STUDIES OF TOXIGENIC AND ZOOPATHOGENIC FUNGI ASSOCIATED WITH THE SPOILAGE OF NIGERIAN POULTRY FEEDS

BY

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ABSTRACT

Six species of known toxigenic and zoopathogenic fungi were isolated from Nigerian poultry feeds. These include *Aspergillus flavus* Link: Fr. (IMI 280819), *A. fumigatus* Fres. (IMI 280822), *A. niger* v. Tieghem (IMI 280823), *A. oryzae* (Ahlburg) Cohn (IMI 280831), *Rhizopus arrhizus* Fischer (IMI 280827) and *Rhizomucor pusillus* Lindt Schipper (IMI 280824).

Growth-temperature range for the fungi was between 15° and 45° C. *Aspergillus fumigatus* and *Rhizomucor pusillus* are thermotolerant with optimum growth 40° C while *Rhizopus arrhizus* had optimum growth at 30° C. For *Aspergillus flavus*, *A. niger* and *A. oryzae* the optimum growth was at 35° C. The pH growth studies showed that all the fungi had good mycelial growth at pH 4-8 with optimal growth at pH 5.5 for *Aspergillus fumigatus*, *A. flavus* and *A. oryzae*. *Aspergillus niger*, *Rhizomucor pusillus* and *Rhizopus arrhizus* had optimal growth at pH 6.0.

Nutritional studies showed that all the fungi were capable of utilizing the various forms of carbon and nitrogen provided to varying extents. Starch and dextrin were excellent sources of carbon for mycelial

growth and sporulation while pectin and carboxymethyl cellulose (CMC) were poorly utilized by all the fungal isolates. The fungal species grew poorly on native cellulose (filter papers) except Rhizomucor pusillus and Rhizopus arrhizus which showed no growth on this carbon source. Apart from tryptophan, all the nitrogen sources supplied were utilized for growth and sporulation by the test fungi though to varying extents. Casein was the best nitrogen source for all the fungi. Feed infusion medium also supported growth and sporulation of all the isolates.

Varying quantities of aflatoxins (B_1, B_2 and G_2) were produced by Aspergillus flavus and A. oryzae on modified Czapek-Dox media. None of the remaining fungal species produced aflatoxin. Peak aflatoxin B_1 production was on the 8th day of incubation by the two toxigenic fungi. Optimum pH and temperature for the production of toxins were pH 5 and 30° C respectively. Major sources of aflatoxins in poultry feeds due to mould infestation in increasing order of importance were: palm kernel, corn and groundnut cake meals. Studies on aflatoxin production on feed concentrates by A. flavus and A. oryzae showed that under suitable

conditions of moisture and temperature, dried brewers grains, wheat offals, palm kernel, corn and groundnut cake meals were suitable substrates for toxin production. Other feed concentrates: fish, blood, oyster shell and bone meals were found to be unsuitable substrates for aflatoxin production. Aflatoxins were not detected in poultry droppings before and after inoculation with the toxin producing fungi.

All the fungal isolates produced extracellular amylases, cellulases, proteases and lipases. The synthesis and activity of these enzymes were affected by external factors such as the pH, incubation temperature and type of carbon source in the growth medium. Optimum activity for all the enzymes produced by the isolates was in acidic media (pH 4-6) and within a temperature range of between 40° C and 50° C.

On the basis of these findings recommendations were made for the control of the toxigenic and zoopathogenic fungi in poultry feeds and other stored agricultural products.

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CERTIFICATION

I certify that this work was carried out by Mr. L.O. Adebajo in the Department of Botany and Microbiology, University of Ibadan.

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INTRODUCTION

Livestock production is currently on the increase in all states of the Federal Republic of Nigeria. This may be due to the increase in demand for poultry products as a result of increasing population (Ogunmodede, 1983; Monu, 1983). The expansion in poultry production has led to a considerable interest being shown in the formulation of high efficiency diets for all classes of poultry based on available feed ingredients (Oluyemi et al; 1976).

In many tropical countries and particularly Nigeria, commercially produced poultry feeds contain: maize (50-70%), groundnut cake meal (13-33%), soya bean meal (3-40%), palm kernel meal, fish meal (4.5-6%), blood meal (0-3.5%), bone meal (0-7.5%), wheat offals (0-84%), dried brewers grains (1-4.4%) and oyster shells (0-5.7%). Other ingredients are: common salt (0.25-0.3%), mineral sources (0.5-4.0%), vitamins such as 'green fowl' (1-8%) and amino acid supplements (Oluyemi and Roberts, 1979). Also included are feed additives such as antioxidants like butylated

hydroxytoluene and preventive medication like coccidiostat and other antibiotics (Saloma et al; 1970; Bunyan et al; 1977; Jeffries et al; 1977). The antibiotics produce beneficial effects that are either slight or transient and only included where conditions are substandard resulting in infections (Foster, 1972; Oluyemi and Roberts, 1979).

With the exception of bone meal and oyster shells which are milled, these ingredients if in solid form are ground before thorough mixing. These operations entail the use of mechanical millers, grinders and mixers respectively. The proportions of mixing the various ingredients is important to obtain an efficient balanced diet hence recomendations of the National Research Council (N.R.C.) of the United States of America is widely adopted (Oluyemi and Roberts, 1979). After mixing together the feed concentrates in the desired proportions, packaging of the compounded feed follows. This in some cases is by the use of multi-wall paper bags capable of preventing produce from absorbing considerable atmospheric moisture (Oyeniran, 1976). The moisture content of poultry feeds like other stored agricultural products is influenced by the

relative humidity of the storage atmosphere until an equilibrium is reached (Pixton, 1976).

Spoilage of livestock feeds during storage due to mould infestation is particulary high in the tropics where the temperatures and high relative humidities provide excellent conditions for fungal growth (Ogundero, 1980b, 1983). Various reports occur in the literature on mould infestation of animal feeds. Bonner and Fergus (1959) working with cattle feeds isolated sixty-four fungal species representing three classes, seven orders and twelve families. Of the sixty-four species identified, sixty-three were obtained from suspected feed samples while thirty-eight were isolated also from non-suspected feeds. Twenty-five of their isolates were known or suspected to be pathogenic or have toxic abilities in livestock. These include: Mucor racemosus, Rhizopus arrhizus, R. nigricans, Haplosporangium decipiens, Candida albicans, C. humicola, C. krusei, Cryptococcus albidus, Aspergillus chevalieri, A. flavus, A. fumigatus, A. nidulans, A. terreus, Monascus purpureus and Penicillium expansum. Others are: Chaetomium olivaceum, Cephalosporium kiliense, Paecilomyces

variota, Scopulariopsis brevicaulis, Trichoderma koningi, Fusarium moniliforme, Cladosporium herbarum, Pullularia pullulans, Mucor flavus and Streptomyces spp. From poultry feeds, Ogundero (1980b) obtained eleven species of fungi out of which Aspergillus fumigatus and Rhizomucor pusillus were more frequently encountered than the other fungi. Three of the isolates namely: Aspergillus fumigatus, Mucor pusillus and Thermoascus aurantiacus were found to be zoopathogenic. Research findings of Christensen and Kaufman (1965) and Scott (1965) have implicated several feed inputs as sources of fungal contaminants in compounded feeds. Another major source of fungal contaminants of livestock feeds is the soil. Taber and Robert (1975) isolated several fungal species from peanuts and peanut soils. Some of their isolates were: Mucor pusillus; Humicola lanuginosa, Talaromyces (Penicillium) dupontii, Thermoascus aurantiacus, all of which are thermophilic and Aspergillus fumigatus which is thermotolerant. Mucor pusillus was however the most commonly isolated of the lot.

From previous reports (Christensen, 1957; Christensen and Kaufman, 1965; and Oyeniran, 1978) the

spoilage of mould-infested stored agricultural products such as feeds and feed concentrates could be manifested in several ways: loss in dry weight, macro and micro nutrients depletion, production of undesirable flavours, colours and odour; changes in the physical structure of the products such as conversion of feed particles into sticky masses. Others include initiation of undesirable biochemical changes such as increase in the free fatty acid (F.F.A) contents of such products and the production of harmful secondary metabolites called mycotoxins.

The degradative roles of fungi associated with stored agricultural products such as poultry feeds and feed concentrates have also been reported. Nagel and Semeniuk (1957) reported that Penicillium chrysogenum, Aspergillus niger and A. flavus were active in decomposing corn organic matter, producing losses of 40% to 45% within a 4-week period. The level of fatty acids in the corn were increased by the three fungi and also by Mucor racemosus and Aspergillus amstelodami. Decrease in organic weight brought about by Aspergillus tamarii, A. glaucus group and Penicillium citrinum had also been recorded in peanuts (Ward and Diener, 1960).

Kuku (1971, 1975), reported on mould deterioration of Nigerian palm kernels which is an important poultry feed input. The results of her work showed that each of the five isolated moulds (Aspergillus flavus, A. chevalieri, A. fumigatus, Paecilomyces varioti and Penicillium steckii) increased or decreased the free fatty acid (F.F.A.) and oil contents of palm kernel samples respectively. Aspergillus flavus however caused the greatest increase in the free fatty acid content (5%) after four weeks of incubation. It also brought about 9.8% decrease in oil content in eight weeks following inoculation. Kuku and Adeniji (1976) also working on palm kernels reported that seven of the moulds isolated (Aspergillus chevalieri, A. flavus, A. niger, A. ruber, Paecilomyces varioti, Penicillium citrinum and Syncephalastrum racemosum) have lipolytic abilities thus breaking down the fats into fatty acids leading to a reduction in oil contents. Earlier reports by Ward and Diener (1960) and Eggins (1963) showed that the liberation of free fatty acids is a major form of deterioration caused by associated moulds on peanuts and palm produce respectively. That thermophilic fungi play significant roles in such

deterioration was reported by Ogundero (1981a). He obtained considerable decreases (23.4-46.5%) in the oil contents and increases (38.9-84.6%) in the F.F.A. contents of the palm products caused by Chaetomium thermophile, Humicola lanuginosa, H. insolens, Torula thermophila and Mucor miehei. Extracellular lipases produced by the fungi played significant roles in all the degradation given above. They have particularly been implicated in the degradation of Nigerian groundnuts and palm produce (Ogundero, 1980a; 1981a; 1981b). Out of the seven fungi isolated from mouldy groundnuts, Humicola grisea, Mucor pusillus, Talaromyces thermophilus and Thermoascus crustaceus were found to be lipolytic. Their lipases hydrolysed both natural and synthetic glycerides to free fatty acids (Ogundero, 1980a).

The production and release of extra-cellular enzymes by fungi are known to be significant in the degradation of feed concentrates. Cellulases are of particular importance in this respect since cellulose forms the bulk of the dry weight of many agricultural products of plant origin (Gascoigne and Gascoigne, 1960). Oso (1978) reported that Talaromyces emersonii

obtained from Nigerian oil palm kernels was able to synthesize the Cx cellulase enzyme confirming involvement in the biodeterioration of stored agricultural produce. While working with twenty-one fungal species, most of which are known to degrade feed concentrates, Rosenberg (1978) observed cellulolytic abilities in Allescheria terrestris, Aspergillus fumigatus, Sporotrichum thermophile, Talaromyces emersonii, Thielavia thermophila, Chrysosporium pruinosum and Sporotrichum pulverulentum. Chrysosporium pruinosum and Sporotrichum pulverulentum were even able to degrade lignocellulose which is more resistant to biodeterioration. Amylases also play significant roles in the degradation of plant products particularly those rich in starch (Burnett, 1962). Their synthesis by fungi and break-down activities have also been reported (Ogundero, 1978; 1979; Oso, 1979).

Synthesis of proteases by storage moulds and their roles in biodeterioration of agricultural products have also been reported. The report of Ogundero (1983) on the zoopathogenic fungus Thermoascus aurantiacus obtained from poultry feeds in Nigeria was on biosynthesis and activity of the extracellular

proteases of this species. The protease activities of Mucor miehei which is known to be associated with some feed concentrates was reported by Lasure (1980). In a recent work, Ogundero and Adebajo (1987) reported the production of polysaccharide degrading enzymes by a toxigenic strain of Aspergillus isolated from Nigerian poultry feeds. They found that the fungus optimally produced amylases within 48hrs of incubation and at 30° C; CM-cellulases and FP-cellulases at 30° C and pH 6.0 while xylanases and dextranases of the same organism had peak production at pH 5.4. The report also showed that the cellulases were produced only on cellulose containing media while other enzymes were produced to various extents on all the carbon sources supplied.

The nutritional and physiological activities of moulds on agricultural products may lead to the depletion of macro and micro-nutrients hitherto present in them. Gregory et al; (1963) reported rapid utilization of glucose and soluble nitrogen from animal fodders by moulds. Fungal activities in feeds and fodder may also alter the availability of micro-nutrients (Lacey, 1975). Apart from the loss in dry

weight and nutrients of stored agricultural produce moulds may cause discolouration of produce. Botryodiplodia theobromae and Macrophomina phaseoli for example are known to discolour feed ingredients such as groundnuts (Oyeniran, 1978). Such produce with undesirable colours are often rejected by manufacturers and consumers due to decrease in the quality.

Associated with a large number of fungi responsible for the spoilage of poultry feeds, feed concentrates and similar agricultural produce are toxic metabolic by-products which when ingested are harmful to livestock even at low concentrations (Campbell and Stoloff, 1974). Such toxic substances are termed mycotoxins and their extraction from animal feedstuffs have been reported (Roberts and Patterson, 1975; Patterson and Roberts, 1979). Some of the already identified mycotoxins include: aflatoxins, aspertoxin citrinin, cytochalasins, fumagillin, gliotoxin, islanditoxin, luteoskyrin, moniliformin, ochratoxins, patulin, rubratoxins, scirpentriol, scopoletin, sporidesmin, sporofusariogenin, sterigmatocystin, T-2 toxin, verrucarins, verrucarol and zearalenone. Others include: aspergillic acid, epicladosporic acid,

helvolic acid, penicillic acid,cyclopiazonic acid and terreic acid (Bamburg et al, 1969; Robb and Novral, 1983) Mycotoxicoses are intoxications in animal and man caused by intake of mycotoxins (Krogh, 1969). Several reports on mycotoxins and the implicated microbes are available in the literature (Bamburg et al, 1969). Mycotoxicoses associated with such mycotoxins have also been reported (Forgacs and Carll, 1962; Bassir 1969; Uraguchi, 1969).

Krogh (1969) reported that necrosis of pharynx mucosa, degeneration of the liver and kidney, the destruction of the spleen, cancer of the liver, inflamation and proliferation of bile ducts are some of such damages. In addition, vulvo vaginitis, cereals toxicosis, ochratoxicosis and alimentary toxic aleukia (A.T.A.) are other mycotoxicoses associated with animals fed with mouldy feeds (Bamburg et al; 1969). In addition, the notorious "turkey X" disease of poultry is reportedly caused by the ingestion of mycotoxins particularly aflatoxins in feeds (Schroeder, 1969).

External applications of some mycotoxins on the skins of experimental animals has proved to be harmful

in some cases. Joffe (1969) reported that the application of sporofusarin, poaefusarin and aflatoxin on the skins of rabbits resulted in oedematous and heamorrhagic or leukocytorrhic reactions. This subsequently led to the death of about 50% of the animal population. A few reports on the effects of mycotoxins even on plants have been made. The three mycotoxins: sporofusarin, poaefusarin and aflatoxin were implicated in the death of the branches of peas, beans and tomatoes grown in liquid substrates in which the toxin producing fungi had previously been grown (Joffe, 1969). However, out of all the mycotoxins already identified, the aflatoxins produced by some strains of Aspergillus flavus, especially on groundnut kernels has attracted most attention of research workers. Halliday (1969) reported aflatoxin contents ranging between 50 and 250 ppb in Nigerian groundunts with 0.4-1.6% mouldiness. Broadbent et al. (1963) and Halliday and Kazure (1970) have also determined the aflatoxin contents in groundnut cake and groundnut materials used as feed concentrates. Lillehoj et al. (1980) and Opadokun et al. (1979) reported aflatoxin

levels below 300 ppb in most of the pre-harvest and post-harvest corn samples analysed.

Although species in the Aspergillus flavus group are known to be principal producers of aflatoxins, other species of Aspergillus and other moulds such as Rhizopus and Penicillium have been reported to produce aflatoxins (Hodges et al; 1964; Kulik and Holaday, 1966; van Walbeek et al, 1968; Wilson et al; 1968). Eight closely related compounds, designated as aflatoxin B_1,B_2, G_1, G_2, M_1, M_2, B_{2a} and G_{2a} have been discovered to be a part of the aflatoxin complex. However, aflatoxin B_1 is the most toxic and the one most commonly found in significant quantities (Schroeder, 1969). Aflatoxins are hepatocacinogens causing liver damage in the form of a necrosis, proliferation of the bile ducts, fibrosis and the development of malignant liver tumors. They are also reported to be responsible for the destruction of the kidney and necrosis of pharynx mucosa (Krogh, 1969; Campbell and Stoloff 1974; Bababunmi et al; 1978). Man and animals such as duck, rainbow trout, guinea-pig, rabbit, dog, rat, turkey poult, pig, monkey, cattle, pheasant, chick, hamster and mink were found to be

sensitive to aflatoxin while the sheep however seems to be almost resistant (Krogh, 1969). Feeding-stuffs and foods meant for livestock and human consumption respectively should therefore be carefully screened to determine their aflatoxin and other mycotoxin contents (Opadokun et al; 1979; Robb and Norval, 1983) In most of the previous studies carried out in Nigeria, the qualitative and quantitative estimation of mycotoxins particularly aflatoxins present in groundnuts and groundnut products were given little attention. Feed ingredients such as fish meal, wheat offals, blood meal, brewers grains and even compound rations such as poultry feeds are yet to be screened to assess the role of microfungi and their mycotoxin. The implication of this to Nigeria will be appreciated if it is realised that, unlike in the technologically advanced countries, there is no serious monitoring of the quality of feeds produced by the variuous feed compounders. The prevailing humid tropical climate enhancing mould deterioration of feeds has further complicated the problem. Although livestock feeds contaminated with aflatoxins may not affect man directly, however, they could be dangerous, indirectly. This is so because it

has been reported that ruminants fed with feeds containing aflatoxins B_1 and B_2 excrete a toxic compound of aflatoxin named M-toxin in the milk. While it has been determined that only 1% or less of the ingested aflatoxin is thus excreted, yet this may constitute a public health problem (Wogan, 1966; Legator, 1966).

Apart from the presence of the toxigenic fungi in the feeds and feed concentrates, zoopathogenic fungi have also been reported (Bonner and Fergus, 1959; Ogundero, 1980, 1981; Ogundero and Adebajo, 1987). These fungi have been implicated in various diseases of livestock and man (Alexopoulos, 1962; Ajello et al; 1976). Notable among such diseases resulting from ingestion of contaminated feeds are pulmonary mycoses such aspergillosis which is a killer disease of young birds (Anisworth and Austwick, 1955). Others include mycotic abortion, gastric ulceration, mycotic mastitis, digestive disturbance and allergic reactions could also result from fungal infections (Lacey, 1975). In addition, fungi may cause diseases in several ways simultaneously. Aspergillus fumigatus for instance, may cause respiratory infection and allergy in animals

(Alexopoulos, 1962) and at the same time produce toxins (Bamburg et al; 1969). Absidia sp. may cause mycotic abortion in cattle and with Mucor pusillus may be involved in gastric ulceration in several farm animals (Lacey, 1975). Also, from the reports made by Carll and Forgacs (1953) and Forgacs et al. (1953), respiratory, nervous and intestinal disturbances have been found to develop along with internal mycoses following the effects of allergins, or toxins or both.

The adverse effects and activities of moulds associated with livestock feeds have serious economic implications either directly or indirectly. They lead to decrease in feed quality, in egg, milk and meat production, high expenditure on health problems of livestock and man and mass loss of livestock due to fungal infections. The removal or neutralization of the undesirable effects brought about by invading fungi on produce has therefore been attempted. For instance detoxification of agricultural produce particularly feed concentrates such as groundnuts and corn has been reported. Coomes et al. (1966) Ciegler and Paterson (1968) Gardner (1971) and Beckwith et al. (1975) attempted a variety of chemical degradation methods

involving the use of acids, bases, oxidizing agents and bisulphite to detoxify contaminated feed concentrates.

Biological control measures have also been attempted to get rid of the effects of toxin producing fungi in agricultural products. Ginterova et al. (1980) brought about detoxification of corn meals contaminated with aflatoxin B_1 by cultivating Pleurotus ostreatus with the aflatoxin producing fungus which is Aspergillus flavus. Tsubouchi et al. (1981) also inhibited aflatoxins B_1 and G_1 production in rice cultures by A. flavus when A. niger was co-inoculated.

Physical method of control has also been used to eliminate the adverse effects of fungi particularly the toxigenic ones from feed concentrates. For example, Conway et al. (1978) reported the detoxification of aflatoxin-contaminated corn by roasting. Huff(1980) also reported the use of a physical method involving the use of density for the segregation of aflatoxin contaminated corn from uncontaminated ones. Shantha and Venkateshaish (1981) reported the detoxification of stored groundnut products by exposure to sunlight.

The prevention of infection in stored products has also been suggested as a method for controlling fungal

activities in produce. McDonald (1969, 1970) reported that mechanical damage is one of the factors enhancing fungal invasion of agricultural produce and therefore should be avoided particularly during cultivation and at harvest time. Removal of pre-harvest infected produce; addition of chemical preservative agents particularly when produce are stored under conditions favouring biodeterioration; the use of fungicides; engagement of other technical methods of control such as refrigeration, irradiation and storage under inert atmosphere; and careful management to ensure that produce are not stored for unnecessarily long periods (Oyeniran, 1978) are some of the control measures already attempted.

In spite of the above attempts at controlling the adverse effects brought about by moulds associated with livestock feeds and similar products, more researches are still necessary before satisfactory solution to the problem could be achieved.

The aims of this study consequently are to identify the fungi associated with the spoliage of feeds and feed inputs and study their physiological and biochemical activities. Attempts were also made to

determine the roles of these microbes in relation to levels of mycotoxins often recorded in poultry feeds, and their ability to produce such toxins. This was done with a view to controlling contamination of livestock feeds by such fungi.

MATERIALS AND METHODS

Collection of Samples

Random samples (approximately 500g each) of poultry feeds and feed concentrates were collected from several feed millers, retailers and storage depots in poultry farms in South Western Nigeria. The compound rations collected include feed mashes for day-old chicks, growers, layers, broiler starters and broiler finishers. The feed concentrate samples collected were corn meal, groundnut cake meal, palm kernel meal, fish meal, blood meal, wheat offals, dried brewers grains, bone meal and oyster shell meal. Each sample was brought to the laboratory at the Department of Botany and Microbiology, University of Ibadan in a clean, labelled polythene bag and kept in the cold room at 5° C. All samples were analysed within two days of collection.

Storage history and nature of feeds collected for investigation.

The feed samples used for the various investigations were obtained from bags stored in the

feed depot and stores of the poultry houses for a period of not more than ten days after milling. The stores were always kept dry and neat thus ensuring that the bagged feeds were protected in all cases from direct contact with moisture.

Investigation on the nature of the feed samples used for the studies showed that they were relatively dry, powdery and not mashy. There was absence of offensive odours in all the feed samples collected. The temperature of the samples ranged between 30° C and 32° C which is equivalent to the ambient temperature. The capacity of the various feed samples to absorb moisture varied from one sample to the other as indicated by their moisture contents which ranged between 8.86% and 18.92% (Tables 1 and 2).

