusarium, Mucor, Neurospora, Paecilomyces, Penicillium, Phoma, Rhizoctonia, Rhodotorula and Verticillium in the non-stackburn White Maize.

(b) Aspergillus, Chaetomuim, Cladosporium, Fusarium, Mucor, Penicillium, Rhodotorula and Verticillium in the stackburn White Maize.

(c) Aspergillus, Cladosporium, Curvularia, Dreschlera, Fusarium, Gliocladium, Mucor, Paecilomyces, Penicillium, Phoma, Scopulariopsis, Stemphylium and Verticillium in the non-stackburn Yellow Maize.

(d) Aspergillus, Cladosporium, Curvularia, Mucor, Penicillium and Verticillium in stackburn Yellow Maize.

8. (a) The occurrence of Aspergillus flavus in all the samples consistently rose to a peak at the 4th month and then declined.

(b) Paecilomyces puntonii and Paecilomyces variotii were consistently present during the first two months and disappeared in the 4th and 6th months.

(c) The rest did not show a consistent pattern of occurrence.

9. The moisture content of non-stackburn and stackburn White maize grains and non-stackburn and stackburn Yellow maize grain increased with storage for 6 months at atmospheric temperature:

(a) Non-stackburn White maize - from 13.7 to 15.1 per cent

(b) Stackburn White maize - from 13.8 to 15.4 per cent

(c) Non-stackburn Yellow maize - from 13.0 to 15.6 per cent.

(d) Stackburn Yellow maize - from 13.6 to 15.8 per cent.

10. A total of 26 fungal species were isolated from the atmosphere of Kaneshie warehouse.

11. The 10 most abundant species in descending order were Aspergillus flavus, Cladosporium herbarum, Aspergillus flavus-oryzae, Mucor sp., Aspergillus fumigatus, Rhodotorula sp., Penicillium expansum, Aspergillus niger, and Penicillium chrysogenum.

12. A total of 27 fungal species were isolated from the atmosphere of the Tema warehouse.

13. The most abundant species in descending order were: Cladosporium herbarum, Aspergillus flavus, Penicillium expansum, Penicillium chrysogenum, Rhodotorula sp., Rhizoctonia solani, Aspergillus parasiticus and Paecilomyces puntonii

14. Grains of four maize varieties kept at 40°C showed the following number of contaminant species.

(a) Abeleehi variety - 15 species
(b) Mixed White variety - 16 species
(c) Obatanpa variety - 18 species
(d) Yellow maize variety - 18 species

15. Aspergillus flavus was the most abundant contaminant species

16. Grains kept at the temperature of 40°C lost considerable amount of moisture over the period of 4 months. The percentage moisture content at the beginning and at the end were:

    (a) Abeleehi variety; from 12.1 to 8.0 per cent
    (b) Mixed white variety; from 14.4 to 8.3 per cent
    (c) Obatanpa variety; from 14.0 to 7.9 per cent
    (d) Yellow variety; from 13.4 to 7.5 per cent

17. Aspergillus clavatus and Aspergillus tamarii cultures incubated at relative humidities ranging from 62.4 to 100% R.H. grew best at humidities of 62.4 to 73.4% R.H. A.flavus grew best at 73.4 to 92.8% R.H., and A. niger grew best at 92.8 and 100% R.H.

18. Over the same range of relative humidities of 62.4 to 100% R.H., sporulation by:

    (a) A. clavatus was best at 85.2 - 100% R.H.
    (b) A. flavus was best at 62.4 - 100% R.H.
    (c) A. niger was best at 62.4 - 92.8% R.H.
    (d) A. tamarii was best at 62.4% R.H.

19. Aspergillus clavatus, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii all grew over the temperature range

20. Growing over the temperature range of 22 to 38°C, the four species sporulated best at the following temperatures:

(a) A. clavatus - 26°C
(b) A. flavus - 34°C
(c) A. niger - 34°C
(d) A. tamarii - 30°C

21. Conidia of each species formed at 62.4, 65.0, 73.4, 85.2,92.8 and 100% R.H. had practically the same germination capacity. Germination of conidia in Potato Dextrose Broth after 12 hours was:

(a) 90.0 - 99.5 per cent for A. clavatus conidia
(b) 80.7 - 94.3 per cent for A. flavus conidia
(c) 83.4 - 98.4 per cent for A. niger conidia
(d) 87.7 - 98.6 per cent for A. tamarii conidia

22. The conidia of all four species did not germinate in distilled water.

23. A. flavus, A. niger and A. tamarii conidia did not germinate in 1.0% Dextrose, 1.0% sucrose and 0.1% Peptone solutions.

24. Conidia of A. clavatus showed 83.7, 69.6 and 72.0 per cent germination in 1.0% Dextrose, 1.0% sucrose and 0.1% Peptone solutions respectively.

24. Conidia of A. clavatus showed 83.7, 69.6 and 72.0 per cent germination in 1.0% Dextrose, 1.0% sucrose and 0.1% Peptone solutions, respectively.

25. Between 22.2 and 6.8 per cent of A. clavatus conidia, and between 11.2 and 57.8 per cent of A. flavus conidia germinated in 12 hours in exudates of White, Obatanpa and Yellow maize grains.

26. A. niger and A. tamarii conidia, did not germinate in the exudates.

27. Conidia stored at 0, 20, 40, 60, 80 and 100% R.H. in light at 30°C for 56 days showed the following patterns of survival:

(a) A. clavatus conidia lived longest at 0, 60 and 100% R.H. and lost viability quickest at 20 and 80% R.H.

(b) A. flavus conidia lost viability quickest at 80% R.H. and survived longest at 0, 20, 40 and 100% R.H.

(c) A. niger conidia survived longest at 100% R.H. and longevity decreased with decreasing relative humidity.

(d) A. tamarii conidia survived longest at 0, 20, 40 and 60% R.H. and lost viability quickest at 100% R.H.

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APPENDIX A

Fungal species isolated from grains of maize (Zea mays L.) from both Kaneshie and Tema warehouses.

Asperillus candidus Link ex Fr.

Asperillus clavatus Desmazieres

Asperillus effusus Tiraboschi

Asperillus flavus Link Fr.

Asperillus flavus-oryzae

Asperillus fumigatus Fresenius

Asperillus glaucus

Asperillus niger van Tieghem

Asperillus ochraceus Welhelm

Asperillus parasiticus

Asperillus sulphureus (Fres.) Thom and Church

Asperillus tamarii Kita

Asperillus ustus (Bainier) Thom and Church

Asperillus wentii Wehmer

Chaetomium sp.

Cladosporium herbarum (Persoon: Fries) Link

Curvularia lunata Boedjin

Dresclera maydis (Nisikado) Subrain and Jain

Fusarium moniliforme Sheldon

Fusarium sp.

Gliocladium sp.

Mucor sp.

Neurospora sitophilia (Montagne) saccardo

Paecilomyces carneus (Duche et Heim) A.it Brown et G.Smith

Paecilomyces puntonii (Vuillemin) Nannizzi

Paecilomyces varotti Bain

Penicillium citrinum Thom

Penicillium chrysogenum

Penicillium cyclopium Westling

Penicillium expansum Link ex S.F. Gray

Penicillium sp.

Phoma glomerata

Rhizotonia solani Kuhu

Rhodotorula sp.

Scopulariopsis sp.

Stemphylium lanngolosum

Verticillium leecanni