Determination of moisture contents and pH of samples

The moisture content was analysed by drying 50g of each sample in triplicates to constant weight at 80° C in an oven for 24 hrs using pre-weighed crucibles. Dried samples were allowed to cool down in a desiccator before weighing after which the % moisture content was calculated.

The pH values of 1:1 (w/v) feed; water suspensions of the samples in triplicates were determined with the aid of an electronic pH meter.

Analysis of aflatoxins content in samples

A modification of the method of Anon (1976) was used. This involved placing 25g of the dried powdery test sample into a 250ml conical flask. Twelve and half grams of diatomaceous earth, 12.5ml of water and 125ml of chloroform were added. The flask was corked and stirred for 30 minutes using a wrist action shaker. The filtrate was collected using a funnel and filter paper. Fifty millilitres of the extract collected above were mixed with 100ml of n-hexane and transferred into a chromatography column containing glass wool, chloroform, sodium sulphate and silica gel. The tap was opened to allow the liquid to flow until it was just above the upper surface of the sodium sulphate layer. The tap was then closed and 100ml of diethyl ether were added and again allowed to flow until it was just above the upper surface of the sodium sulphate layer. The column was finally eluted with 150 ml of the mixture of chloroform and methanol 97: 3(v/v).

The eluate obtained was collected in the round bottomed flask of the rotary evaporator and evaporated almost to dryness at a temperature not exceeding 50° C. Two millilitres of chloroform were then added to re-dissolve for spotting. Five, 10, 15, 20 and 25ul of the extract obtained was spotted on thin-layer chromatography (TLC) plate along with similar concentrations of standard aflatoxins (B_1, B_2, G_1 and G_2). The chromatogram was developed in the dark using 5% (v/v) acetone in chloroform. The plates were dried and examined under longwave ultra-violet light. Presence of aflatoxins in the sample was indicated by a purple blue fluorescence with the same rate of flow (R_f) as that of the qualitative standard.

Visual estimation of each aflatoxin was carried out by comparing the intensities of fluorescence of the extract spots with that of the standard solution spots (Anon, 1976). The content in micrograms of aflatoxin per kg of sample i.e. parts per billion (ppb) was subsequently calculated according to Anon. (1976).

Isolations and identification of isolates

Waksman's direct inoculation method (Crisan, 1964) was used. Two grammes of each feed sample were placed

in 11.5cm diam. Sterile Petri-dishes on 20ml of agar medium. Ten-fold serial dilution method of Waksman and Fred (1922) was also carried out to estimate the relative abundance of each fungal species in the sample. For this method, 20g of feed sample were added to 200ml of sterile water in a litre flask and vigorously shaken to obtain a suspension. Ten-fold dilutions were subsequently prepared from the suspension by adding 10ml portions to 100ml, 1000ml and 10,000ml of sterile water. One ml of each dilution was introduced into Petri-dishes into which sterilized molten media were then added. The direct hyphal isolation technique (Cooney and Emerson, 1964) was also used to isolate fungi from mouldy feeds. The media used for the isolations were:

Malt extract agar (MEA). This was prepared by suspending 50g of dehydrated 'Oxoid' malt extract agar in one litre of distilled water,

Potato dextrose agar (PDA): prepared by dissolving 39g of dehydrated 'Bact' potato dextrose agar in one litre of distilled water,

Yeast extract agar (YEA): Sucrose, 20g; yeast extract, 5g; $NaNO_3$, 0.2g; KCl, 0.05g; sodium glycerophosphate,

0.05g; K_2SO_4, 0.03g; $FeSO_4 . 7H_2O$, 0.01g; agar, 15g; distilled water, 1000ml,

Malt-yeast-extract agar (MYEA): Malt extract, 20g; yeast extract, 2.5g; $NaNO_3$, 0.2g; KCl, 0.05g; sodium glycerophosphate, 0.05g; K_2SO_4 0.03g; $FeSO_4.7H_2O$, 0.01g; agar, 15g; distilled water, 1000ml,

Czapek-Dox agar (CDA): Sucrose, 30g; $NaNO_3$, 2g; K_2HPO_4, 1g; $MgSO_4.7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4.7H_2O$, 0.01g, yeast extract, 0.5g; agar, 15g; distilled water, 1000ml. The pH of all media was adjusted to pH 6.8 before autoclaving them at 121° C for 15min. Incubation of the inoculated plates was carried out at each of the following temperatures: 15°, 25°, 30°, and 40° C. All the Petri-dishes were examined daily for fungal growth and spores of individual species were transferred to fresh agar plates as soon as they appeared.

The isolated fungal species were examined under stereoscopic microscope to study their vegetative characteristics. Tentative identifications were made by reference to Raper and Fennel (1965) and Raper and Thom (1949). The fungal cultures were subsequently sent to the Commonwealth Mycological Institute, Kew, England for confirmation .

Physiological Studies

The test fungi used for the physiological experiments were: Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, Rhizomucor pusillus and Rhizopus arrhizus. The growth medium used had the following composition: D-glucose, 10g; KNO_3, 3.5g; $MgSO_4 . 7H_2O$, 0.75g; KH_2PO_4, 1.75g; distilled water, 1000ml. For inoculum, the fungi were grown for 3 to 5 days on MYEA medium in Petri-dishes at 30° C. Each flask of the growth medium was inoculated with one disc of agar plus mycelium (5mm diam) of the test fungus obtained by using a sterile cork borer. After the required period of incubation, the mycelium produced was harvested with oven-dried and weighed filter papers (Whatman No.1 15cm diam). The filter papers with mycelia were dried in an oven at 80° C for 24h, cooled in a desiccator and weighed. The difference in weight gave the weight of the mycelium.

Growth temperature relations of isolates

Thirty millilitre-portions of the growth medium were dispensed into each of several 250-ml conical flasks and autoclaved. The flasks (3 for each fungus)

were inoculated with the test fungi and incubated for 6 days at each of the temperatures: 15°, 20°, 25°, 30°, 35°, 40°, 45° and 50° C. Preliminary experiments showed that peak mycelia production was on the 6th day of incubation. The mycelia produced were harvested, dried and weighed.

Effects of pH on growth

The pH of the growth medium was adjusted to values between 4.0 and 8.0 with 0.2N NaOH or 0.2N HCl where appropriate. These were stabilized with citric-acid-phosphate buffers (Child et al; 1973) and dispensed in 30ml portions into each of several 250ml conical flasks. The contents of each flask were sterilized by millipore filtration and the flasks (3 for each fungus) inoculated with test fungi. All the flasks were incubated for 6days at 30° C after which the mycelia produced were harvested and dried weights determined.

Carbon-source utilization for growth and sporulation

The carbon sources used were glucose, fructose, galactose, sorbose, xylose, sucrose, lactose, cellobiose, raffinose, maltose, pectin, soluble starch, dextrin and carboxymethyl cellulose. The appropriate

weights of each carbon source to yield 0.8g carbon/l was incorporated into the growth medium as the sole carbon source. Because of the complex nature of pectin, starch, dextrin and carboxymethyl cellulose, 10g/l of basal medium were used. The media were dispensed in 30ml portions into each of several 250-ml conical flasks. Due to the thermolabile nature of the simple sugars, they were autoclaved at 115° C for 10minutes. The flasks (6 for each fungus) were inoculated with the test fungi and incubated at 30° C for 6days. The control flasks were without any carbon source. At the end of incubation period, the dry weights of mycelia produced were determined for each fungus. Sporulation was also determined by direct cell count technique with the aid of a haemocytometer slide.

Utilization of native cellulose (filter papers).

This was determined using the modified method of Garrett (1962). Wads (10per flask) of Whatman No 1 (9cm diam) filter papers were oven dried, weighed and put in each of several 250-ml conical flasks before autoclaving. Eggins and Pugh's nutrient solution (Eggins and Pugh, 1962) of the following composition: $(NH_4)_2 SO_4$, 0.5g; KH_2PO_4, 1.0g; KCl, 0.5g; $CaCl_2.2H_2O$,

0.1g; $MgSO_4.7H_2O$, 0.2g; Yeast extract 0.5g; Asparagine, 0.5g; Distilled water, 1000ml, was separately autoclaved at 121° C for 15minutes and 20ml portions was aseptically added to each flask. The flasks (3 for each fungus) were inoculated with the test fungi and incubated at 30° C. Control flasks were inoculated with blank agar discs. After 21 days of incubation, the filter papers plus the mycelia produced were oven-dried at 80° C and weighed. The wads were not washed and loss in weight was taken to be due to the utilization of the native cellulose by the test fungi during respiration. The ability of each fungus to utilize cellulose was expressed as net loss in weight of cellulose after subtracting the values of the control flasks.

Nitrogen-source utilization for growth and sporulation

The inorganic nitrogen sources used were KNO_3, $NaNO_3$, NH_4NO_3, $Mg(NO_3)_2$, NH_4Cl, and $(NH_4)_2SO_4$ while the organic nitrogen sources (amino acids) were: glycine, DL-methionine, B-alanine, guanine, L-glutamic acid, L-asparagine, DL-tryptophan, casein hydrolysate, amino-n-butyric acid and L-aspartic acid. The appropriate weight of each nitrogen source to yield 0.5g nitrogen/l

was incorporated into the growth medium as the sole nitrogen source. The media were dispensed in 30ml portions into each of 250ml flasks and the pH was adjusted to 6.5 before autoclaving at 115° C for 10minutes. The flasks (6 for each fungus) were inoculated with the test fungi and incubated at 30° C for 6 days. The control flasks were without any nitrogen source. At the end of the incubation period, the dry weights of mycelia in three flasks were determined for each fungus. Sporulation in the remaining three flasks was determined by direct cell counts.

Mycelial growth and sporulation on feed infusion medium

Livestock feed infusion medium used was prepared by boiling 100g layers mash with 1000ml distilled water, cooling and squeezing out the liquid content from muslin cloth (Ogundero, 1983). The medium was dispensed in 30 ml portions into each of 250ml conical flasks and sterilized by autoclaving at 121° C for 15minutes. The flasks were inoculated with test fungi and incubated at 30° C for 6 days. At the end of the incubation period, the dry weights of mycelia in three flasks were determined for each fungus. Sporulation in

the remaining three flasks was determined by direct cell counts.

Aflatoxin Studies

Test for the production of aflatoxins by isolates

A modified Czapek-Dox medium was used. The medium contained: Sucrose, 30.0g; $NaNO_3$, 2.0g; K_2HPO_4, 1.0g; $MgSO_4.7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4.7H_2O$, 0.01g; yeast extract, 0.5g; distilled water, 100ml. A 2%(w/v) finely ground toxin-free yellow maize variety (Farz 7), or toxin-free white maize variety (Farz 34) was incorporated into the medium. Inclusion of ground maize in a growth medium is known to enhance aflatoxin production by microfungi (Schroeder, 1969). The medium was dispensed in 30 ml portions into each of 250ml conical flasks and sterilized by autoclaving at 121° C for 15 minutes. The flasks (3 for each fungus) were inoculated with agar and mycelia discs (5mm diam) of the test fungi. Uninoculated flasks served as control. The flasks were incubated at 30° C for 6 days after which the culture filtrates were analysed for aflatoxins as previously described. The results were reported as micrograms of aflatoxins per litre of medium.

Aflatoxin B_1 production with time by Aspergillus flavus and A. oryzae

The two test fungal species were used because the results of previous experiments showed them to be aflatoxin producers. The modified Czapek-Dox medium supplemented with finely ground yellow maize variety (Farz 7) was dispensed in 30ml portions into several 250 ml conical flasks and sterilized by autoclaving at 121° C for 15 minutes. Each flask was inoculated with an agar and mycelia disc (5mm diameter) of the test fungus. The inoculated flasks were incubated at 30° C. Harvesting of mycelia produced was carried out every 48 hours and the culture filtrates were analysed for aflatoxin B_1 content as previously described. Changes in the pH of the growth medium during the period of incubation were also recorded.

Effects of pH on aflatoxin B_1 production

The pH of the modified Czapek-Dox medium supplemented with ground yellow maize variety (Farz 7) was adjusted to values between 3 and 8 with 0.2N NaOH or 0.2N HCL where appropriate. These were stabilized with citric-acid-phosphate buffers (Child et al, 1973). The various media were dispensed in 30ml portions into each of 250 ml conical flasks. Sterilization was carried out by autoclaving at 121° C for 15 minutes. The pH values after autoclaving were regarded as the experimental pH. Three flasks were inoculated with each fungus and incubated at 30° C for 6 days. At the end of incubation, the culture filtrates were analysed for aflatoxin content.

Effects of incubation temperature on aflatoxin B_1 production.

Flasks of the modified Czapek-Dox medium supplemented with ground yellow maize variety (Farz 7) were inoculated with Aspergillus flavus or A. oryzae and incubated for 6 days at each of the temperatures: 15°, 20° 25°, 30°, 35°, 40° and 45° C. At the end of

the incubation period, the culture filtrates were analysed for aflatoxin B_1 production.

Utilization of feed concentrates as substrates for aflatoxin production by Aspergillus flavus and A. oryzae.

This study was carried out to determine which of the feed concentrates enhances aflatoxin production by the toxin producing fungi. The feed concentrates used for this study were: corn, groundnut cake, palm kernel, fish and blood meals. Others were wheat offals, dried brewers grains, oyster shell and bone meals. Each feed concentrate sample was dried in the oven at 80° C for 24hrs after which aflatoxins in 25g portions was determined. Another 25g portions were placed in each of several 250 ml conical flasks and autoclaved at 121° C for 20 minutes. Sterile distilled water (13.5ml) was later added aseptically to the content of each flask to obtain a moisture content of 35%(w/w). Three flasks for each feed concentrate were then separately inoculated with 2ml of spore suspension ($20x10^6$spores/ml) of each test fungus. Control flasks were inoculated with sterile water. All flasks were

incubated at 30° C for 6 days after which the contents were analysed for aflatoxin B_1 production.

Analysis of fresh poultry droppings for aflatoxins

The detection of aflatoxins in poultry feeds and the possibility of the toxins being egested with the droppings necessitated this experiment. Poultry droppings are used in making compost, as farm yard manure (F.Y.M.) and fish feeds (Phillips, 1956; Akinyosoye, 1976). Fresh poultry droppings were collected from poultry farms and oven dried at 50° C for 48hrs. Test for aflatoxins was carried out on 25g of the dried sample (in triplicates) using the previously described methods.

Test for aflatoxins in poultry droppings previously inoculated with toxigenic fungal species (Aspergillus flavus and A. oryzae).

Twenty five grams of dried poultry droppings were placed into each of several 250ml conical flasks and autoclaved at 121° C for 15 minutes. Sterile distilled water (13.5ml) was later added aseptically to the content of each flask to obtain a moisture content value of 35%(w/w). Three flasks were separately inoculated with 2ml of spore suspension ($20x10^6$ spores/ml) of each test fungus. Control flasks were inoculated with sterile water. All flasks were incubated at 30° C for 6 days after which the contents were analysed for aflatoxins.

Extracellular enzyme production and assay:

Amylase production and activity

A starch-yeast extract medium (SYE) described by Chapman et al. (1975) was used in this study. The medium contained: starch, 5g; yeast extract, 2g; K_2HPO_4, 1g; $MgSO_4.7H_2O$, 0.5g; distilled water, 1000ml. It was dispensed in 30ml portions into each of 250ml conical flasks and sterilized at 121° C for 15minutes.

Each flask was inoculated with the test fungus and incubated at 30° C for 6 days. The culture filtrates were obtained through glass wool and then centrifuged. The amylase activity of the filtrate was determined using the dinitrosalicylic acid (DNSA) reagent method of Bernfeld (1951). To 9ml of 1% soluble starch prepared in 0.2m phosphate buffer (pH6.9) was added 1ml of culture filtrate. The reaction mixture was incubated for 1h at 45° C after which 1ml of the filtrate/starch reaction mixture was transferred into a test-tube. To this was added 3ml of DNSA reagent (prepared by adding 1g of DNSA to 20ml of 2N NaOH and mixed with 20g of potassium sodium tartrate dissolved in 100ml of distilled water). The mixture was boiled for 5 minutes in water bath and cooled under running tap water. The absorbance was read at 540nm using an Sp6-250 spectrophotometer against a reagent blank. The reducing sugars released by the action of the culture filtrate on starch molecules was read off from a standard curve constructed using increasing values (0.1-2.0mg/ml) of maltose. The results are reported as amount of maltose (mg/ml) released by culture filtrate.

Amylase production with time on SYE medium

The inoculated SYE media were incubated at 30° C. Mycelial growth of the organism was allowed to proceed for 24hours after which harvesting was done daily for 10days and the culture filtrate analysed for amylase activity using the DNSA method earlier described.

Effects of carbon sources on amylase production

For this experiment SYE medium used contained either 1% glucose or dextrin, starch, pectin and carboxymethyl cellulose (CMC) as the only carbon source. Livestock feed infusion broth prepared by boiling 100g of layers mash with 1000ml distilled water was also used. The media were dispensed in 30ml portions into each of 250ml conical flasks and sterilized by autoclaving at 121° C for 15minutes. The glucose medium was sterilized at 115° C for 10minutes. Each flask was inoculated with the test organism and incubated at 30° C for 6 days after which the culture filtrates were analysed for amylase activity.

Effects of pH of assay medium on amylase activity

Three flasks of sterile SYE media for each test fungus were inoculated and incubated at 30° C for 6 days. At the end of the incubation period, the amylase activity of the culture filtrates was determined at different pH levels. One per cent soluble starch solution was prepared and buffered to different pH (3-8) with citric acid phosphate buffers. To 9ml of the buffered starch solution was added 1ml of culture filtrate and the mixture incubated for 1h at 45° C. The amylase activity was then determined by the DNSA reagent method.

Effects of temperature on amylase activity

Three flasks of sterile SYE medium for each test fungus were inoculated and incubated at 30° C for 6 days. At the end of incubation period, the amylase activity of the culture filtrates was determined at 20°, 30°, 40°, 45°, 50°, 55° and 60° C.

Cellulase production

An oat-meal chaff medium (OCM) described by Ogundero (1978) was used. It was prepared by soaking

Quaker oats in water for 24hrs after which the starch contents were flushed out by squeezing the flakes collected in a piece of muslin cloth followed by rinsing in several changes of tap water. The chaffs obtained were dried in the oven at 80° C for 48h and a 2% (w/v) suspension of these in distilled water served as the growth medium. The medium was dispensed in 30ml portions into 250ml conical flasks and sterilized by autoclaving at 121° C for 15 minutes. Each flask was inoculated with the test organism and mycelial growth was allowed to continue for 6 days at 30° C.

Cellulase assay

At the end of the incubation period, the cultures were filtered through glass wool. The cellulase activities of the filtrates were determined using the method of Reese and Mandels (I963). The assay medium was 0.55% carboxymethyl cellulose-sodium salt in 0.2M acetate/acetic acid (pH5.5). Nine ml of this medium were incubated with lml of the fungus filtrate for lh at 45° C. Filtrates of uninoculated flasks were also assayed to serve as control. The amount of reducing sugars (maltose and glucose) released by the culture

filtrates from CMC was estimated by adding 3ml of dinitrosalicylic acid (DNSA) reagent to 1ml of the filtrate CMC reaction mixture. The absorbance was determined at 540nm using an Sp6-250 spectrophotometer. Transmittance of the spectrophotometer was set at 100% with the control sample. The amount of reducing sugars released was read off from a standard curve prepared with aqueous solutions (0.1-2.0 mg/ml) of glucose. The results are reported as total reducing sugars (mg/ml) released by culture filtrate.

Cellulase production in relation to time

Inoculated flasks of oat-chaff medium were incubated at 30° C and at 24h intervals, three flasks of each fungus were removed, filtered and the celluloytic activities of the culture filtrates determined. This was done for 10days.

Effects of carbon sources on cellulase production

A similar experiment on the effects of carbon sources on amylase production was repeated. At the end of the incubation period, the culture filtrates were assayed for cellulase activity.

Effects of pH of assay medium on cellulase activity

Three flasks of sterile oat-chaff medium for each test fungus were inoculated and incubated at 30° C for 6 days. At the end of the incubation period, the cellulase activity of the culture filtrates was determined at different pH levels. The assay medium of 0.55% CMC was prepared and buffered to different pH (3-8) with citric acid-phosphate buffers. Cellulolytic activities were determined by incubating 1ml of fungus filtrate with 9ml of each of the CMC solutions for 1h at 45° C and the amount of reducing sugars released estimated by the DNSA reagent method.

Effects of temperature of assay medium on cellulase activity

Three flasks of sterile oat-meal chaff medium for each test fungus were inoculated and incubated at 30° C for 6days. At the end of incubation period, the cellulase activity of the culture filtrates was determined at 20°, 30°, 40°, 50°, 60°, 70° and 80° C.

Protease production

For this experiment, a poultry feed medium described by Ogundero (1983) was used. It contained: Poultry feed, 15g; casein, 4.0g; distilled water, 1000ml. The mixture was thoroughly shaken before 30ml portions were dispensed into several 250ml conical flasks. All flasks were then sterilized by autoclaving at 121° C for 15 minutes. Inclusion of casein in a growth medium for protease synthesis is known to enhance extracellular protease production by micro-fungi (Lasure, 1980). Each flask was inoculated with a 5mm diameter of agar and young mycelium disc of test fungus. Uninoculated flasks served as control. Mycelial growth was allowed to continue for the desired period before the culture filtrates were collected and assayed for protease activity.

Protease assay

Growth of the test fungi was allowed to proceed for 6 days at 30° C before the cultures were filtered through glass wool to obtain the culture filtrates. The protease activities of filtrates were determined as follows. One-ml of culture filtrate was added to an

assay mixture containing casein (4ml of 1% w/v) in 0.05 M phosphate buffer (pH 6.0) and incubated for 1h at 45° C (McDonald and Chen, 1965). At the end of the incubation period, an equal volume of 5% (w/v) trichloroacetic acid (TCA) was added to stop the reaction and to precipitate excess proteins. These were filtered through two layers of glass fibre papers and the absorbance of the filtrates determined with a Beckman 25 Dual Beam Spectrophotometer at 280nm. Transmittance of the spectrophotometer was set at 100% with the control sample. The amount of tyrosine released was read off from a standard curve prepared with aqueous solutions (0.1-2.5moles/ml) of tyrosine. The results are reported as micromoles of tyrosine solubilized per millilitre.

Protease production in relation to time

Inoculated flasks of the poultry feed-casein medium were incubated at 30° C. At 24h intervals, three flasks of each fungus were removed, filtered and the protease activities of the culture filtrates determined.

Effects of pH of assay medium on protease activity

Inoculated flasks of the poultry-feed-casein medium were incubated at 30° C for 6 days. The culture filtrates were obtained and used to assay the effects of pH on protease activity. The assay medium used was buffered to different pH (3-8) with citric-acid-phosphate buffers (Child et al; 1973).

Effects of temperature of assay medium on protease activity

Three flasks of sterile poultry-feed-casein medium for each test fungus were inoculated and incubated at 30° C for 6 days. At the end of the incubation period, the protease activity of the culture filtrates was determined at 20°, 30°, 40°, 50° and 60° C.

Lipase production

The basal medium used for this study was a 2%(w/v) suspension of dried oat-meal chaffs supplemented with 5g/litre of yeast extract. The medium was dispensed in 30ml portions into 250 ml conical flasks and sterilized at 121° C for 15 minutes. The pH of the medium after

autoclaving was 6.8. Each flask was inoculated with the test fungus and incubated at 30° C for 6 days.

Lipase assay

After the incubation period, the cultures were filtered through glass wool to obtain the culture filtrates. The extracellular lipase activity of the culture filtrates was measured using the method of Somkuti and Babel (1968). The assay medium contained: groundnut oil, 1ml; sodium taurocholate, 0.4g; 0.1 M $CaCl_2$, 1ml; acetate buffer 6ml, 0.1M (pH 5.8) all in a 250ml Erlenmeyer flask. Three ml of each of the culture filtrate were added and the reaction mixture incubated at 45° C for 2h. At the end of the incubation period, 40ml of absolute ethanol were added and the liberated fatty acids titrated with 0.02N NaOH with two drops of phenolphthalein (1g/500ml of 50% ethanol) as indicator. The assay mixture containing 3 ml of boiled extract served as the control. The amount of free fatty acids (ffa) liberated by the lipases of the fungi during the incubation period were expressed as micromoles of ffa mg protein per ml in 2h (Somkuti and Babel, 1967).

Lipase production in relation to time

Inoculated flasks of the basal medium were incubated at 30° C. At 24h intervals, three flasks of each fungus were removed, filtered and the culture filtrates analysed for lipase activity using the method of Somkuti and Babel (1968).

Effects of pH of assay medium on lipase activity

Inoculated flasks of the basal medium were incubated at 30° C for 6 days. The pH of the groundnut oil assay mixture was adjusted to different levels (3-8) with 0.2N HCl or 0.2N NaOH where appropriate and then stabilized with citric acid-phosphate buffers. The culture filtrates of each of the test fungi were then added and incubated at 45° C for 2h. The lipase activity was subsequently determined.

Effects of temperature of assay medium on lipase activity

Inoculated flasks of the basal medium were incubated at 30° C for 6 days ,after which the culture filtrates were obtained. The filtrate-groundnut-oil assay mixtures subsequently prepared were incubated at 30° , 35° , 40° , 45° , 50° , 55° and 60° C for 2h the lipase activity determined.

RESULTS

Sample analysis

Results on the analysis of poultry feed concentrates and compound ration samples collected on monthly basis from March 1984 to January 1985 are presented on Tables 1 and 2 respectively. Results on Table 1 show that the pH values recorded for the feed concentrates varied from one feed ingredient to another and also from month to month. For the corn meal samples the values ranged from pH 5.2 recorded in April, July and November to pH 5.6 recorded for March and September samples. For the months of May, June, August and January, pH values of 5.5, 5.3, 5.3 and 5.4 respectively were obtained. Groundnut cake meal samples had pH values ranging between 5.4 recorded in May, June and September and pH 5.8 recorded for samples collected in March and August 1984. The pH values recorded for the months of April, July, November and January were 5.7, 5.6, 5.6 and 5.6 respectively. The pH values obtained for palm kernel samples which were

TABLE 1

Moisture content(%) aflatoxin content (ppb) and pH of feed concentrates samples. Figures are means of 3readings from samples collected in each month.

Feed ingredient	Sample No:	1	2	3	4
	Date of collection:	March 1984	April 1984	May 1984	June 1984
	pH	5.6±0.12	5.2±0.04	5.5±0.09	5.3±0.10
	Moisture content(mc)	12.80±0.16	14.02±0.33	16.00±0.14	16.08±0.09
Corn meal	Aflatoxin B_1	10±0.0	50±0.0	60±10.0	120±10.0
	B_2	10±0.0	20±5.0	10±0.0	100±10.0
	G_1	0	0	40±5.0	20±5.0
	G_2	10±5.0	0	10±5.0	40±5.0
	pH	5.8±0.05	5.7±0.06	5.4±0.04	5.4±0.03
	Moisture content(mc)	13.02±0.04	13.89±0.09	14.80±0.11	15.05±0.03
Groundnut	Aflatoxin B_1	120±20.0	110±10.0	110±0.0	320±30.0
cake meal	B_2	20±0.0	40±5.0	40±5.0	100±5.0
	G_1	100±10.0	100±20.0	100±10.0	200±10.0
	G_2	10±0.0	10±0.0	0	40±0.0
Palm-kernel.	pH	NS**	NS	NS	NS
meal	Moisture content	"	"	"	"
	Aflatoxin B_1	"	"	"	"
Fish meal*	pH	NS	NS	NS	6.0±0.10
	Moisture content	"	"	"	11.80±0.18
Blood meal*	pH	NS	6.5±0.06	6.4±0.04	6.4±0.08
	Moisture content	"	14.76±0.21	15.48±0.36	15.49+0.51
Wheat offals*	pH	6.7±0.31	6.7±0.06	6.7±0.10	6.8±0.06
	Moisture content	13.94±0.19	13.96±0.19	13.88±0.11	13.98±0.16
Dried brewers*	pH	5.7±0.3	5.7±0.06	5.8±0.06	5.7±0.06
grains	Moisture content	9.62±0.08	9.74±0.03	9.72±0.11	9.86±0.07
Oyster shell*	pH	8.8±0.09	NS	NS	NS
meal*	Moisture content	10.04±0.07	"	"	"
Bone meal*	pH	6.4±0.06	6.4±0.08	6.7±0.11	6.5±0.06
	Moisture content	10.58±0.14	10.59±0.11	10.84±0.08	11.20±0.0

* No aflatoxin detected in samples.

TABLE 1 (Contd)

Moisture content(%) aflatoxin content (ppb) and pH of feed concentrates samples. Figures are means of 3readings from samples collected in each month.

Feed ingredient	Sample No:	5	6	7	8	9
	Date of collection:	July 1984	August 1984	Sept.1984	Nov.1984	Jan.1984
	pH	5.2±0.07	5.3±0.04	5.6±0.13	5.2±0.09	5.4±0.02
	Moisture content(mc)	17.63±0.42	18.92±0.71	16.09±0.13	13.66±0.08	11.20±0.26
Corn meal	Aflatoxin B_1	123.3±5.8	153.3±5.8	100±20	10±0.0	10±0.0
	B_2	100±10.0	83.3±5.8	20±0.0	16.7±5.8	10±0.0
	G_1	40±5.0	20±5.0	10±0.0	0	10±5.0
	G_2	0	0	0	0	0
	pH	5.6±0.05	5.8±0.03	5.4±0.06	5.6±0.03	5.6±0.04
	Moisture content(mc)	16.44±0.40	16.90±0.31	14.96±0.08	13.08±0.51	12.60±0.82
Groundnut	Aflatoxin B_1	326±11.5	570±17.3	110±10.0	120±20.0	100±0.0
cake meal	B_2	100±0.0	80±0.0	20±5.0	20±10.0	10±10.0
	G_1	150±20.0	100±0.0	80±10.0	60±0.0	56.7±5.8
	G_2	40±5.0	40±0.0	10±0.0	0	0
Palm-kernel.	pH	5.2±0.06	NS	5.2±0.05	5.0±0.02	NS
meal	Moisture content	13.02±0.08	"	11.50±0.02	10.92±0.09	"
	Aflatoxin B_1	20±0.0	"	20±0.0	20±5.0	"
Fish meal*	pH	6.2±0.1	5.8±0.06	6.3±0.10	6.3±0.06	NS
	Moisture content	12.06±0.51	10.64±0.48	9.74±0.09	8.86±0.32	"
Blood meal*	pH	6.3±0.06	6.3±0.06	6.5±0.05	6.3±0.15	6.3±0.06
	Moisture content	16.60±0.44	16.37±0.33	15.21±0.42	15.37±0.31	12.48±0.34
Wheat offals*	pH	6.4±0.12	6.8±0.20	6.6±0.11	6.6±0.22	6.7±0.06
	Moisture content	14.70±0.17	14.62±0.09	14.60±0.13	13.98±0.08	12.04±0.18
Dried brewers*	pH	5.7±0.16	5.7±0.12	5.7±0.06	5.8±0.11	5.7±0.06
grains	Moisture content	9.86±0.04	10.04±0.21	10.74±0.20	9.82±0.08	9.80±0.05
Oyster shell*	pH	8.2±0.05	8.4±0.07	9.0±0.10	8.2±0.10	8.4±0.10
meal*	Moisture content	11.43±0.11	10.90±0.09	10.98±0.05	9.23±0.09	9.24±0.16
Bone meal*	pH	6.5±0.09	6.8±0.23	6.3±0.08	6.4±0.10	6.5±0.12
	Moisture content	11.66±0.24	11.80±0.18	10.88±0.09	10.21±0.17	10.24±0.36

* No aflatoxin detected in samples

** No sample collected.

collected only in July, September and November were 5.2, 5.2 and 5.0 respectively. The results also show that pH values of 6.0, 6.2, 5.8, 6.3 and 6.3 were recorded for the fish meal samples collected in June, July, August, September and November respectively. For the blood meal samples, the pH ranged from 6.3 which was recorded in July, August, November and January samples and 6.5 recorded for April and September samples. The wheat offals sample collected in July had the lowest pH value (6.4) for this feed concentrate while pH 6.8 recorded in June and August was the highest. For the dried brewers grains samples pH 5.7 was recorded for each of the months except in May and November when the pH of sample was 5.8. Oyster shell meal samples had comparatively higher pH values ranging from 8.2 recorded for July and November samples to 9.0 which was obtained only in September. The pH values for March, August and January oyster shell samples were 8.2, 8.4 and 8.4 respectively. The bone meal samples had pH values ranging between 6.3 in September and 6.8 in August. On the whole, for all the feed concentrates, the lowest pH value was 5.2 recorded for some corn meal samples and palm kernel meal samples.

The highest was pH 9.0 recorded for the September sample of oyster shell meal.

Results of the moisture content analysis showed that the corn meal samples had values ranging from 11.20% in January and 18.92% in August. For the groundnut cake meal, 12.60% recorded in January was the lowest while 16.90% obtained in August was the highest. The results also showed that 13.02%, 11.50% and 10.92% were the moisture contents of palm kernel meal samples collected in July, September and November respectively. Out of the five months during which fish meal samples were analysed, the highest moisture content (12.06%) was recorded in July and the lowest (8.86%) in November. For June, August and September the respective % moisture contents were: 11.80, 10.64 and 9.94. For the blood meal samples, the highest %moisture content (16.60) was recorded in July and the lowest (12.48) recorded in January. The results also showed that wheat offals samples analysed in July, August and September recorded highest % moisture content values of 14.70, 14.62 and 14.60 respectively while 12.04, 13.94, 13.96 and 13.88 recorded for January, March, April and May samples respectively were

the lowest. The dried brewers grains samples analysed in September and March had % moisture contents of 10.74 (highest) and 9.62 (lowest) respectively. For the oyster shell meal samples the % moisture contents (11.43, 10.90 and 10.98) recorded in July, August and September respectively were the highest while 9.23 and 9.24 recorded in November and January were the lowest. Bone meal samples had highest % moisture contents in June, July and August when 11.20, 11.66 and 11.80 respectively were recorded. The month with lowest % moisture content was November with 10.21% Generally for all the feed concentrate samples, highest % moisture contents were recorded either in July or August except for dried brewers grains samples with highest % moisture content in September. Similarly, the results showed that the lowest % moisture content was obtained in January for all the feed concentrate types except for the oyster shell meal with the November sample having the lowest % moisture content.

Results on aflatoxin contents of the feed concentrates showed that aflatoxins B_1, B_2, G_1 and G_2 were present in corn meal but all the four types of toxin were not present together in any of the samples

analysed. Aflatoxins B_1 and B_2 were both detected in all the corn meal samples. The results also showed that aflatoxin G_1 was absent in March, April and November samples while aflatoxin G_2 was present only in March, May, June and July samples. Generally, the months when samples had high aflatoxin contents were May, June, July, August and September when the aflatoxin B_1 contents were 60, 120, 123, 153 and 100 parts per billion (ppb) respectively. Low levels of aflatoxins was recorded in November, January and March samples when the aflatoxin B_1 content was only 10 ppb.

Results obtained for the groundnut cake meal samples showed that while aflatoxins B_1, B_2 and G_1 were present in all the samples analysed, aflatoxin G_2 was present only in May, November and January samples. The months when samples had high aflatoxin content were June, July and August when aflatoxin B_1 contents of 320, 326 and 570ppb were recorded respectively. Low aflatoxin B_1 content in groundnut cake meal samples was recorded in September, November, January, March, April and May when 110, 120, 100, 120, 110 and 110 ppb were recorded respectively. For the palm kernel meal samples, only aflatoxin B_1 was detected and the

quantity was 20ppb for each month. No aflatoxin was detected in samples of the remaining feed concentrates. On the whole, the results showed that the lowest aflatoxin B_1 content (10ppb) was recorded for the corm meal samples analysed in March, November and January while the highest of 570 ppb was recorded for the groundnut cake meal sample collected and analysed in August. For aflatoxin B_2, the lowest value (10ppb) was recorded in corn meal samples analysed in March, May and January and from the groundnut cake meal sample obtained in January. Highest aflatoxin B_2 content (100ppb) was recorded in corn and groundnut cake meal samples analysed in June and July. The results also showed that the highest aflatoxin G_1 content of 150ppb was obtained in groundnut cake meal sample analysed in July while the lowest value of 10ppb was recorded in corn meal samples analysed in September and January. For aflatoxin G_2, the highest level (40ppb) was recorded in groundnut cake meal samples analysed in June, July and August and corn meal samples analysed in June and July.

Results on the analysis of the compound rations are presented in Table 2. The pH values obtained for

the chicks mash ranged from 6.4 recorded for the June sample and 6.9 recorded for July and November samples. The pH values recorded for March, April, May, August, September and January samples were 6.7, 6.7, 6.8, 6.8, 6.8 and 6.8 respectively. The results also showed that the growers' mash sample of June had the lowest pH value (6.4) while the highest value of 6.8 was recorded in samples analysed in July, November and January. For the broiler starters mash the minimal pH (6.3) was recorded for the May sample while the highest value of 6.8 was recorded for April sample. Samples analysed in June and March had pH 6.7. The broiler finishers mash samples had the lowest value pH (5.9) recorded in March, July and September while the highest pH value (6.4) was recorded in April and August samples. For the layers mash samples, the pH ranged from 6.3 recorded in June and 6.6 obtained in April, September and January. On the whole, results on the pH of compound rations showed that 5.9 recorded for broiler finishers mash analysed in March, July and September was the lowest while the highest was 6.9 recorded for July and November samples of chicks mash.

TABLE 2

Moisture content(%), aflatoxin content (ppb) and pH of compound feeds samples.
Figures are means of 3 readings from samples collected in each month.

Compound feed	Sample No. / Date of Collection	1 March 1984	2 April '84	3 May 1984	4 June '84	5 July '84	6 Aug. 1984	7 Sep. 1984	8 Nov. 1984	9 Jan. 1985
Chicks mash	pH	6.7±0.06	6.7±0.06	6.8±0.11	6.4±0.09	6.9±0.15	6.8±0.06	6.8±0.06	6.9±0.15	6.8±0.13
	Moisture content	14.04±.19	15.02±0.31	15.44±034	16.84±0.11	17.31±0.72	16.69±041	16.00±0.06	15.40±0.22	14.42±0.11
	Aflatoxin B_1	0.0	0.0	10±0.0	10±0.0	10±0.0	10±0.0	0.0	0.0	0.0
Growers mash	pH	6.7±0.06	6.6±0.12	6.5±0.21	6.1±0.23	6.0±0.20	6.5±0.06	6.7±0.06	6.8±0.06	6.8±0.15
	Moisture content	13.22±0.22	14.82±0.09	14.98±0.41	15.50±0.57	15.90±0.11	1580±0.09	15.03±041	14.66±0.31	13.27±0.21
	Aflatoxin B_1	0.0	0.0	0.0	10±0.0	15±0.0	10±0.0	10±10	10±0.0	0.0
Broiler starters mash	pH	6.7±0.06	6.8±0.06	6.3±0.06	6.7±0.21	NS*	NS	NS	NS	NS
	Moisture content	12.44±0.32	14.88±0.18	14.96±023	16.30±051	"	"	"	"	"
	Aflatoxin B_1	0.0	0.0	0.0	15±5.0	"	"	"	"	"
Broiler finishers mash	pH	5.9±0.22	6.4±0.20	6.0±0.0	6.2±0.0	5.9±0.22	6.4±0.16	5.9±0.09	6.3±0.16	6.3±0.13
	Moisture content	14.87±0.21	14.88±0.33	15.91±027	15.94±0.30	17.60±038	1624±0.43	1580±0.19	1504±0.29	13.93±0.05
	Aflatoxin B_1	0.0	0.0	10±10	10±0.0	20±10	10±0.0	10±0.0	10±10	0.0
Layers' mash	pH	6.4±0.20	6.6±0.10	6.4±0.10	6.3±0.00	6.5±0.21	6.5±0.13	6.6±0.05	6.4±0.21	6.6±0.17
	Moisture content	14.36±0.09	14.94±0.21	16.88±0.35	18.36±0.31	1743±052	1725±0.29	1666±0.37	1430±0.10	13.78±0.09
	Aflatoxin B_1	10±0.0	10±0.0	10±0.0	40±10.0	20±0.0	25±5.0	25±10.0	25±0.0	0.0

* NS : No sample collected.

The % moisture contents of the compound ration samples ranged from 13.22% recorded for the growers mash samples analysed in January and 18.36% obtained for the layers mash in June. Generally, highest % moisture contents in samples were recorded in June, July and August for all the feed types while November, January and March samples had the lowest moisture contents.

Results on the aflatoxin contents showed that only aflatoxin B_1 was detected in the compound rations. The levels of the toxin ranged from 10ppb observed in several samples to 40 and 50 ppb obtained in layers mash sample analysed in June and broiler finishers mash sample analysed in July respectively. All the June, July and August samples contained the toxin although at low levels. Most of the samples analysed in November, January, March and April had no detectable amount of aflatoxin B_1.

Isolations

Six fungal species were isolated from the feeds and feed concentrate samples collected. The species were:-

Aspergillus flavus Link: Fr. (IMI 280819), A. fumigatus Fres. (IMI 280822) A. niger v. Tieghem (IMI 280823), A. oryzae (Ahlburg) Cohn (IMI 280831) Rhizopus arrhizus Fischer (IMI 280827) and Rhizomucor pusillus Lindt Schipper (IMI 280824)

The results presented on Table 3 show the average number of colonies of each fungal species per gram of feed concentrate that grew on the agar medium at 15°, 25°, 30° and 40° C. At 15° C, only a few colonies of Aspergillus flavus were observed from the plates inoculated with fish meals. No fungal colonies grew from the other feed ingredients. At 25° C, large number of colonies of all the fungi were obtained except Rhizomucor pusillus which was not present from all the feed concentrates. Aspergillus fumigatus colonies were not isolated from wheat offals, blood and bone meals at 25° C. At the same incubation temperature (25° C), no colonies of A. niger grew on plates inoculated with palm kernel meal, blood meal, wheat offals, dried brewers grains, oyster shell and bone meals. The results also showed that no colonies of A. oryzae were isolated from blood, oyster shell and bone meals. Similarly, no Rhizopus arrhizus colonies grew on plates

Table 3

Average number of colonies/g of feed ingredient obtained on malt-yeast-extract agar at different incubation temperatures. Each value is an average of estimates from nine Petri dishes.

Fungus	Incubation temperature (°C)	Corn meal	Ground-nut cake meal	Palm Kernel meal	Fish meal	Blood meal	Wheat offals	Driend brewers grains	Oyster shell meal	Bone meal
	15°C	1.0×10^{2}	0	0	2.1×10^{2}	0	0	0	0	0
Aspergillus	25	2.2×10^{6}	3.1×10^{5}	3.2×10^{3}	2.4×10^{5}	1.3×10^{4}	1.0×10^{3}	2.1×10^{4}	1.2×10^{2}	1.0×10^{2}
flavus	30	3.5×10^{6}	4.9×10^{6}	3.6×10^{4}	1.9×10^{6}	2.2×10^{4}	2.0×10^{3}	3.2×10^{4}	2.3×10^{4}	3.9×10^{4}
	40	8.0×10^{2}	3.3×10^{3}	9.0×10^{2}	4.0×10^{2}	2.0×10^{2}	3.1×10^{2}	8.5×10^{2}	1.0×10^{2}	0
	15	0	0	0	0	0	0	0	0	0
A. fumigatus	25	1.0×10^{2}	1.7×10^{2}	3.2×10^{2}	3.6×10^{2}	0	0	2.1×10^{2}	1.7×10^{2}	0
	30	4.0×10^{2}	4.6×10^{3}	9.0×10^{2}	2.9×10^{2}	4.5×10^{2}	2.1×10^{2}	2.9×10^{2}	1.0×10^{2}	2.0×10^{2}
	40	7.5×10^{4}	3.5×10^{4}	6.0×10^{4}	4.8×10^{4}	3.2×10^{4}	4.4×10^{2}	3.6×10^{2}	3.8×10^{2}	4.9×10^{2}
	15	0	0	0	0	0	0	0	0	0
A. niger	25	3.2×10^{2}	1.3×10^{3}	0	4.4×10^{3}	0	0	0	0	0
	30	9.5×10^{2}	4.0×10^{3}	1.2×10^{2}	6.2×10^{3}	0	0	1.5×10^{2}	0	0
	40	2.0×10^{2}	1.0×10^{2}	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0
A. oryzae	25	2.4×10^{5}	1.7×10^{5}	4.1×10^{2}	2.4×10^{2}	0	1.3×10^{2}	2.0×10^{2}	0	0
	30	2.1×10^{5}	5.0×10^{5}	6.2×10^{4}	6.0×10^{4}	0	3.7×10^{4}	2.0×10^{4}	0	0
	40	2.3×10^{2}	3.4×10^{2}	5.3×10^{2}	2.1×10^{2}	0	0	0	0	0
	(°C)									
	15	0	0	0	0	0	0	0	0	0
Rhizomucor	25	0	0	0	0	0	0	0	0	0
pusillus	30	9.1×10^{2}	3.2×10^{2}	2.1×10^{3}	1.9×10^{3}	2.0×10^{2}	1.0×10^{2}	2.3×10^{2}	0	0
	40	5.4×10^{5}	9.0×10^{5}	8.8×10^{4}	6.4×10^{4}	4.9×19^{4}	3.7×10^{2}	2.8×10^{3}	0	2.8×10^{3}
	15	0	0	0	0	0	0	0	0	0
Rhizopus	25	3.4×10^{3}	3.0×10^{3}	0	1.2×10^{2}	2.0×10^{3}	2.0×10^{3}	0	0	0
arrhizus	30	6.9×10^{5}	9.0×10^{4}	8.4×10^{5}	3.3×10^{4}	2.9×10^{5}	2.5×10^{4}	0	0	1.3×10^{2}
	40	2.1×10^{2}	1.2×10^{3}	0	1.0×10^{2}	2.4×10^{2}	2.2×10^{2}	0	0	0

inoculated with dried brewers grains, oyster shell, bone and palm kernel meals. For most of the fungal species, the largest number of colonies grew at 30° C. However, no colony of A. niger grew on plates inoculated with wheat offals, oyster shell, bone and blood meals. At the same temperature (30° C), A. flavus was not isolated from blood, oyster shell and bone meals while no colony of Rhizomucor pusillus grew from oyster shell and bone meals. The results also showed that plates inoculated with dried brewers grains and oyster shell meal gave no colony of Rhizopus arrhizus. At 40° C, A. flavus was not isolated from bone meal while A. niger was isolated only from corn and groundnut cake meals. A. oryzae colonies were not isolated from wheat offals, dried brewers grains, blood, oyster shell and bone meals. No Rhizomucor pusillus colony was obtained from oyster shell meal at the same temperature (40° C). For Rhizopus arrhizus, no colony count was recorded on plates inoculated with dried brewers grains, oyster shell, bone and palm kernel meals. Generally, the results showed that large number of fungal colonies were isolated from feed concentrates like corn, groundnut cake, fish, and palm kernel meals.

TABLE 4

Average number of fungal colonies/g of feed obtained on malt-yeast-extract agar at different incubation temperatures. Each value is an average of estimates from nine Petri-dishes.

Fungus	Incubation Temperature (°C)	Chicks mash	Growers Mash	Broiler starters mash	Broiler finishers mash	Layers mash
Aspergillus flavus	15	0	0	0	0	0
	25	3.1×10^{5}	4.0×10^{4}	2.1×10^{5}	1.9×10^{5}	3.0×10^{5}
	30	2.9×10^{6}	3.1×10^{6}	2.0×10^{6}	1.8×10^{6}	2.4×10^{6}
	40	6.2×10^{2}	7.1×10^{2}	5.1×10^{2}	6.0×10^{2}	4.4×10^{2}
A. fumigatus	15	0	0	0	0	0
	25	1.0×10^{2}	1.2×10^{2}	1.0×10^{2}	1.0×10^{2}	1.5×10^{2}
	30	2.4×10^{2}	2.5×10^{3}	1.9×10^{2}	2.0×10^{2}	4.2×10^{2}
	40	4.0×10^{3}	6.2×10^{3}	5.5×10^{3}	7.1×10^{2}	4.4×10^{4}
A. niger	15	0	0	0	0	0
	25	2.0×10^{2}	3.0×10^{2}	4.6×10^{2}	9.6×10^{2}	2.9×10^{2}
	30	2.0×10^{3}	2.5×10^{3}	2.5×10^{3}	2.4×10^{3}	6.0×10^{2}
	40	1.5×10^{2}	1.0×10^{2}	1.0×10^{2}	1.0×10^{2}	2.2×10^{2}
A. oryzae	15	0	0	0	0	0
	25	1.9×10^{4}	2.6×10^{3}	9.6×10^{3}	6.6×10^{4}	6.0×10^{4}
	30	6.0×10^{4}	4.8×10^{4}	2.5×10^{5}	9.2×10^{5}	4.0×10^{5}
	40	4.0×10^{2}	3.0×10^{2}	8.1×10^{2}	2.2×10^{2}	4.0×10^{2}
Rhizomucor pusillus	15	0	0	0	0	0
	25	0	0	0	0	0
	30	1.4×10^{2}	1.2×10^{2}	3.0×10^{2}	1.6×10^{2}	7.4×10^{2}
	40	4.4×10^{4}	2.6×10^{4}	4.9×10^{4}	4.0×10^{4}	8.4×10^{5}
Rhizopus arrhizus	15	0	0	0	0	0
	25	2.4×10^{2}	4.2×10^{2}	1.2×10^{2}	88	1.6×10^{3}
	30	5.2×10^{3}	4.9×10^{2}	7.2×10^{2}	5.6×10^{3}	5.5×10^{3}
	40	2.0×10^{2}	1.8×10^{2}	1.5×10^{2}	1.3×10^{2}	9.1×10^{2}

Comparatively low number of fungal colonies were obtained from oyster shell and bone meals.

The average number of fungal colonies isolated from the compound poultry feeds at 15°, 25°, 30° and 40° C are presented in Table 4. At 15° C no fungal colony grew on plates inoculated with any of the compound feeds. Growth of colonies of all the fungal isolates were observed at all other temperatures except Rhizomucor pusillus which gave no colony count at 25° C from all the compound feeds. The results also showed that largest number of colonies were obtained for the test species at 30° C except Aspergillus fumigatus and Rhizomucor pusillus which had highest number of colonies at 40° C.

Physiological Studies:

Growth temperature relations of isolates

The optimum temperature range of the isolated fungi was 30° -40° C (Fig. 1). At 15° C, no growth was recorded for Rhizomucor pusillus but the remaining five fungal species had slight growth. At 20° C all the isolated fungal species including Rhizomucor pusillus had slight growth. Comparatively, Rhizopus arrhizus

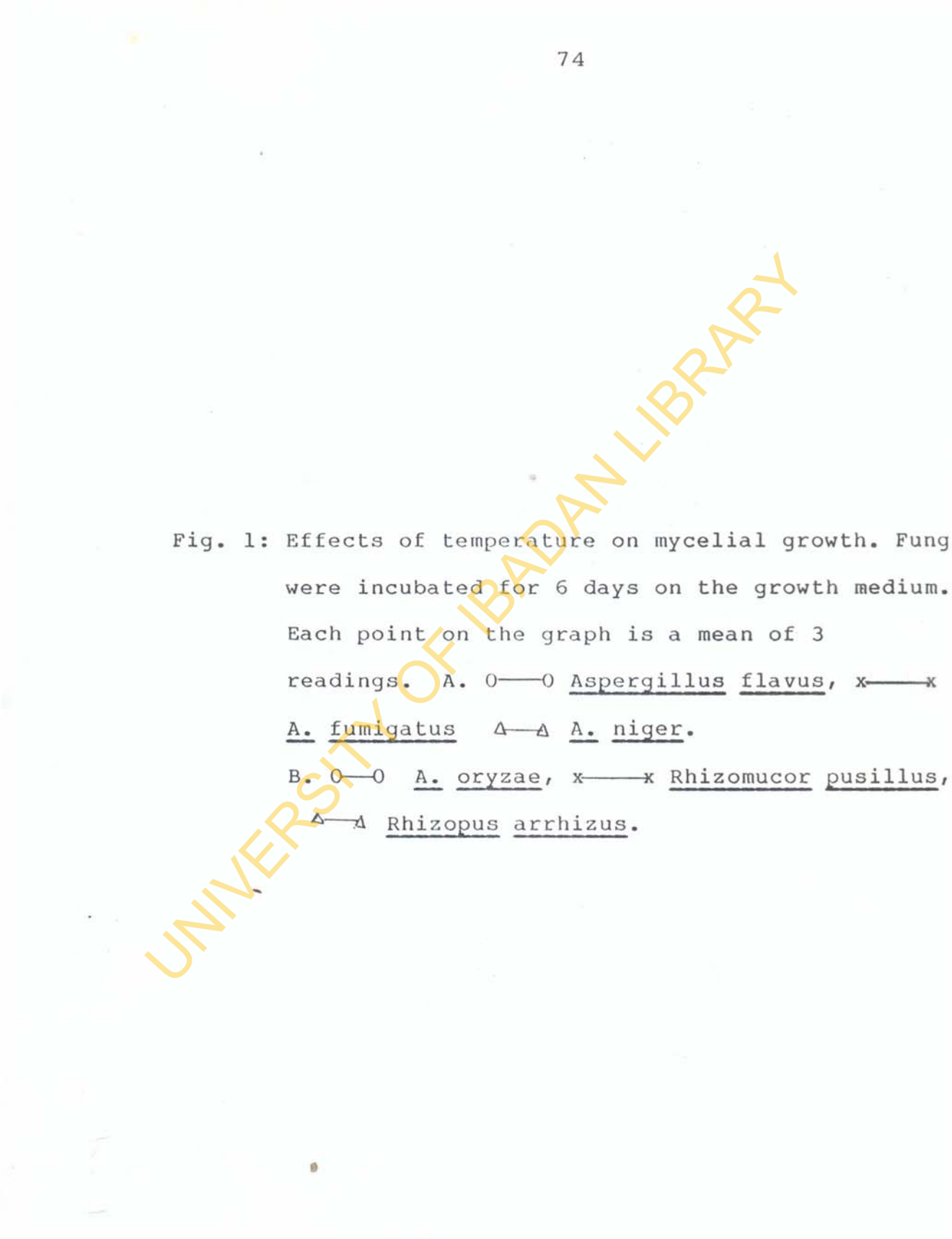

Fig. 1: Effects of temperature on mycelial growth. Fung were incubated for 6 days on the growth medium. Each point on the graph is a mean of 3 readings. A. O——O *Aspergillus flavus*, x——x *A. fumigatus* Δ——Δ *A. niger*. B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*.

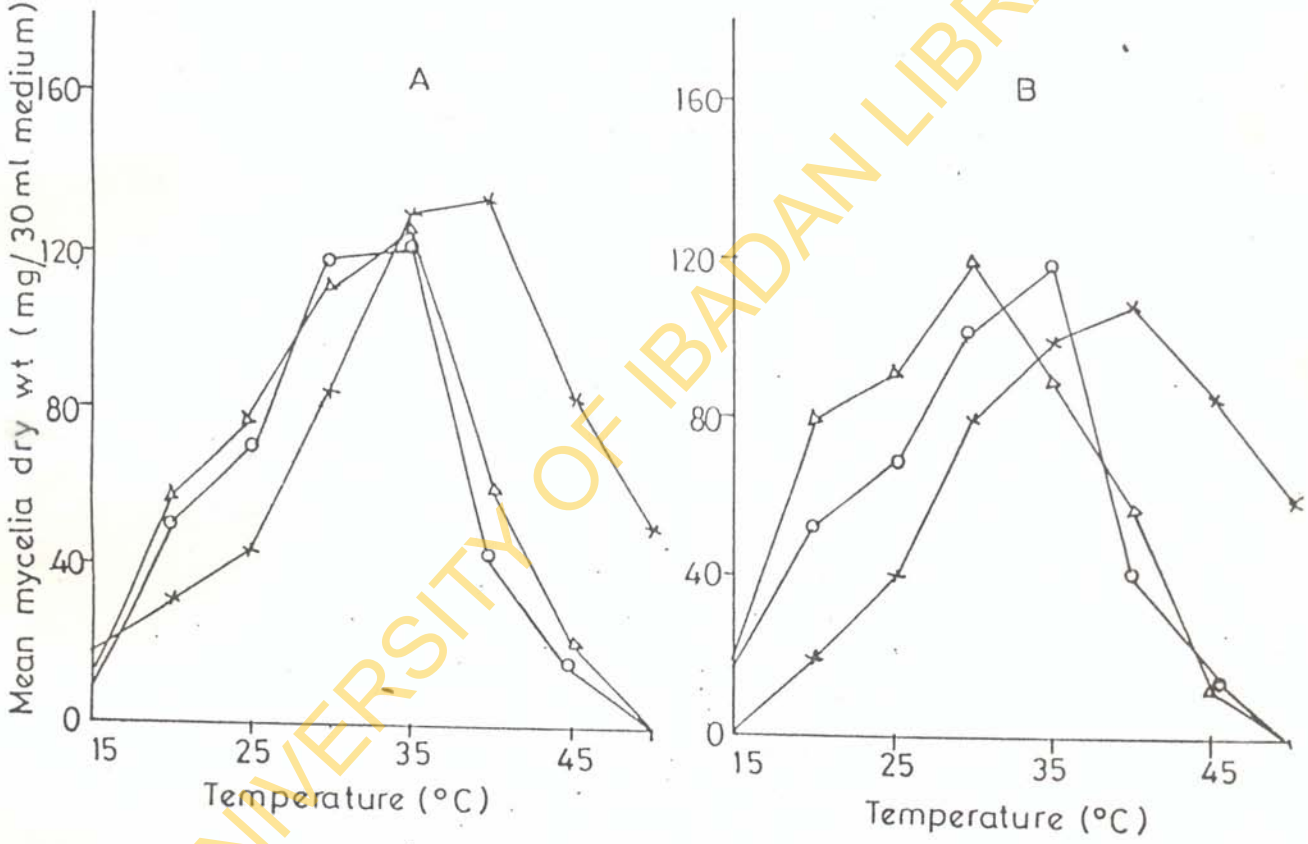

Fig. 1

recorded the best growth at 20° C. On the other end only A. fumigatus and Rhizomucor pusillus grew at 50° C. Growth was however recorded for all the isolated fungal species at 45° C although it was slight for all except A. fumigatus and Rhizomucor pusillus both of which had fairly good growth. The results also showed that Rhizopus arrhizus had its best growth at 30° C; A. flavus, A. niger and A. oryzae at 35° C while 40° C was the optimum temperature for the mycelial growth of A. fumigatus and Rhizomucor pusillus. For all the species, increase in growth was observed as the incubation temperature increases until the peak was attained at the optimum temperature. Further increase in temperature resulted in decrease in mycelial growth.

Effects of pH on growth

All the fungal isolates grew within the pH range (4-8) provided, although at varying degrees (Fig.2). The optimum pH range for the isolates was 5.5 to 6.0. The best mycelial growth was recorded for A. flavus, A. fumigatus and A. oryzae at pH 5.5. The remaining species: A. niger, Rhizomucor pusillus and Rhizopus arrhizus had peak mycelial growth at pH 6.0. Generally, the growth for each fungus increased with

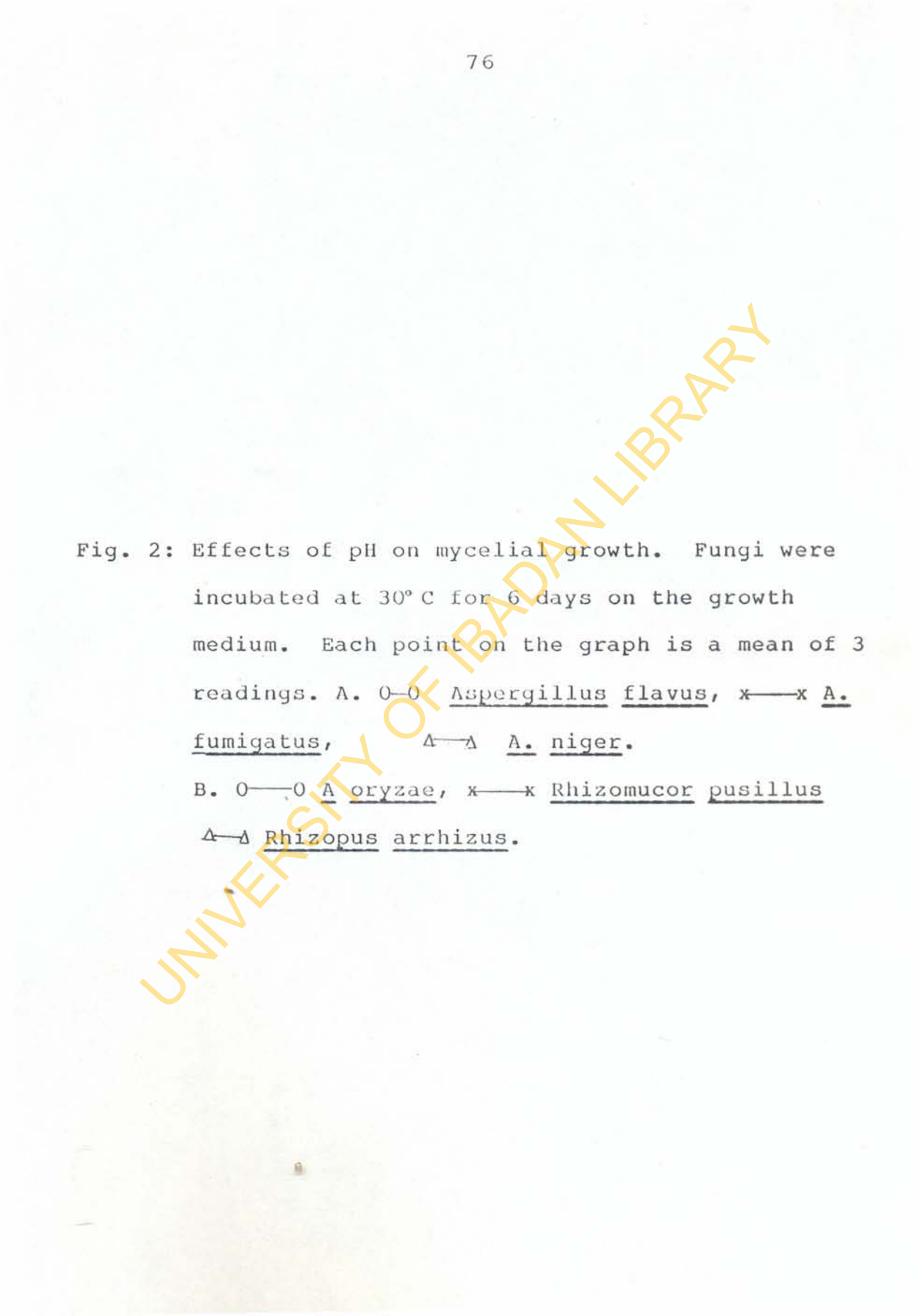

Fig. 2: Effects of pH on mycelial growth. Fungi were incubated at 30° C for 6 days on the growth medium. Each point on the graph is a mean of 3 readings. A. O—O Aspergillus flavus, x——x A. fumigatus, Δ—Δ A. niger.

B. O——O A oryzae, x——x Rhizomucor pusillus Δ—Δ Rhizopus arrhizus.

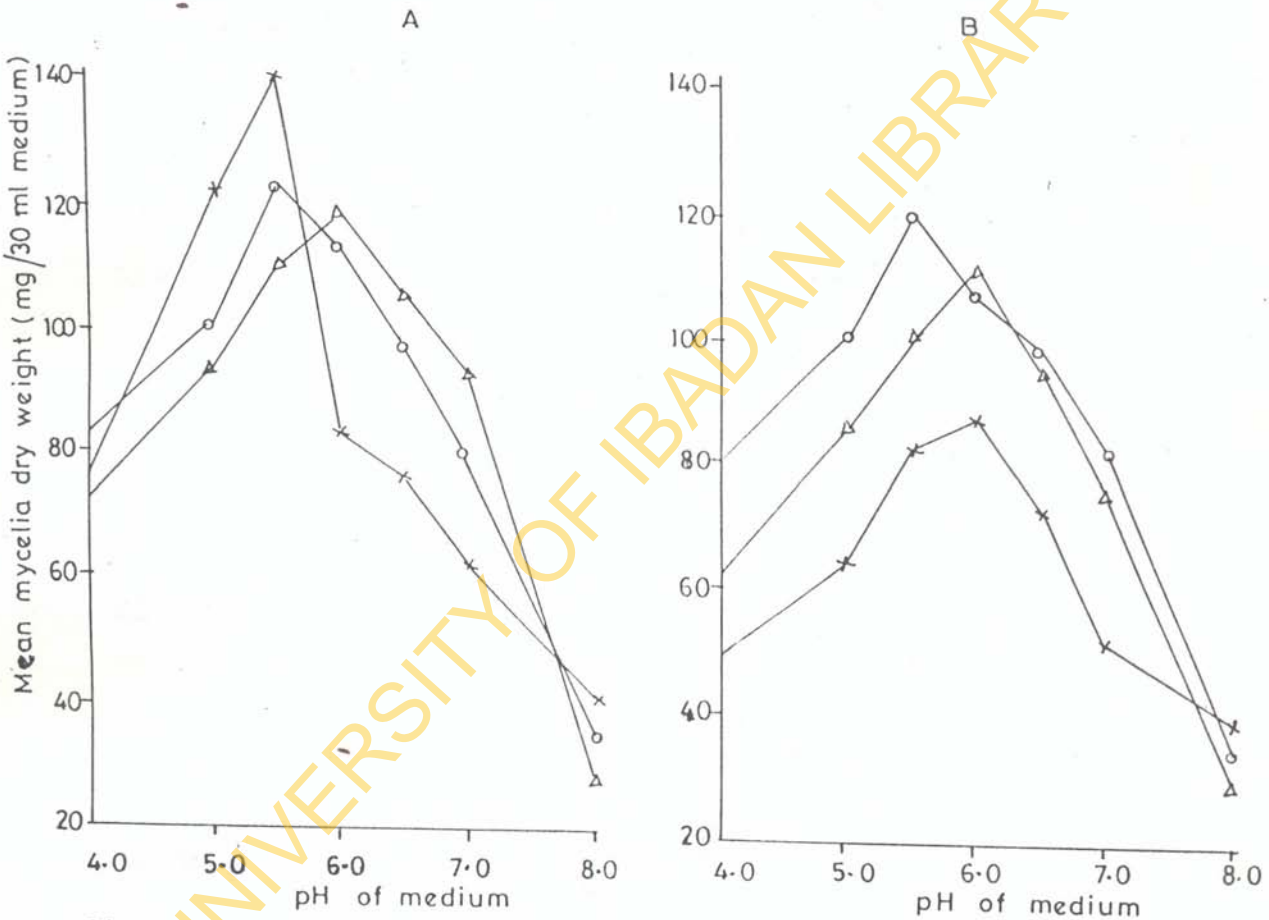

Fig. 2.

increase in pH until the optimum pH value was attained. Above the optimum pH, decrease in the growth was observed for all the fungi.

Carbon-source utilization for growth and sporulation

All the carbon sources supplied except sorbose supported growth and sporulation of the isolated fungi (Table 5). However, Aspergillus fumigatus and A. niger had very slight growth but no sporulation on sorbose. Starch and dextrin were the best carbon sources for mycelial growth and sporulation of all the isolated fungi. The two carbon sources had very similar effects on all the fungal isolates. Comparatively, Rhizopus arrhizus recorded highest mycelial growth on starch and dextrin while A. fumigatus recorded the highest amount of sporulation on the two carbon sources. For mycelial growth, A. fumigatus, A niger, A. flavus, Rhizomucor pusillus and A. oryzae ranked next after Rhizopus arrhizus in decreasing order of growth recorded. Also in the decreasing order of the values recorded for sporulation on starch and dextrin after A. fumigatus were: A. flavus, Rhizopus arrhizus, A. niger, A. oryzae and

TABLE 5

Carbon-source utilisation for growth and sporulation. Fungi were incubated at 30° C for 6 days. *Figures (mg mycelia dry wt. /30ml medium) are means of 3 readings with standard deviations. **Figures (spores $\times 10^6$/ml) are means of 3readings with standard deviations

Carbon source	Aspergillus flavus		Apergillus fumigatus		Aspergillus niger		Aspergillus oryzae		Rhizomucor pusillus		Rhizopus arrhizus	
	Dry wt.(mg)*	Spores** $x10^6$/ml	Dry.wt(mg)	Spores $x10^6$	Dry wt.(mg)	Spores $x10^6$	Dry wt.(mg)*	Spores** $x10^6$/ml	Dry.wt(mg)	Spores $x10^6$	Dry wt.(mg)	Spores $x10^6$
Glucose	83.7±2.2	12.6±4.2	71.0±2.4	11.8±1.4	89.3±2.1	12.4±1.3	90.6±2.7	12.6±2.1	70.7±1.7	14.4±1.2	121.3±3.0	20.6 2.1
Fructose	79.5±1.7	12.1±1.9	64.8±1.7	10.2±0.6	86.2±3.2	12.2±2.1	71.4±3.3	11.8±1.7	66.8±3.1	14.1±0.8	110.7±4.1	21.±1.0
Galactose	64.8±1.1	7.4±1.3	39.2±3.4	4.1±0.3	48.0±2.9	5.8±0.4	61.7±3.3	9.4±0.6	68.4±2.2	12.2±1.3	71.5±3.1	14±1.0
Sorbose	0.0	0.0	22.9±1.1	0.0	24.1±0.2	0.0	0.0	0.0	22.9±1.1	0.0	24.1±0.2	0.0
Xylose	79.9±4.3	12.4±1.2	69.4±1.2	10.9±1.1	78.0±0.8	10.2±0.6	74.2±3.7	12.2±0.8	62.6±3.1	12.8±0.6	54.4±1.6	10.8±0.9
Sucrose	46.6±2.1	3.8±0.6	41.9±3.6	3.1±0.2	55.2±1.4	7.1±0.7	40.3±2.2	4.2±0.2	48.7±1.7	6.4±1.2	52.7±2.5	8.4±0.8
Lactose	43.3±1.8	4.2±0.2	32.0±2.1	4.1±0.1	45.8±0.9	6.2±0.8	48.1±0.8	5.1±0.1	30.6±3.1	3.1±0.4	47.1±1.9	4.1±0.2
Cellobiose	56.4±0.8	8.1±0.6	62.3±2.5	8.4±1.2	58.9±2.4	8.1±0.6	67.4±3.7	10.4±1.3	49.0±1.4	6.8±1.3	56.6±1.4	8.8±1.3
Raffinose	52.7±2.0	7.0±1.1	59.0±1.7	6.3±0.8	47.1±0.8	2.8±1.1	57.3±1.3	6.8±0.4	42.6±1.1	4.8±0.8	48.5±3.4	5.6±0.7
Maltose	77.2±1.9	10.5±1.4	60.4±3.1	10.8±0.9	81.2±2.2	10.4±1.7	77.5±2.6	10.2±0.6	53.0±0.8	8.7±1.1	97.2±4.7	14.1±1.5
Pectin	33.7±0.6	2.2±0.3	39.1±1.4	2.1±0.2	30.7±2.0	1.9±0.3	32.3±2.7	1.9±043	26.6±0.9	0.8±0.1	29.4±2.9	2.0±0.1
Starch	112.0±2.6	28.8±2.6	140.9±2.6	30.8±1.7	130.3±2.7	26.7±1.8	99.2±3.0	24.8±0.6	106.4±2.6	18.4±2.3	158.5±3.1	26.9±1.3
Dexrin	122.4±4.1	28±1.0	148.4±3.1	30.1±2.1	124.1±3.0	27.1±2.1	120.5±3.2	25±91.1	119.2±3.2	20.2±1.9	162.4±2.7	28.2±1.9
CMC	29.7±1.1	1.1±0.2	38.8±3.0	3.2±0.3	31.5±0.9	1.3±0.6	26.2±1.3	1.4±0.3	18.7±1.1	0.2±0.1	30.1±1.3	0.2±0.1
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Rhizomucor pusillus with the least amount of sporulation. Glucose, fructose, maltose and xylose were also good carbon sources for the growth and sporulation of all isolates. Galactose also promoted abundant growth and sporulation comparable to those recorded for glucose, fructose, maltose and xylose. However, galactose was poorly utilized both for mycelial growth and sporulation by Aspergillus fumigatus and A. niger. Apart from sorbose, the poorest utilized carbon sources of the lot were pectin and carboxymethyl cellulose (CMC). They gave the least mycelial growth and sporulation in all the isolated fungi. The flask containing any of the two carbon sources could easily be identified by the scanty fungal growth. The performance of A. fumigatus on pectin and CMC though very poor was comparatively the highest among the isolated fungi. Rhizomucor pusillus on the other hand recorded the least mycelial growth and sporulation on pectin and CMC. The sporulation of Rhizopus arrhizus on CMC was also very poor, being the same with that recorded for Rhizomucor pusillus.

Utilization of native cellulose

Four of the isolated fungi were able to utilize the cellulose (filter papers) provided for mycelial growth and sporulation thereby bringing about considerable loss in weights of the filter papers. This growth of the four fungal species on the filter papers was at various extents. Rhizomucor pusillus and Rhizopus arrhizus showed no sign of growth on the filter papers. A comparison of the weight losses of the filter papers inoculated with the two fungal species and with those of the control experiments (at p = 0.1) showed no significant differences. Out of the four fungal species that brought about significant loss in weights of the filter papers, A. fumigatus was the most effective. It brought about a weight loss of 89.3mg. Other fungi in decreasing order of effectiveness were A. niger (67.5mg), A. flavus (58.8mg) and A. oryzae (56.3mg) (Table 6). Visual estimation of mycelial growth on the filter papers showed that A. fumigatus had a very abundant growth, A. niger had an abundant growth while light growth was recorded for A. flavus and A. oryzae.

TABLE 6

Utilization of native cellulose (filter papers) by isolates. Cultures were incubated at 30°C for 21 days. Figures are means of 3 readings with standard deviations.

Test fungus	Net loss in wt(mg) of filter papers	Visual estimation of fungal growth on filter papers.
Aspergillus flavus	58.8±2.9	+
A. fumigatus	89.3±2.1	+++
A. niger	67.5±1.3	++
A. oryzae	56.3±1.9	+
Rhizomucor pusillus	0.0	- *
Rhizopus arrhizus	0.0	-
Control	0.0	-

* - No growth

\+ Light growth

++ Abundant growth

+++ More abundant growth.

Nitrogen-source utilization for growth and sporulation

The results presented in Table 7 show that casein was the best nitrogen source for mycelial growth and sporulation of all the isolated fungi. Glutamic acid and aspartic acid were also very good nitrogen sources for the growth and sporulation of the isolates. The two nitrogen sources ranked next after casein in order of effectiveness. Other good nitrogen sources were KNO_3, NH_4NO_3, asparagine, alanine and glycine all of which promoted abundant growth and sporulation in all the test species. Sodium nitrate ($NaNO_3$), NH_4Cl and Mg $(NO_3)_2$ were also well utilized for growth and sporulation by all the isolates although they were not as effectively utilized as the nitrogen sources previously mentioned. The results also showed that $(NH_4)_2SO_4$ was fairly utilized by some of the isolates but it is not a good nitrogen source for Rhizomucor pusillus, A. niger and A. fumigatus. Guanine was also fairly well utilized by all the fungal isolates with the exception of Rhizomucor pusillus. Amino-n-butyric acid was also utilized by all the isolated fungi but to various extents. It was well utilized for mycelial growth and sporulation by A. niger, fairly utilized by

TABLE 7

Nitrogen-source utilization for growth and sporulation. Fungi were incubated at 30°C for 6 days. *Figures (mg mycelia dry wt./30ml medium) are means of 3 readings with standard deviations. **Figures (spores x 10^6/ml) are means of 3 readings with standard deviations.

Carbon source	Aspergillus flavus		A	fumigatus	A	niger	A.	oryzae	Rhizomucor	pusillus	Rhizopus	arrhizous
	Dry wt.(mg)*	Spores** x10^6/ml	Dry.wt(mg)	Spores x10^6	Dry wt.(mg)	Spores x10^6	Dry wt.(mg)*	Spores** x10^6/ml	Dry.wt(mg)	Spores x10^6	Dry wt.(mg)	Spores x10^6
KNO_3	110.3±4.2	22.5±1.4	122.0±2.6	24±1.0	126.6±2.2	23.2±0.8	120±4.7	22.7±1.3	88.6±2.2	14.2±1.3	129.9±2.0	31.±1.0
$NaNO_3$	77.5±1.9	19.±1.0	87.3±1.9	20.3±0.7	90.8±3.0	20.1±1.3	69.9±1.1	18.2±0.8	72.3±1.7	15.1±0.8	97.6±3.4	21.8±0.7
NH_4NO_3	101.7±3.1	21.8±0.8	99.4±2.7	22.4±0.4	120.2±1.6	21.±1.0	91.2±3.0	19.7±1.7	88.1±0.9	13.8±1.6	86.7±1.96	19.6±1.2
$Mg(NO_3)_2$	66.8±0.7	12.4±0.4	91.5±3.6	20.2±0.3	121.7±0.4	20.6±1.1	82.4±0.9	18.4±2.1	55.7±3.3	9.6±0.8	80.3±4.8	18.2±2.4
NH_4Cl	80.2±1.1	20.1±0.8	71.6±4.2	16.1±1.1	108.5±2.7	19.4±0.8	86.9±1.3	19.1±0.6	66.6±2.2	11.8±1.3	77.4 ±1.0	14.2±0.8
$(NH_4)_2SO_4$	68.1±0.7	14.±1.0	51.4±2.5	4.1±0.4	44.7±1.1	7.2±0.4	69.3±0.4	13.8±1.3	38.5±0.7	4.1±0.6	77.6±3.2	14.9±1.1
Glycine	79.9±1.7	18.8±0.3	92.6±1.0	19.8±0.8	81.2±3.2	19.8±1.6	89.5±0.7	20.2±1.4	77.2±2.2	13.6±0.8	89.5±1.1	20.2±1.6
Methionine	39.2±1.3	5.2±0.1	42.8±225	5.2±0.1	51.5±1.6	9.4±0.7	37.7±0.8	6.4±0.9	40.8±1.0	7.1±1.3	46.4±0.7	5.8±0.5
Alanine	88.8±2.4	20.4±0.8	93.0±1.8	20.7±0.8	124.8±3.1	21.8±1.8	99.6±2.9	21.7±1.3	84.4±1.4	12.4±0.6	111.6±4.3	30.±2.0
Guanine	66.8±1.9	13.8±054	80.4±3.6	17.4±1.2	79.2±2.2	10.2±0.6	68.2±1.7	14.2±1.1	49.5±0.7	8.2±1.1	70.1±1.1	12±1.0
Glutamic acid	134.4±3.7	30.1±0.3	141.7±1.2	30.6±1.4	144.3±2.7	31.8±1.1	136.2±2.7	29.7±0.9	95.3±3.9	15.9±0.8	117.7±3.0	30.4±1.6
Asparagine	107.8±3.2	20.8±1.1	127.8±2.7	23.7±0.6	111.2±1.4	20.2±0.9	97.8±2.5	20.4±1.3	84.6±4.7	12.4±1.5	99.9±1.1	20.8±1.6
Tryptophan	0.0	0.0	22.6±0.6	0.0	28.4±0.5	0.01±0.01	0.0	0.0	0.0	0.0	0.0	0.0
Casein	151.8±3.1	32.2±1.3	14.3.4±1.3	33.7±1.8	139.2±2.4	32	147.8±1.9	30.2±2.1	110.6±1.3	19.6±2.2	1324.±1.8	31.8±2.8
Amino-n-butyric acid	58.6±0.9	6.9±0.2	67.6±1.8	9.8±0.5	88.4±1.7	17	55.4±2.6	8.0±1.0	40.9±0.7	6.2±0.8	59.9±1.9	9.4±1.3
Aspartic acid	119.4±2.6	23.2±1.7	136.7±2.5	28.7±1.3	110.6±2.0	21	134.7±1.7	24.5±1.4	99.6±0.8	18.4±1.4	108.0±3.4	28.2±2.5
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

A. fumigatus but poorly utilized for the mycelial growth and sporulation of Rhizomucor pusillus. The results also showed that methionine was poorly utilized both for the mycelial growth and sporulation of all the isolates. The poorest utilized of all the nitrogen sources supplied was tryptophan with only A. fumigatus and A. niger recorded very slight mycelial growth on it. A very slight amount of sporulation (0.01×10^6 spores/ml) was recorded for A. niger when supplied with tryptophan. No other fungal isolate sporulated on this nitrogen source. In general, excluding the casein, a comparison of the growth and sporulation values of the isolated fungi on the organic nitrogen sources with those of the inorganic sources (at $p=0.1$) showed no significant differences.

Mycelial growth and sporulation on feed infusion medium

According to the results presented in Fig. 3, all the isolated fungi utilized feed infusion medium for mycelial growth and sporulation. Rhizopus arrhizus most effectively utilized the medium both for mycelial growth and sporulation. Other fungi in decreasing order of utilization of the medium were: A. flavus,

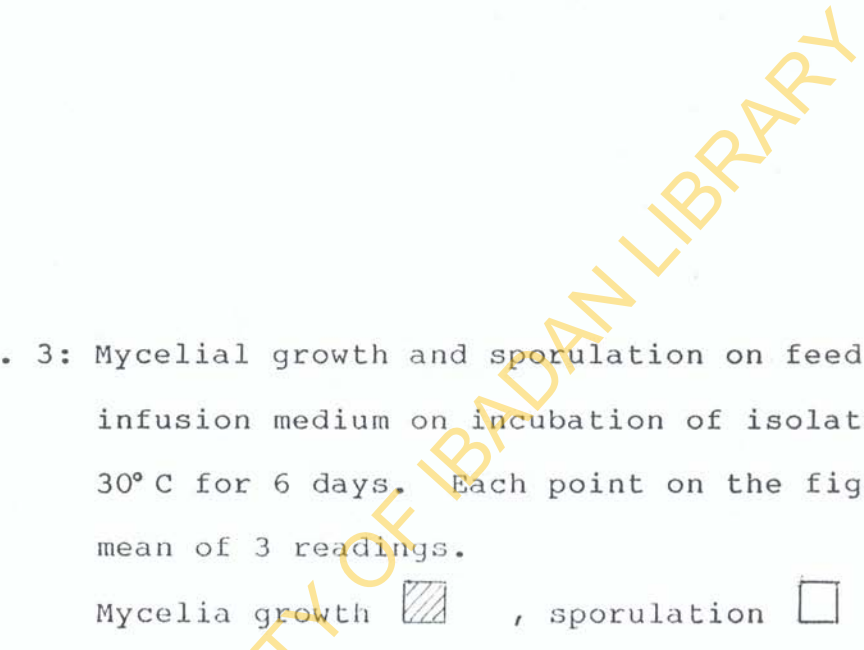

Fig. 3: Mycelial growth and sporulation on feed infusion medium on incubation of isolates at 30° C for 6 days. Each point on the figure is a mean of 3 readings.

Mycelia growth ▨ , sporulation □ .

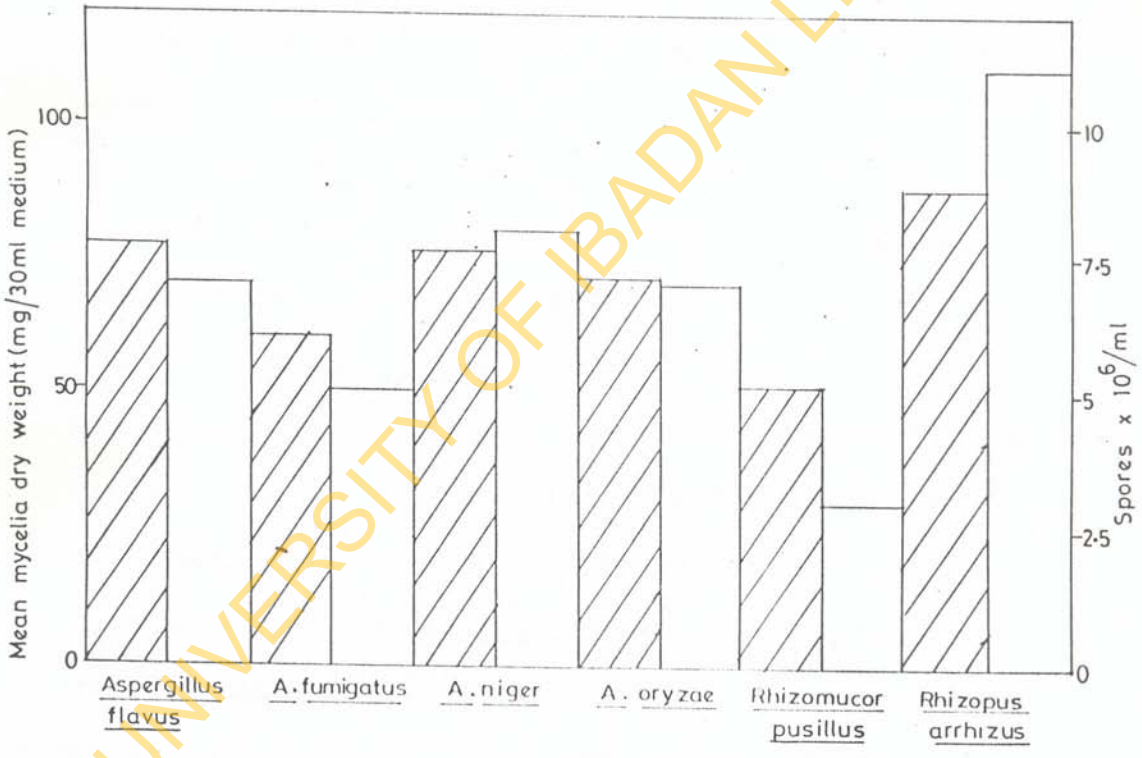

Fig. 3

A. niger, A. oryzae, A. fumigatus and Rhizomucor pusillus. The mycelial growth and sporulation of the last two fungal isolates above were obviously poor when assessed quantitatively and by visual estimation.

Aflatoxin studies:

Production of aflatoxins

Aflatoxins (B_1, B_2, and G_2) were detected in measurable quantities in the culture filtrates of Aspergillus flavus and A. oryzae. No aflatoxin was detected in the culture filtrates of the remaining four isolated fungi and the control (Table 8). The results showed that A. flavus produced more aflatoxins B_1, B_2 and G_2 on the medium supplemented with the yellow maize variety (Farz 7) than on the medium supplemented with the white maize variety (Farz 34). Similar results were obtained for A. oryzae except that the medium with white maize variety enhanced the production of aflatoxin B_2 more than the medium supplemented with yellow maize variety. In fact, the ratio of the level of aflatoxin B_2 production by A. oryzae in the two media was 1:4.48 in favour of the white maize containing medium. Comparatively, A. flavus produced

TABLE 8

Production of aflatoxins on two types of modified Czapek-Dox media. Fungi were incubated at 30°C for 6 days. Figures (µg/l of aflatoxins in the culture filtrates) are means of 3 readings with standard deviations.

Fungus	Aflatoinxs (ug/l) in:							
	Medium supplemented with yellow maize variety (Farz 7)				Medium supplemented with white maize variety (Farz 34)			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Aspergillus flavus	670±20	516±28	0.0	347±34	655±19	486±26	0.0	242±22
A. fumigatus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A oryzae	643±16	86±12	0.0	458±17	625±15	385±25	0.0	255±15
Rhizomucor pusillus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhizopus arrhizus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

]more aflatoxins B_1 and B_2 than A. oryzae in the two growth media while the latter produced larger amounts of aflatoxin G_2 than A. flavus in the two types of media. Excluding the production of aflatoxin B_2, a comparison of the levels of toxins produced by the two toxigenic fungi showed no significant differences (at $p = 0.1$).

Aflatoxin B_1 production with time by A. flavus and A. oryzae

Detection of aflatoxin was first recorded in culture fitrates of A. flavus and A. oryzae on the 2nd and 4th days after incubation respectively (Fig. 4). The levels of toxin production in both fungal isolates increased sharply from the inoculation time up to the 6th day after which the rate of increase slowed down considerably. The peak of toxin level in culture filtrates of the two fungal species was recorded on the 8th day after incubation. A slight decrease or almost the same amounts of toxin recorded on the 8th day were recorded in the culture filtrates of the two test organisms on the 10th day. For each of the two test

Fig. 4: Aflatoxin B_1 production and pH changes with time on modified Czapek-Dox medium. Cultures were incubated at 30° C. Each point on the graph is a mean of 3 readings.

Aspergillus flavus: Aflatoxin B_1 production O——O, pH of medium O-----O Aspergillus oryzae: Aflatoxin B_1 production x——x, pH of medium x-----x

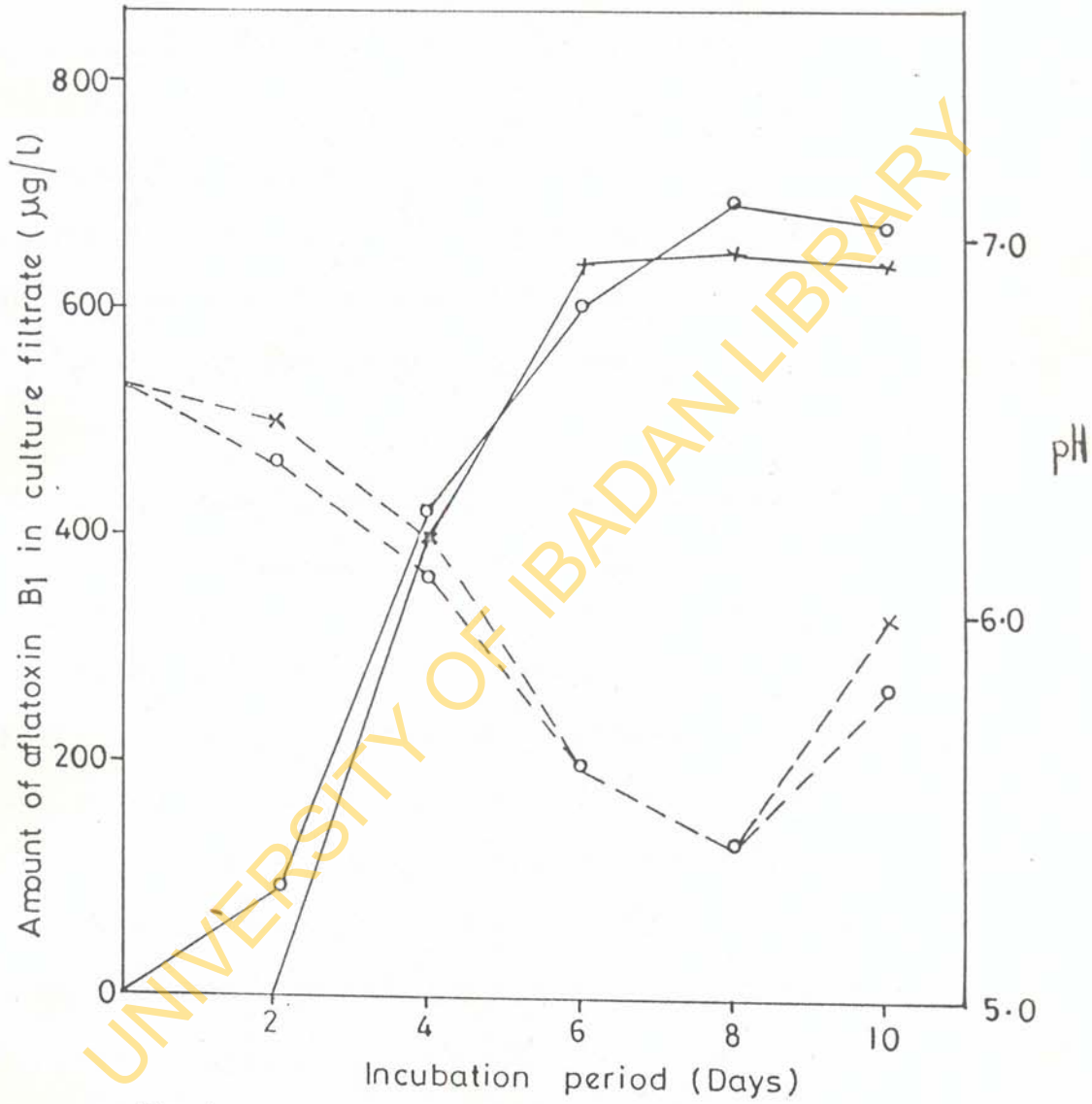

Fig. 4.

fungi, an initial lowering and then a rise of the pH levels of the growth medium occurred with time of incubation (Fig.4). The lowest pH value (5.4) was recorded for the two test fungi on the 8th day. This coincided with the peak of toxin level in the culture filtrates. By the 10^{th} (final) day of incubation, the pH of the growth medium had increased to 5.8 and 6.0 for A. flavus and A. oryzae respectively.

Effects of pH of growth medium on aflatoxin B_1 production by A. flavus and A. oryzae

Aflatoxin B_1 was detected in all the culture filtrates of the two test fungi at all levels of pH (3-8) provided, although in different amounts (Fig. 5). The aflatoxin levels first increased with rise in pH from pH 3 to pH 5 when the peak was attained. Further increase in pH brought about decrease in the levels of toxin detected in culture filtrates. At pH 3 and 4 the amounts of aflatoxin detected in culture filtrates of A. oryzae were more than that detected in the culture filtrates of A. flavus. However the latter fungus accumulated more toxin in the culture filtrates at pH 5 to 8.

Fig. 5: Effects of pH of growth medium on aflatoxin B_1 production. Fungi were incubated at 30° C for 6 days at different pH levels. Each point on the graph is a mean of 3 readings. Aspergillus flavus O———O, A. oryzae x———x.

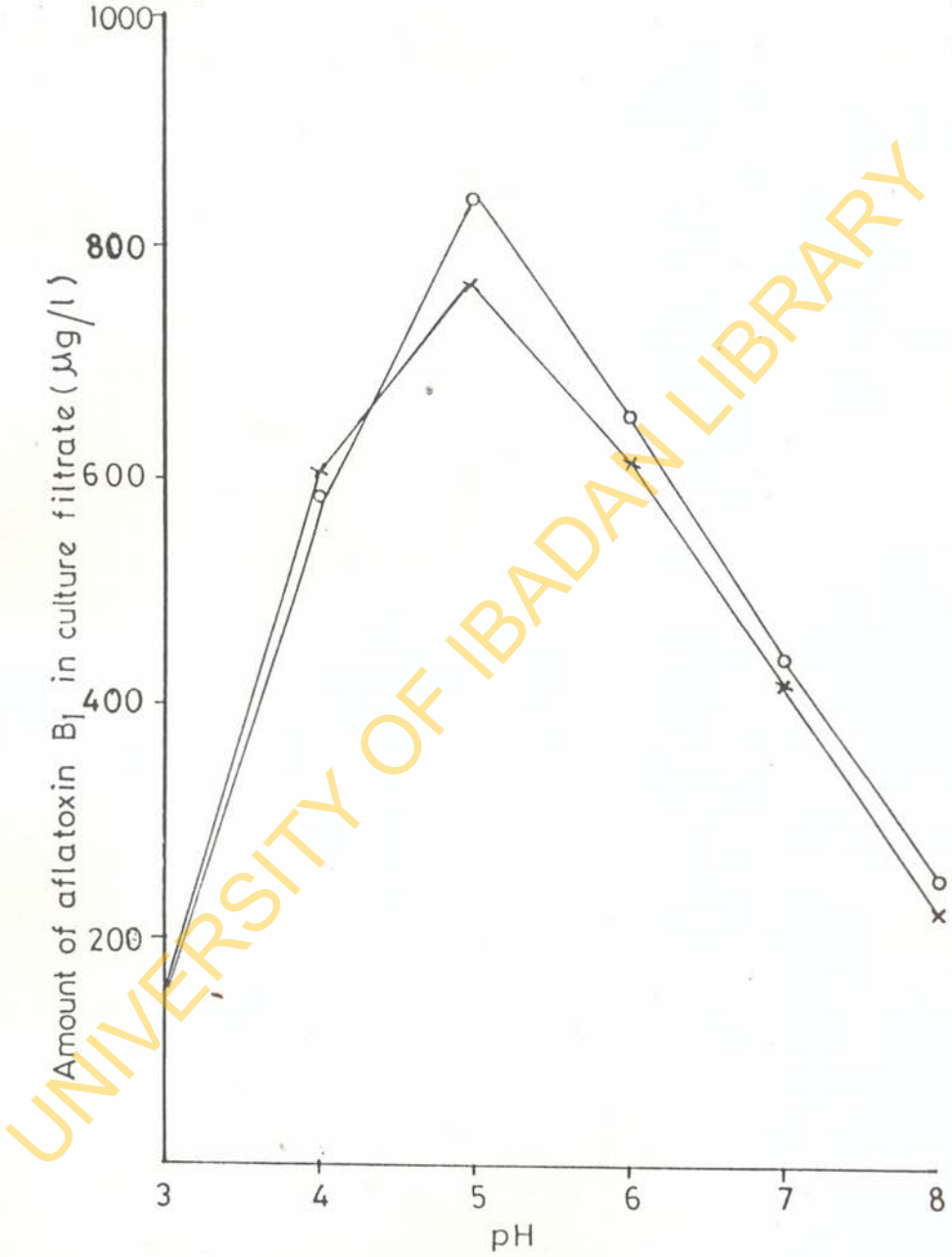

Fig. 5

Fig. 6: Effects of incubation temperature on aflatoxin B_1 production by test fungi after 6 days of incubation. Each point on the graph is a mean of 3 readings. Aspergillus flavus O——O, *A. oryzae* x——x

Effects of incubation temperature on aflatoxin B_1 production by A. flavus and A. oryzae

The results showed that aflatoxin B_1 was produced in considerable quantities at temperatures between 25° and 35° C (Fig. 6). No aflatoxin B_1 was detected in culture filtrates of the two test fungi incubated at 15° and 45° C. For the two fungi increase in temperature brought about a corresponding increase in level of toxin until peak was attained for the two test organisms at 30° C. Further increase in temperature brought about decrease in amounts of toxin accumulated in the culture filtrates. At 20°, 25°, 30° and 35° C the levels of toxin in culture filtrates of A. flavus were more than the levels recorded for A. oryzae. The two fungal species however ended up with same level of toxin in their culture filtrates at 40° C.

Utilization of feed concentrates as substrates for aflatoxin B_1 production by A. flavus and A. oryzae

Results of this experiment are shown in Table 9. Corn, groundnut cake and palm kernel meals samples when collected had 20, 140 and 10 parts per billion (ppb) of aflatoxin B_1. The remaining feed concentrates

TABLE 9

Utilization of feed concentrates as substrates for aflatoxin B_1 production by Aspergillus flavus and A. oryzae. Flasks were incubated at 30°C for 6 days. Figures (aflatoxin B_1 contents in ppb) are means of 3 readings with standard deviations.

Feed concentrate	Aflatoxin B_1 content (ppb) in:			
	sample on collection (initial)	sample inoculated with A. flavus	Sample inoculated with A. oryzae	Control sample
Corn meal	20±0	850±30	780±20	15±5
Groundnut cake meal	140±10	3,080±60	2,900±100	130±10
Palm kernel meal	10±0	300±20	300±30	0
Fish meal	0	0	0	0
Blood meal	0	0	0	0
Wheat offals	0	220±40	260±20	0
Dried breweres grains	0	140±10	80±10	0
Oyster shell meal	0	0	0	0
Bone meal	0	0	0	0

had no detectable aflatoxin B_1 on collection. At the end of the incubation period, corn meal, groundnut cake meal, palm kernel meal, wheat offals and dried brewers grains inoculated with A. flavus had 850, 3,080, 300, 220 and 140 ppb of aflatoxin B_1 contents respectively. The remaining feed concentrates (fish, blood, oyster shell and bone meals) had no detectable aflatoxin B_1 at the end of the incubation period. Similarly, corn meal, groundnut cake meal, palm kernel meal, wheat offals and dried brewers grains inoculated with A. oryzae had 780, 2,900, 300, 200 and 80ppb of aflatoxin B_1 contents respectively at the end of the incubation period. No aflatoxin B_1 was detected in the remaining feed concentrates (fish, blood, oyster shell and bone meals). For the control, only corn and groundnut cake meals had 15 and 130 ppb of aflatoxin B_1 contents respectively after the incubation period. This gave a reduction of 5 and 10ppb respectively when compared to the aflatoxin B_1 levels in the initial samples. No aflatoxin B_1 was detected in palm kernel meal in the control flasks. The results also showed that groundnut cake meals had the highest levels of aflatoxin B_1. This was followed by corn meal, palm kernel meal, wheat

offals and dried brewers grains in decreasing order of toxin levels. Except in palm kernel meal and wheat offals, A. flavus brought about the accumulation of more toxin than A. oryzae. The amount of toxin produced by A. oryzae on wheat offals was more than the amount produced by A. flavus on the same substrate. Both test fungi produced the same amount of toxin on palm kernel meal.

Analysis of fresh poultry droppings for aflatoxins

No aflatoxin was detected in all the samples analysed.

Test for toxins in poultry droppings previously inoculated with toxigenic fungal species

No toxin was detected in all the samples inoculated either with A. flavus or A. oryzae.

Extracellular enzyme production and assay:

Amylase production with time on SYE medium

The results of this experiment presented in Fig. 7 show that all the isolated fungi produced amylase extracellularly. The amount of the enzyme produced by

Fig. 7: Amylase production with time on SYE medium. Fungi were incubated at 30° C Each point on the graph is a mean of 3 readings. A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ——Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*.

the fungal species increased with the period of incubation until a peak was reached at different days for the isolates. For Aspergillus flavus, A. fumigatus and A. oryzae, the peak amylase production was attained on the 6th day of incubation at 30° C. A. niger and Rhuzopus arrhizus recorded peak activity on the 4th day while same was recorded for Rhizomucor pusillus on the 5th day of incubation at 30° C. The amylolytic activity for all the isolates decreased gradually after peak activity was attained.

Effects of different carbon sources on amylase production

The results presented in Table 10 showed that amylase was produced by all the isolates in all the media types. However, the largest amount of amylase was produced by each of the fungi on the medium that contained starch as the sole carbon source. Other media containing different carbon sources enhanced amylase production in decreasing order as follows: Feed infusion medium, CMC, pectin, dextrin and glucose.

Effects of pH of assay medium on amylase activity

Amylase activity was recorded for all the fungal isolates with the pH range (3-8) used. Optimum pH for amylase activity of each of the test fungal species was in the acidic medium (Fig. 8). For Aspergillus flavus and A. niger, highest amylase activity was recorded at pH 5. The remaining four fungal species had optimum amylase activity at pH 6. At values above the optimum pH, a decline in amylase activity was recorded for all the species.

Effects of temperature of assay medium on amylase activity

The results presented in Fig. 9 showed that no amylase activity was observed at 20° C for all the species. At 30° C, amylase activity was observed for all the species and this rose gradually to a peak at 45° or 50° C. The optimum activity of the amylases of A. flavus, A. niger, A. oryzae and Rhizopus arrhizus was obtained at 45° C while amylases of A. fumigatus and Rhizomucor pusillus recorded peak activity at 50° C. At higher temperatures, amylase activity decreased sharply for all the species and eventually ceased at 60° C.

TABLE 10

Effects of carbon sources on amylase production. Fungi were incubated at 30°C for 6 days and amylase activity determined. Figures (mg/ml of maltose released by culture filtrate) are means of 3 readings with standard deviations.

Test fungus	Carbon Sources					
	Glucose	Dextrin	Starch	Pectin	CMC	Feed infusion medium
Aspergillus flavus	0.05±0.01	0.10±0.03	0.62±0.03	0.22±0.01	0.40±0.10	0.48±0.07
A. fumigatus	0.12±0.04	0.30±0.09	0.90±0.07	0.44±0.03	0.40±0.08	0.66±0.03
A. niger	0.24±0.07	0.26±0.06	1.20±0.04	0.60±0.04	0.72±0.06	1.00±0.04
A. oryzae	0.04±0.02	0.14±0.04	0.60±0.03	0.26±0.07	0.42±0.01	0.46±0.04
Rhizomucor pusillus	0.08±0.02	0.24±0.03	0.66±0.07	0.16±0.02	0.30±0.02	0.50±0.0
Rhizopus arrhizus	0.20±0.03	0.30±0.09	1.10±0.08	0.14±0.05	0.22±0.07	0.90±0.08

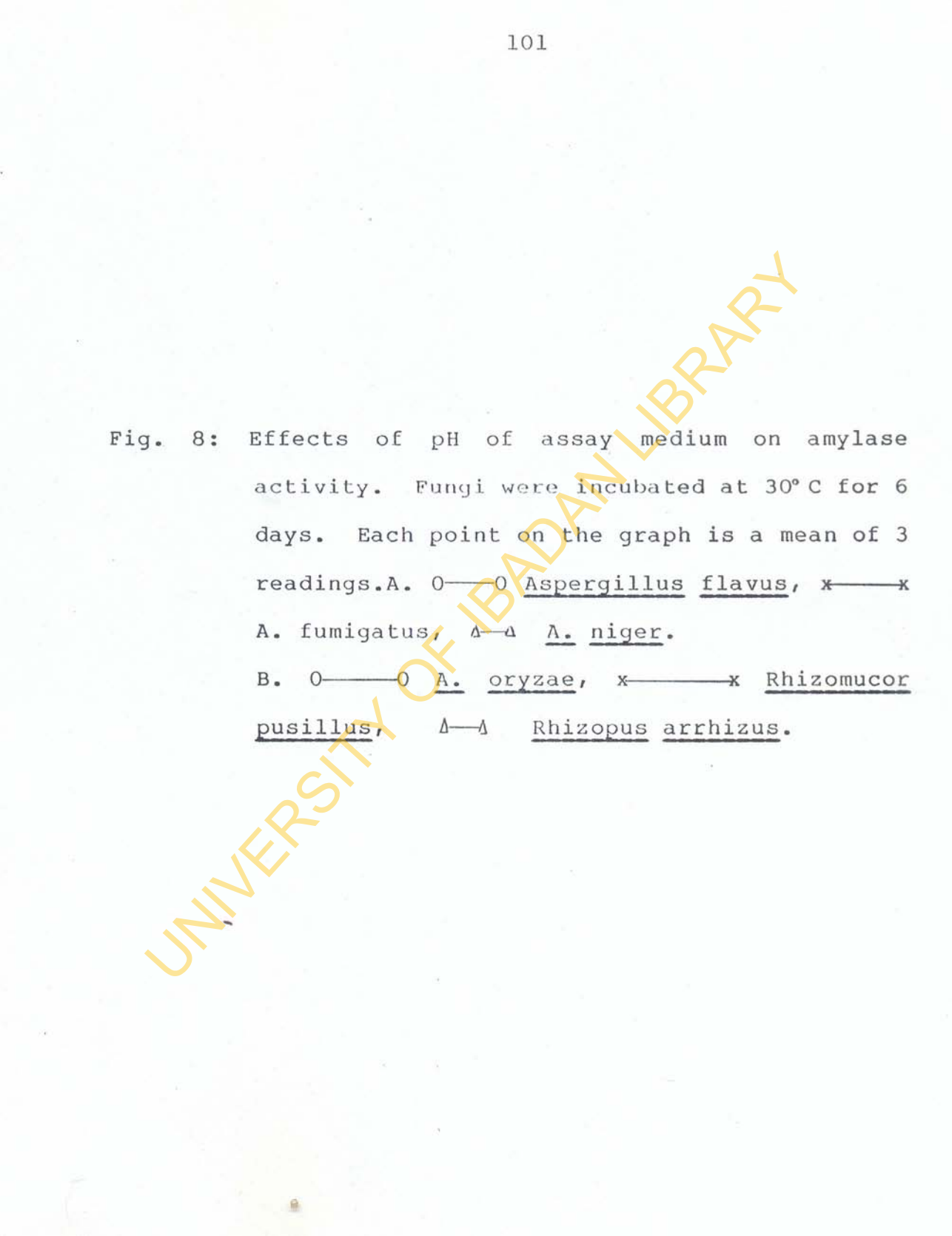

Fig. 8: Effects of pH of assay medium on amylase activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings. A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ—Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ—Δ *Rhizopus arrhizus*.

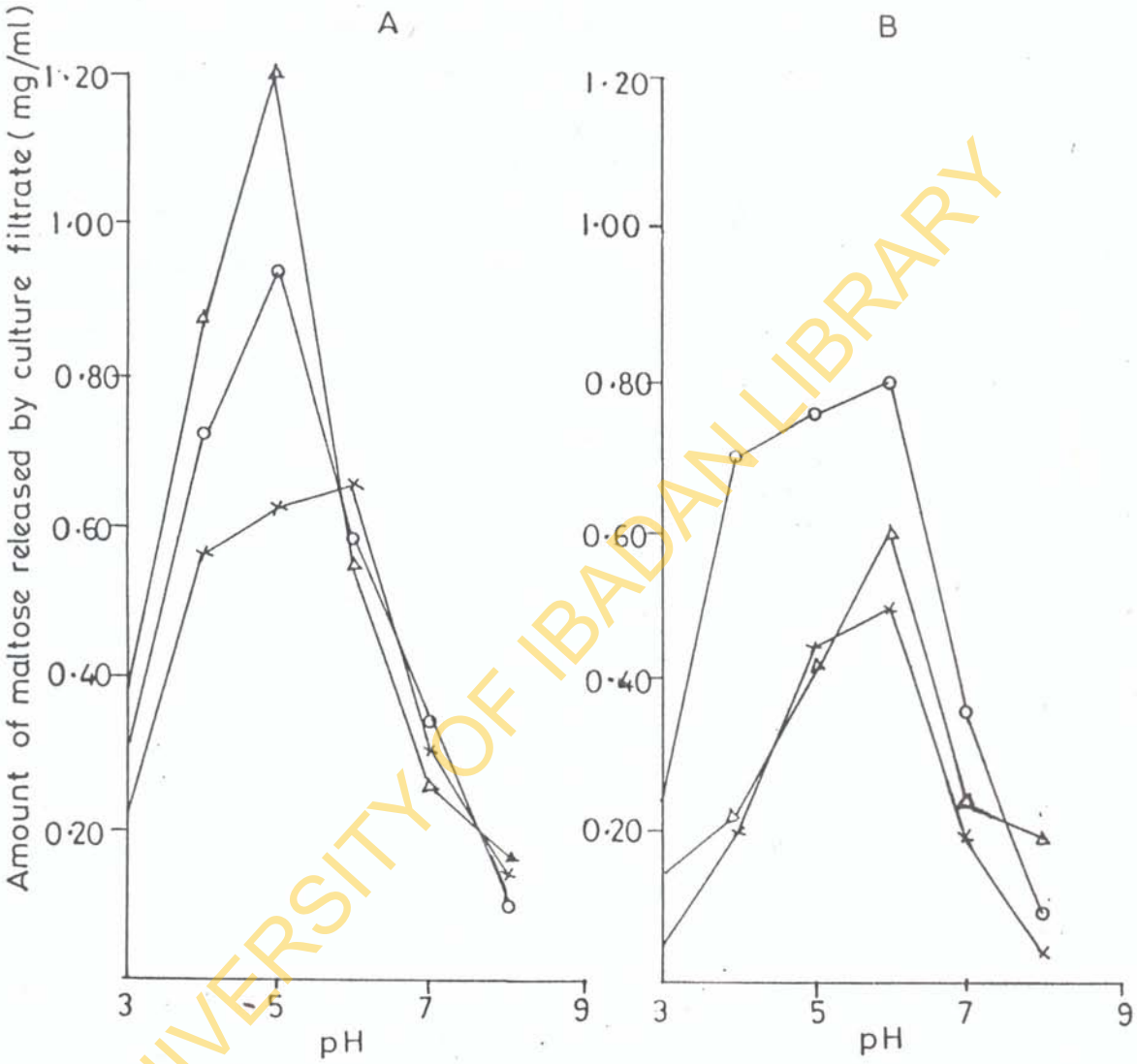

Fig. 8.

Cellulase production with time on oat-meal chaff medium

The results showed that Aspergillus fumigatus had the highest cellulase activity while Rhizomucor pusillus had the lowest (Fig. 10). For all the species, cellulase production first increased with the time of incubation until the peak was attained.

Fig. 9: Effects of temperature of assay medium on amylase activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings. A. O——O Aspergillus flavus, x——x A. fumigatus, Δ——Δ A. niger.

B. O——O A. oryzae, x——x Rhizomucor pusillus, Rhizopus arrhizus Δ——Δ

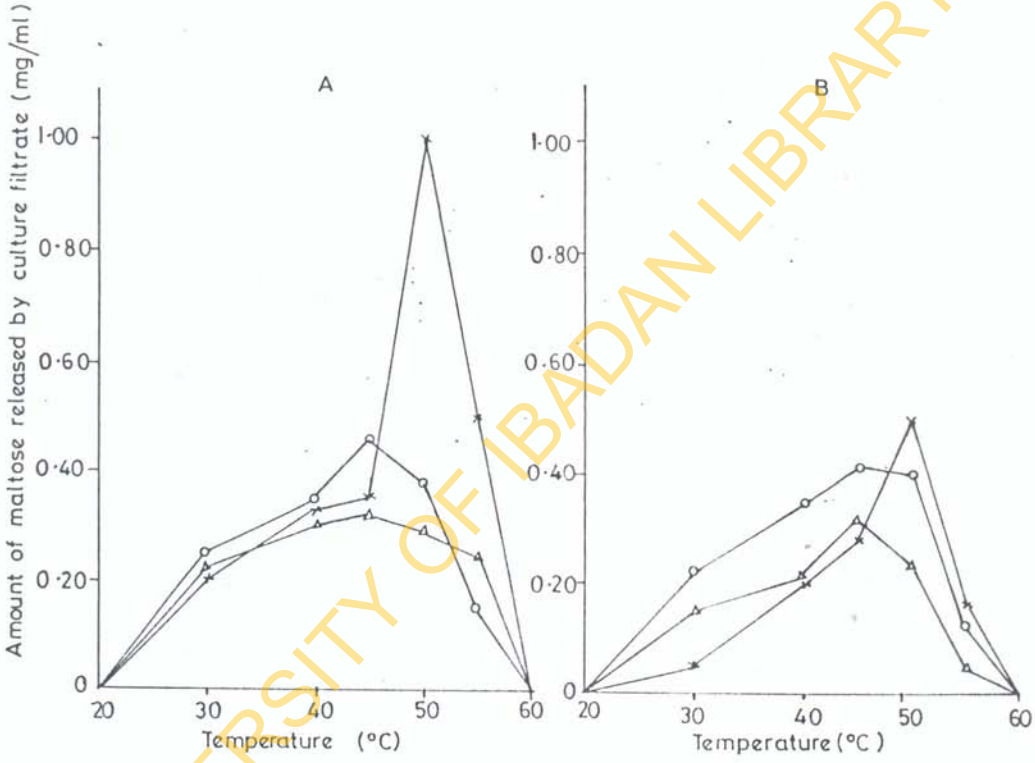

Fig. 9:

Fig. 10: Cellulase production with time on oat-meal chaff medium (OCM). Fungi were incubated at 30° C. Each point on the grap is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ——Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*

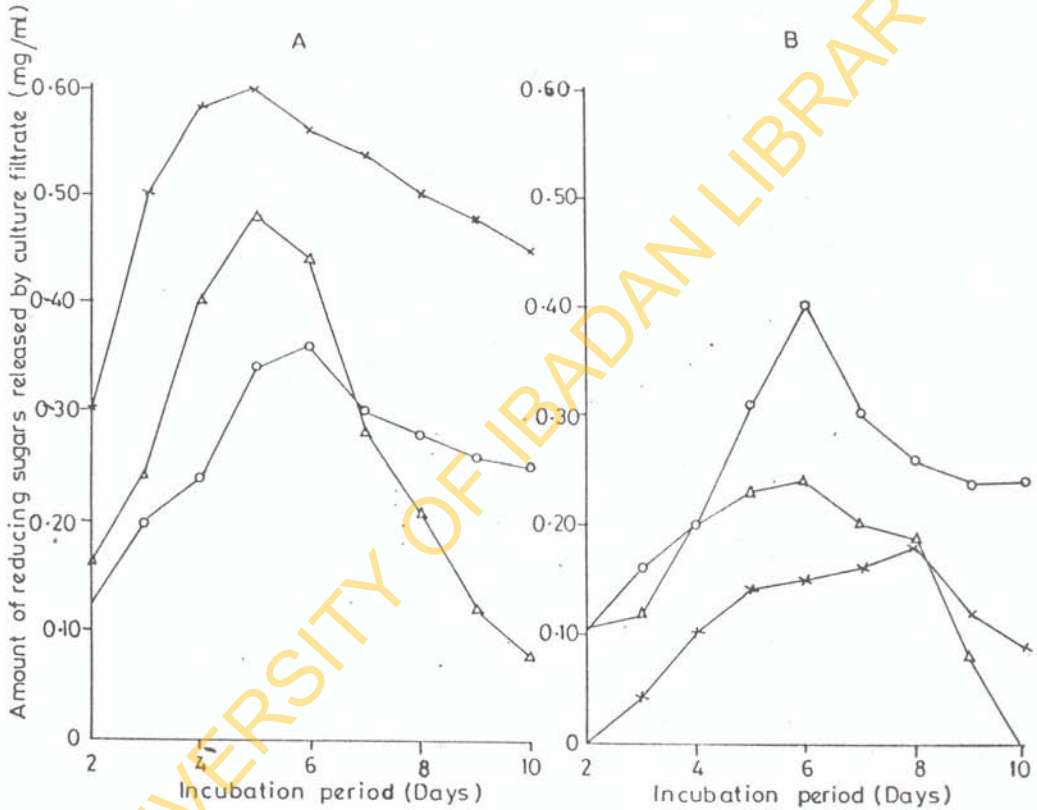

Fig. 10:

Aspergillus fumigatus and A. niger recorded peak production of cellulases on the 5th day of incubation, A. flavus, A. oryzae and Rhizopus arrhizus on 6th day and Rhizomucor pusillus on the 8th day of incubation. After the peak production day for each fungal species, a decrease in the cellulase activity of the culture filtrates was observed.

Effects of different carbon sources on cellulase production

According to the results shown in Table 11, no cellulase production was recorded for Rhizomucor pusillus and Rhizopus arrhizus on media with glucose, dextrin and starch as sole carbon sources. Cellulases were produced by the remaining fungal species on all the media types provided. The feed infusion medium and CMC medium proved to be the best media for cellulase synthesis by all the isolates. While cellulase production by A. flavus, A. niger and Rhizopus arrhizus was greater on CMC medium than on feed infusion medium, it was greater on feed infusion medium for A. fumigatus and A. oryzae. For Rhizomucor pusillus, equal amounts

TABLE 11

Effects of carbon sources on cellulase production Fungi were incubated at 30°C for 6 days and cellulase activity determined. Figures (mg/ml of total reducing sugars released by culture filtrate) are means of 3 readings with standard deviations.

Tes fungus	Carbon Sources					
	Glucose	Dextrin	Starch	Pectin	CMC	Feedinfusion medium
Aspergillus flavus	0.01±0.01	0.10±0.01	0.01±0.01	0.02±0.01	0.34±0.04	0.28±0.02
A. fumigatus	0.01±0.01	0.01±0.01	0.01±0.01	0.04±0.01	0.72±0.03	0.80±0.05
A. niger	0.01±0.01	0.01±0.01	1.01±0.01	0.05±0.01	0.60±0.02	0.50±0.02
A. oryzae	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.40±0.03	0.48±0.08
Rhizomucor pusillus	0.0	0.0	0.0	0.01±0.01	0.10±0.03	0.10±0.02
Rhizopus arrhizus	0.0	0.0	0.0	0.01±0.01	0.14±0.02	0.20±0.04

of cellulases were produced on CMC and feed infusion media.

Effects of pH of assay medium on cellulase activity

Although cellulase activity was recorded for the isolated fungal species within the pH range (3-8) used, acidic medium enhanced greatest activity (Fig 11). For all the fungal species, increase in cellulase activity was recorded with increase in pH until the peak was attained at pH 5 for A. flavus and A. oryzae and at pH 6 for A. fumigatus, A. niger, Rhizomucor pusillus and Rhizopus arrhizus. Further increase above the optimum pH brought about decrease in cellulase activity for all the fungal organisms.

Effects of temperature of assay medium on cellulase activity

From the results presented in Fig. 12, cellulases of the isolated fungal species were active within a wide temperature range (20° -70° C). No cellulase activity was recorded for A. fumigatus and Rhizomucor pusillus at 20° C and also none was recorded for A. niger, A. oryzae and Rhizopus arrhizus at 70° C. While

Fig. 11: Effects of pH of assay medium on cellulase activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ—Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ—Δ *Rhizopus, arrhizus*.

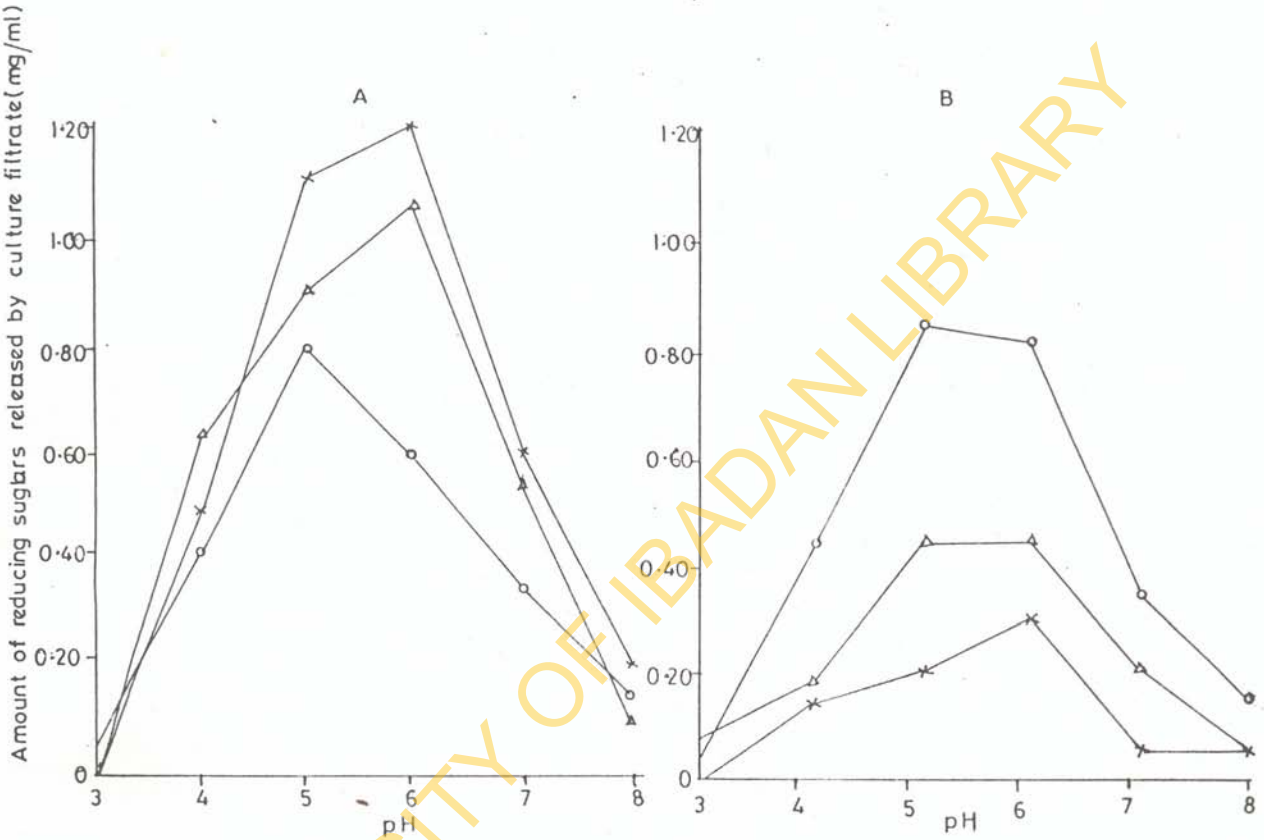

Fig. 11

Fig. 12: Effects of temperature of assay medium on cellulase activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ—Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*.

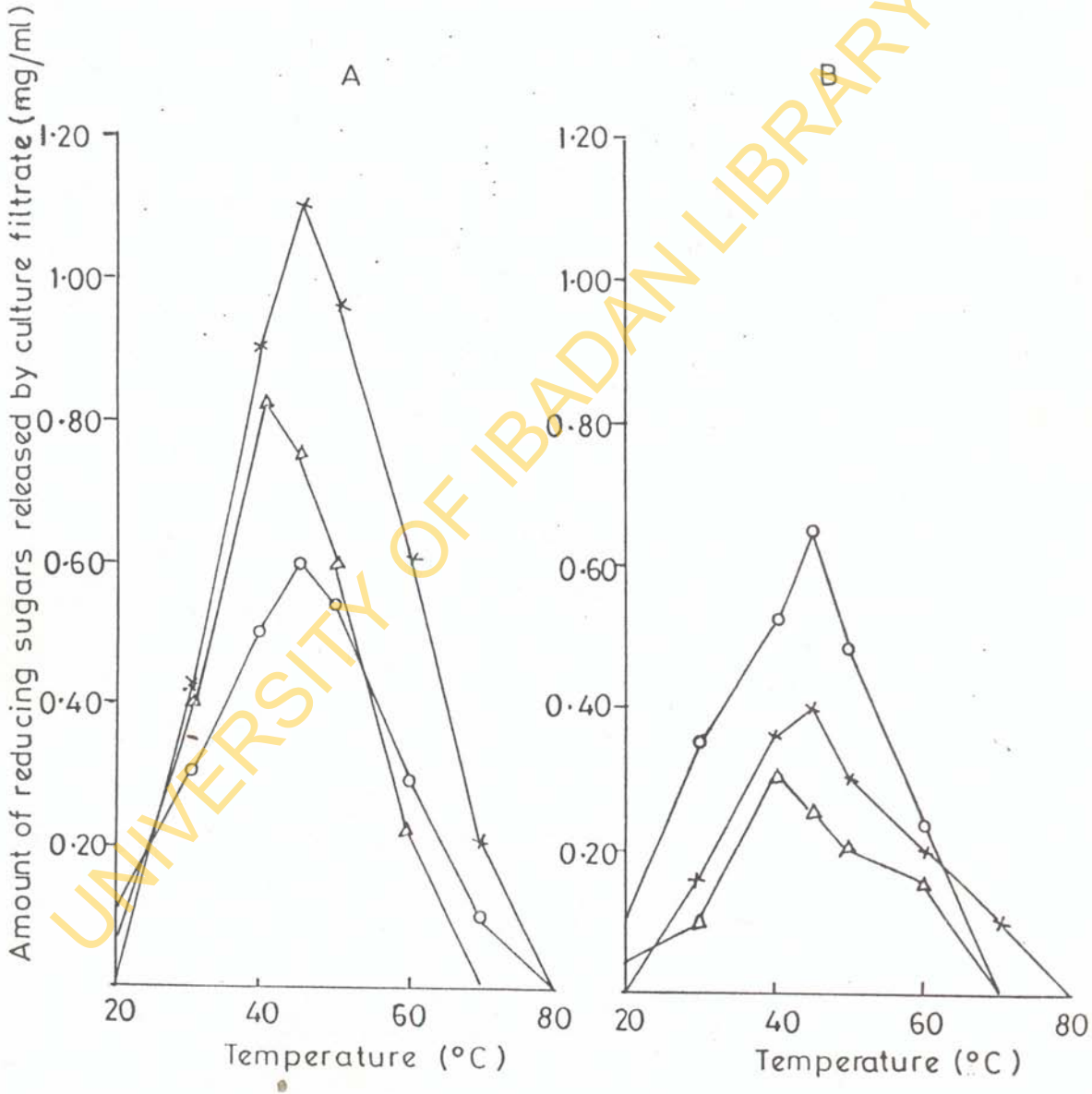

Fig. 12

cellulases of A. niger and Rhizopus arrhizus attained peak activity at 40° C, those of the remaining fungal isolates recorded peak activty at 45° C. Above the optimum temperature, a decrease in the cellulase activity was obtained for all the fungal species.

Protease production with time on poultry feed-casein medium

The protease production by all the isolated fungi increased with time of incubation until a peak was attained (Fig. 13). Rhizopus arrhizus recorded peak production of proteases on the 5th day of incubation; Aspergillus flavus, A. niger and A oryzae on the 6th day and A. fumigatus on the 7th day. Rhizomucor pusillus however did not attain peak protease production until the 8th day of incubation. For all the fungal isolates, a decrease in protease activity of their culture filtrates was observed after the peak production day.

Effects of pH of assay medium on protease activity

According to the results presented in Fig 14; acidic medium favoured higher protease activity of

Fig. 13: Protease production with time on poultry feed-casein medium. Cultures were incubated at 30° C. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ—Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ—Δ *Rhizopus arrhizus*.

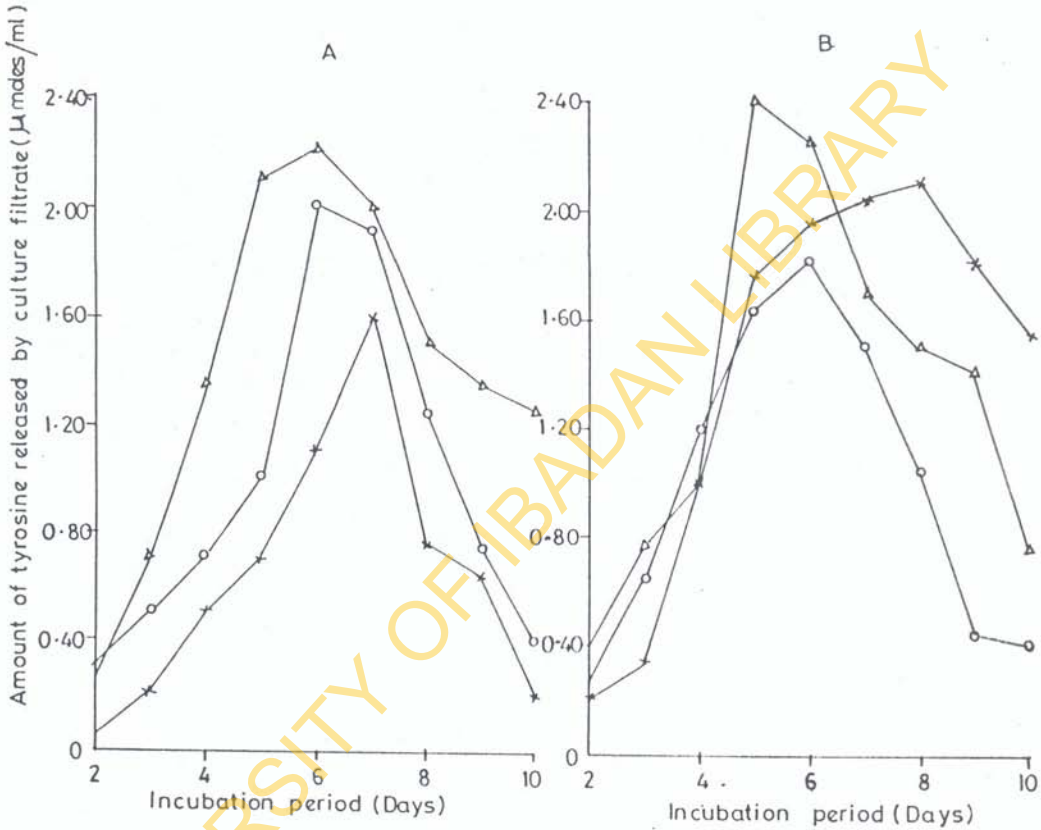

Fig. 13

Fig. 14: Effects of pH of assay medium of protease activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ——Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*

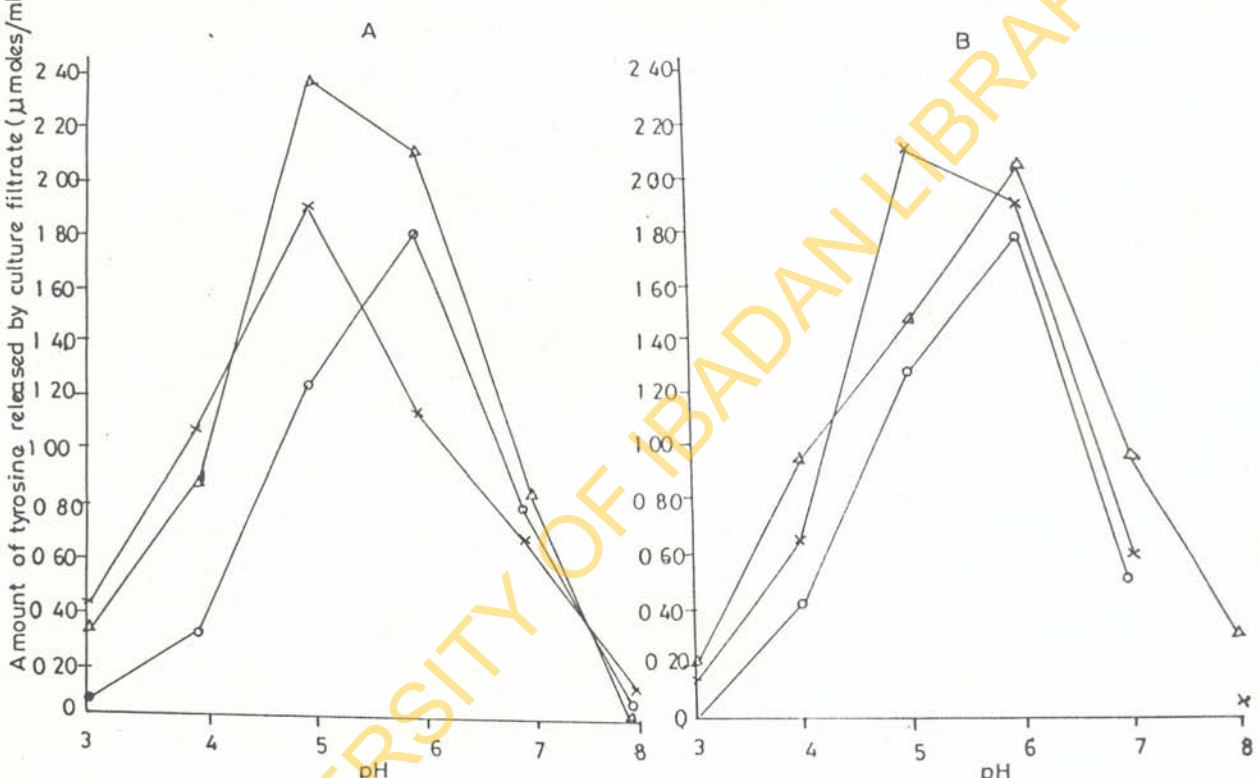

Fig . 14

culture filtrates of all the fungal isolates. Proteases of A. fumigatus, A. niger and Rhizomucor pusillus had peak activity at pH 5. For the remaining species: A. flavus, A. oryzae and Rhizopus arrhizus, peak protease activity was recorded at pH 6. Above the optimum pH, a decrease in protease activity was observed for all the isolated fungi.

Effects of temperature of assay medium on protease activity.

In this study, it was observed that 40° C was the opitmum temperature for the protease activity of culture filtrates of all the test species except for the protease of Rhizopus arrhizus which recorded peak activity at 30° C (Fig.15). At 60° C, protease activity was recorded only in culture filtrates of A fumigatus, A niger and Rhizomucor pusillus.

Lipase production with time on oat-meal chaff medium

Lipase activity of fungal culture filtrates first increased with time of incubation until a peak was attained. Aspergillus flavus, A. niger and A. oryzae recorded peak production of lipases on the 5th day of

Fig. 15: Effects of temperature of assay medium of protease activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ——Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*.

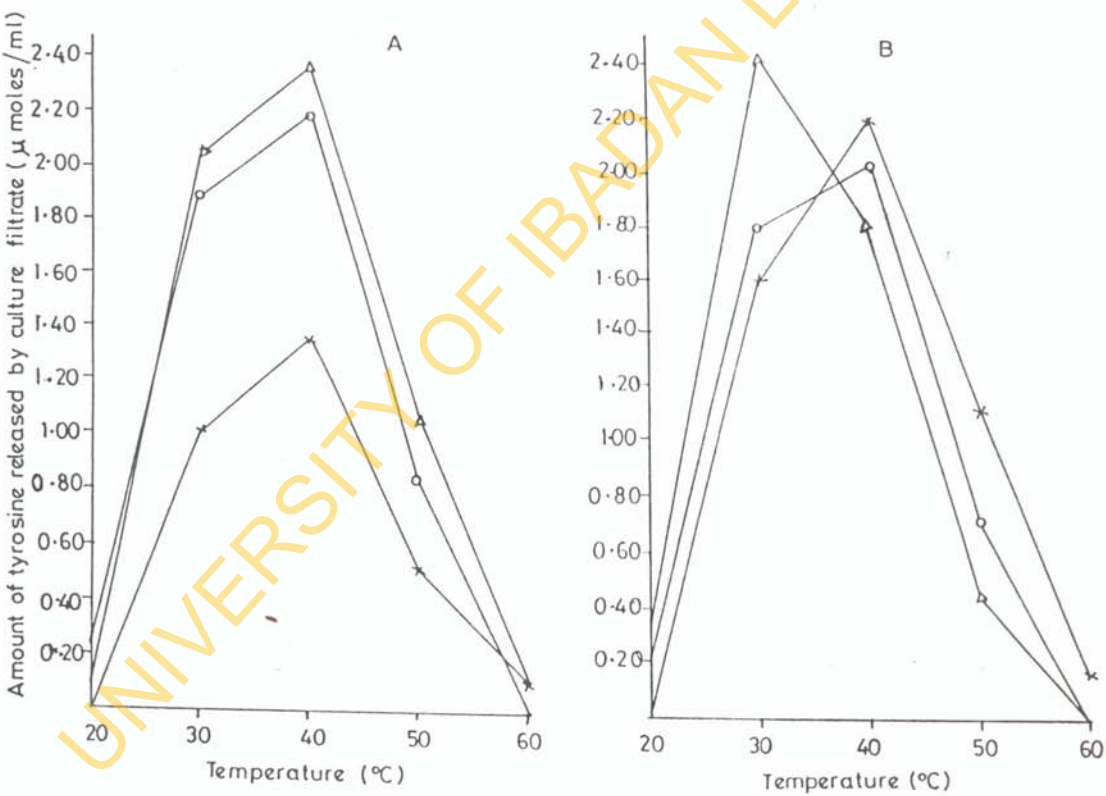

Fig 15

Fig. 16: Lipase production with time on oat-meal chaff medium. Fungi were incubated at 30° C. Each point on the graph is a mean of readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ—Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ—Δ *Rhizopus arrhizus*.

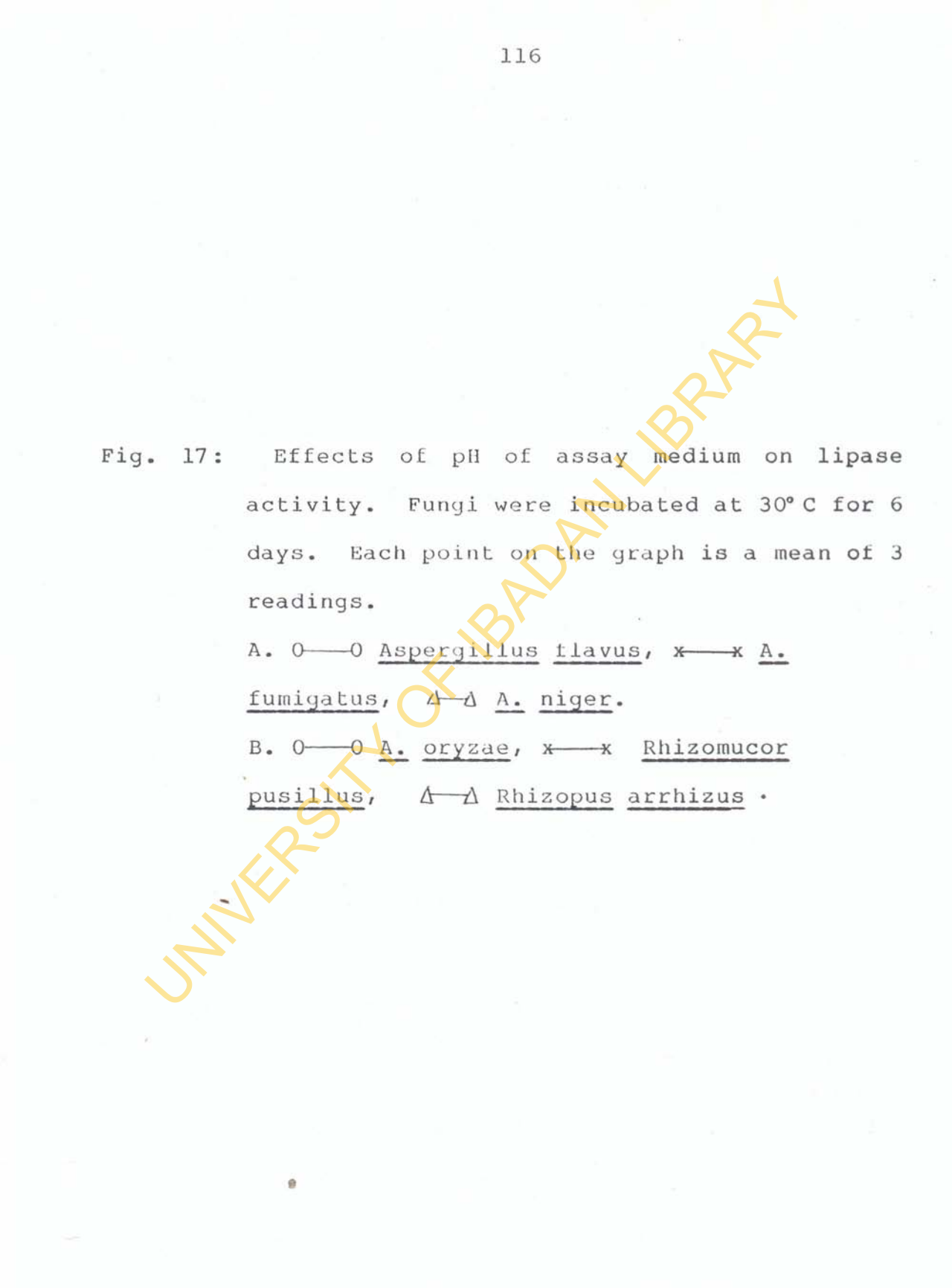

Fig. 17: Effects of pH of assay medium on lipase activity. Fungi were incubated at 30° C for 6 days. Each point on the graph is a mean of 3 readings.

A. O——O *Aspergillus flavus*, x——x *A. fumigatus*, Δ——Δ *A. niger*.

B. O——O *A. oryzae*, x——x *Rhizomucor pusillus*, Δ——Δ *Rhizopus arrhizus*.

incubation, Rhizopus arrhizus on the 6th day, and A. fumigatus and Rhizomucor pusillus on the 7th day (Fig. 16). After the peak production day for each species, a decrease in the lipase activity of the culture filtrates was obtained.

Effects of pH of assay medium on lipase activity

Lipases of all the species were more active in acidic medium than in alkaline medium (Fig. 17). Aspergillus niger had peak activity at pH 5 and almost the same amount of activity at pH 6. For the remaining fungal species, pH 6 was optimal for lipase activity. Above the optimum pH decrease in lipase activity was recorded for all the fungal isolates.

Effects of temperature of assay medium on lipase activity

The results observed showed that lipases of A. niger and Rhizopus arrhizus had optimal activity at 40° C (Fig. 18). The peak lipase activity for the remaining four fungal species was recorded at 45° C. Above the optimal temperature for each isolate a

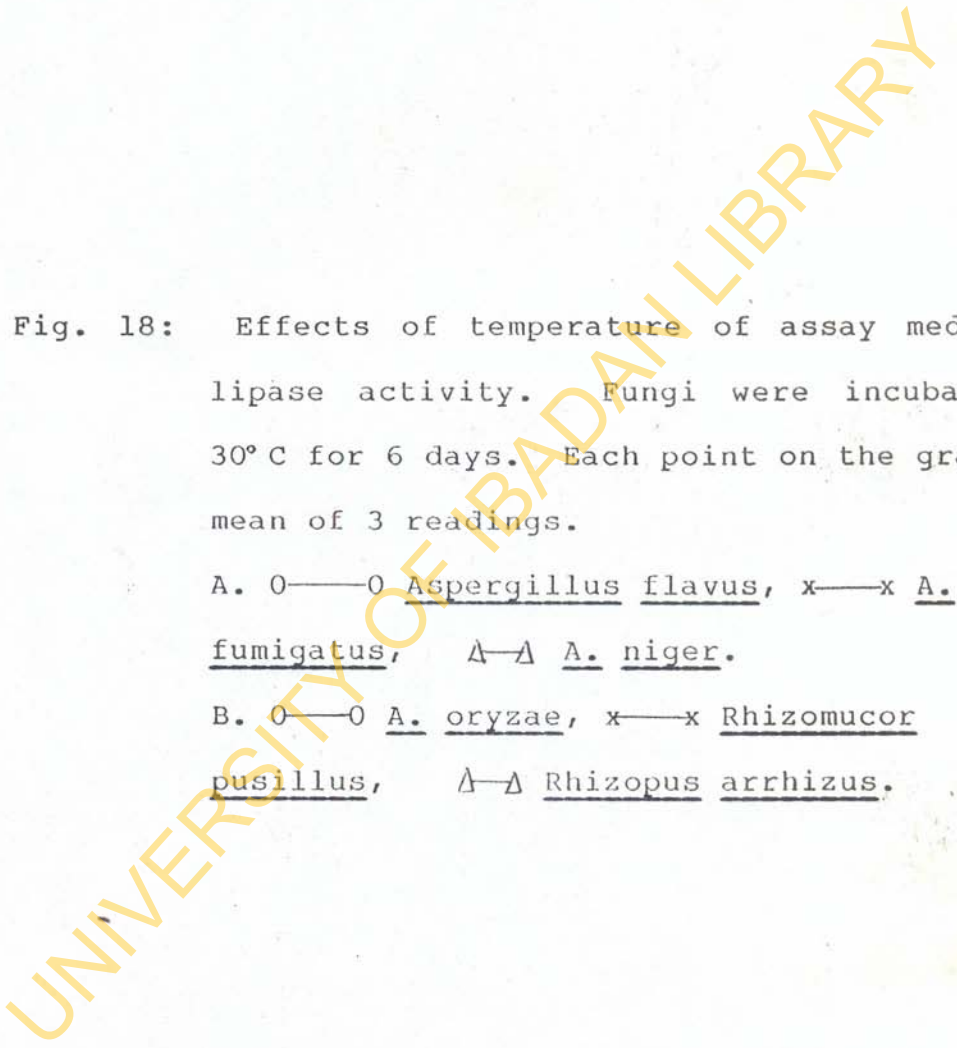

Fig. 18: Effects of temperature of assay medium on lipase activity. Fungi were incubated at 30° C for 6 days. Each point on the grap is a mean of 3 readings.

A. O——O Aspergillus flavus, x——x A. fumigatus, Δ—Δ A. niger.

B. O——O A. oryzae, x——x Rhizomucor pusillus, Δ—Δ Rhizopus arrhizus.

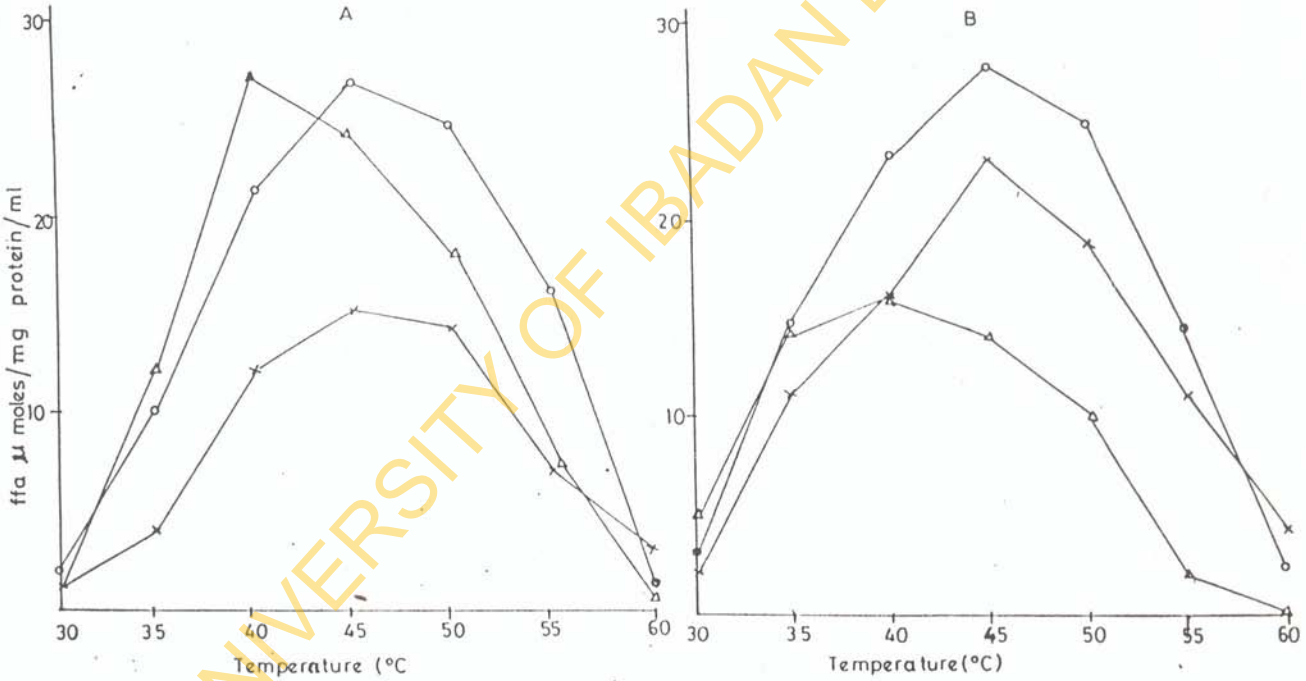

Fig. 18

decrease in lipase activity was recorded. Only Rhizopus arrhizus recorded no lipase activity at 60° C.

DISCUSSION

The fungi associated with stored agricultural products had been identified as one of the factors responsible for their deterioration and spoilage. The agricultural products, both of plant and animal origin serve as substrates for the various moulds or fungi which in most cases are saprophytes. Reports on the fungal invasion of stored produce particularly feeds and feed concentrates have been made. For instance, Bonner and Fergus (1959) identified the fungal flora of twenty-six cattle feed samples, sixteen of which had histories associated with disorders of livestock. Ogundero (1980b) isolated eleven fungal species from poultry feeds out of which four were thermophilic. Broadbent, (1969a, 1969b) working on maize (which is often a major poultry and livestock feed concentrate) isolated fungal species such as Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. tamarii, Penicillium decumbens and Mucor pusillus as the major micro-flora. Other fungal species had been reported to be associated with stored groundnuts which is also a feed component. Some of such fungi according to

Broadbent et al. (1972) include: Aspergillus chevalieri, A. penicilloides, A. ruber, A. amstelodami, A. flavus, A. tamarii, Macrophomina phaseoli and Botryodiplodia theobromae.

Two of the major factors that influence the above colonization and subsequent growth of fungi in feeds and feed concentrates and other agricultural products are the pH and moisture levels of the produce. Excluding the oyster shell meal samples which had pH values of between 8.2 and 9.0, samples of other feed concentrates like corn, groundnut cake, palm kernel, fish, blood, wheat offals, dried brewers grains, and bone meals had pH values of between 5.2 and 6.8. It is known that fungi like other microorganisms are affected by pH of the substrate within which they are growing. From previous reports (Cochrane, 1958), fungi had better mycelial growth in the acidic medium than in alkaline medium. The pH of most of the feed concentrates therefore is conducive to the colonization and subsequent growth of several fungi. Furthermore, the wide pH range within which the isolated fungi can thrive also suggests that even the oyster shell meal samples with alkaline pH is prone to

infestation by these microorganisms. Similarly, fungi are known to bring about changes in the pH of the substrate within which they grow (Chi and Hanson, 1964; Ruperez and Leal, 1979). This could partly account for the slight changes in pH of the various feed concentrate samples collected during the period of study. For the compound feeds, the pH values (5.9-6.9) recorded is favourable for fungal growth. This favourable pH range of the compound feeds coupled with the fact that the composite feed concentrates have several nutritional components (Oyenuga, 1968) could be responsible for the abundance of each fungal isolate in all the compound feeds.

The % moisture content of stored products has been reported as an important factor enhancing mould deterioration (Oyeniran 1978). Broadbent (1969b) reported that deterioration of maize and similar products by moulds is expected when the moisture content is above the maximum (about 12.5%) for 'safe' storage. The present studies show that for all the feed concentrates analysed, moisture content levels ranged between 8.86% recorded for the fish meal samples in November and 18.92% recorded for the corn meal

samples collected in August. This gives a wide range of 10.06%. The results for each type of feed concentrate is equally noteworthy. For the corn meal samples, the % moisture content values starting from March show an increasing trend up to August after which a downward trend was observed from September to January (Table 1). These results followed climatic trends. Such seasonal variation in moisture content of produce were recorded for palm-kernels by Adesuyi and Cornes (1970), for market samples of maize and groundnuts by Oyeniran et al. (1973) and for maize by Opadokun et al. (1979). This effect is usually caused by the climate affecting the initial drying of the produce or by the produce absorbing moisture during the wet months (Oyeniran 1976). The % moisture contents obtained for the corn meal samples in the present study were generally higher than the figures obtained by Opadokun et al. (1979) but less than those reported by Broadbent (1969b). The possible reasons for the differences in reports could be the variation in relative humidity of storage atmosphere, differences in the number of samples taken and the restriction of sampling between June and early September by Broadbent

(1969b). Differences in the post harvest storage periods and possible exposure of some samples to moisture could also account for the dissimilarity in the reports. Results of the % moisture content of the groundnut cake meal samples also followed climatic trends. The highest figure was 16.90% obtained in August while 12.60% recorded in January was the lowest, thus giving a range of 4.30%. For the palm kernel meal samples, the % moisture contents obtained also supported the view that the relative humidity of storage atmosphere affects the moisture content of produce. This effect of climate on moisture content of produce was also supported by the % moisture contents of fish meal samples, collected. For the blood meal samples,highest % moisture content (16.60) was recorded in July while the lowest (12.48) was obtained in January. This gave a range of 4.12. These results also supported earlier reports that higher moisture contents are obtained when the relative humidity of the storage atmosphere is high. Furthermore, the results on wheat offals samples showed that the highest % moisture content of 14.70 was recorded in July while the lowest (12.04) was recorded in January. The low

range (2.66) obtained suggests that % moisture content of the feed concentrate is not readily influenced by the climate. Samples of dried brewers grains had the highest % moisture content (10.74) in September and the lowest (9.62) in March. This is unlike the results for the other feed concentrates which had the highest % moisture contents in July or August and the lowest in January. The low range of 1.12% is also unique. This deviation in the results against the general observation for other feed concetrates could be due to the effectiveness of the initial drying of the produce or to the structural and or chemical properties which probably made the produce to absorb and give away moisture at comparatively lower rates than what was obtained in other feed concentrates. The oyster shell meal samples had highest % moisture content of 11.43 in July and the lowest (9.23) in November. The low range of 2.20 could also be an indication of little influence of climate on the moisture content. For the bone meal samples, the highest and lowest % moisture content values of 11.80 and 10.21 were obtained in August and November respectively. The low range of 1.59 is also suggestive of little influence of the

climatic factors on the sample moisture content. The general results of the feed concentrates moisture contents showed that corn, groundnut cake, palm kernel, blood and fish meals were probably more readily influenced by the climatic conditions. The moisture content of wheat offals, oyster shell meal, bone meal and dried brewers grains may not readily be affected by the climate. The last two feed concentrates were least influenced by climatic factors.

Results on the % moisture contents of the compound rations also followed climatic trends as observed with the feed concentrates. The generally high % moisture contents recorded (12.44-18.36) could partly be responsible for the large number of fungal colonies isolated from all the compound rations (Table 4). With the exception of the moisture content value (12.44%) recorded for the broiler starters mash collected in March, all other other samples had values in excess of the 12.50% regarded as the 'safe' moisture level (Broadbent, 1969b). The implication of this is that poultry feeds in South Western Nigeria are predisposed to fungal and other microbial deterioration even during the dry season when the relative humidity is

comparatively lower than what is obtained during wet season.

The significance of aflatoxins in foods, feeds and feed concentrates have been emphasized (Bababunmi et al., 1978; Patterson and Roberts, 1979, Rauch et al; 1988). Although other mycotoxins are being discovered, the aflatoxins retain a position of major importance because of their high toxicity and their common natural occurrence in significant quantities in several foods and feed crops (Schroeder, 1969, Miller et al; 1985). In the present work, significant quantities of the principal aflatoxins (B_1, B_2, G_1 and G_2) were detected mainly in corn and groundnut cake meals. The amounts of aflatoxins recorded in most of the corn meal samples were at very low to moderate levels (10 to less than 160ppb). Similar results have been reported for most samples analysed by Broadbent (1969b) and Opadokun et al. (1979). Lillehoj et al. (1980) however, obtained higher aflatoxin levels (above 300ppb) in most of the pre-harvest corn samples analysed. Results obtained from the analysis of groundnut cake meal samples in the present study showed that very low to relatively high levels (10-600ppb) of aflatoxins were recorded. These

results were similar to those recorded by Halliday (1969) except that in the current work some samples contained aflatoxin in excess of 250ppb. In another report (Joffe, 1969) the amounts of aflatoxin B_1 alone produced by most isolates of A. flavus from groundnut kernels were far in excess of 1,000ppb. The differences in levels of aflatoxins recorded in the present study and those previously reported could be due to factors such as the moisture contents of the substrate (Chang and Markakis, 1981, Salunkhe et al; 1987) the strains of the associated toxin producing fungi (Austwick and Ayerst, 1963) and the ambient temperature (Joffe, 1969). The period of storage could also have an effect. The present results suggest that when aflatoxin is present at all in a sample, B_1 is certainly present and most likely to have the highest level. This assertion is supported by the findings of Joffe (1969) which indicated that all Aspergillus flavus which produce any toxin at all produced aflatoxin B_1. This frequent occurrence of aflatoxin B_1 and more so at high levels in some feed concentrates and other agricultural products is of great significance. This is because aflatoxin B_1 is the most

toxic component of the aflatoxin complex (Schroeder, 1969). Apart from the palm kernel meal samples which had only traces of aflatoxin B_1, no detectable amount of aflatoxin was recorded in any other feed concentrate. These results confirmed that the production and accumulation of aflatoxins varied with substrates some of which are unsuitable for toxin production by A. flavus group (Schroeder 1969).

Analysis of the compound rations indicated that only aflatoxin B_1 was present and even then it was at very low and safe levels (below 30 ppb) for most samples. Possible reasons for this could be the dilution effect on the maize and groundnut components by the other feed concentrates which lack aflatoxins or were unsuitable for their production. The addition of fungistatic additives such as coccidiostat and butylated hydroxytoluene (Oluyemi and Roberts, 1979) to the compound rations at production time could possibly retard toxin production by associated microbes.

The six fungal isolates from the current studies have health and economic implications on livestock and man respectively. The fungi were often implicated in the deterioration and spoilage of several stored

agricultural products including animal feeds (Cristensen, 1957; Christensen and Kaufman, 1965). Toxin production by most of these fungal species and their activities as animal pathogens have also been reported. Aspergillus flavus and its related species A. oryzae are probably the most notorious of the lot because of the potency of aflatoxins they produce (Schroeder, 1969; Krogh, 1969, Blaha et al; 1986). Bamburg et al. (1969) reported that Aspergillus fumigatus is also toxigenic, being responsible for the production of fumagillin, gliotoxin and helvolic acid as its major toxic metabolites. The involvement of the same fungus in aspergillosis which is a well known respiratory disease of animals particularly young birds has been reported (Ainsworth and Austwick, 1955; Acha and Szyfres, 1980). It has also been estimated that 75% of mycotic abortions in cattle are due to Aspergillus especially A. fumigatus (Acha and Szyfres, 1980). Although Aspergillus niger is rarely implicated as a pathogen, however, it may be associated with a form of aspergillosis clinically different from that caused by A. fumigatus. It (A. niger) has also been reported to be associated with mastitis in cattle

(Ainsworth and Austwick, 1955). Mucor pusillus which is also one of the isolated fungi has been associated with gastric ulcerations in farm animals (Lacey, 1975). Furthermore, the reports made by Kulik and Holaday (1966) indicated that Rhizopus arrhizus could also be involved in toxin production.

Studies on the effect of environmental conditions such as temperature and pH on the mycelial growth of the isolated fungi became necessary to determine the optimum conditions under which deterioration of feeds take place. The knowledge of the optimum growth conditions for the fungal isolates could then be used for safe storage of feeds and other agricultural produce susceptible to microbial attack. The optimum growth temperatures recorded in the present investigations for the fungal isolates were 30° C for Rhizopus arrhizus and 35° C for Aspergillus flavus, A. niger and A. oryzae. Rhizomucor pusillus and A. fumigatus had optimum growth at 40° C. All the current results on the growth-temperature relations of the isolated fungi illustraed in Fig. 1 are consistent with the reports of previous workers. For instance, Olutiola (1976) reported similar growth-temperature

pattern for the A. flavus group. Studies conducted by Broadbent (1969a) also supported the present findings that Rhizopus arrhizus and A. niger are mesophiles recording optimum mycelial growth at or about room temperature. That Aspergillus fumigatus is a thermotolerant fungus growing well up to 45° C or higher has been reported by Raper and Fennel (1965).The reports of Cooney and Emerson (1964) and Ogundero (1980b) showed that Mucor pusillus is a thermophile growing less vigorously at room temperature than at higher ones.

Optimal growth for all the isolates was recorded in slightly acidic medium (Fig. 2). This is in agreement with earlier reports made by Cochrane (1958) and Child et al. (1973) that fungi have best mycelial growth in acidic media.

Analysis of feeds and feed concentrates showed the presence of several carbon sources some of which include glucose, fructose, pentoses, sucrose, raffinose, galactose, starch, dextrin, galactan, cellulose and pentosans (Oyenuga, 1968). Reports on the utilization of these carbon sources by fungi has been made (Chi and Hanson, 1964; Sorenson and

Hesseltine, 1966). Pisano and Anastasia (1958) and Oso (1974) reported that such carbon sources were utilized to varying extents by the fungi used. With the exception of sorbose, all the carbon sources supplied into the growth medium were utilized by the fungal isolates for mycelial growth and sporulation. This utilization was also found to be in various extents. Only Aspergillus fumigatus and A. niger recorded scanty growth on sorbose. None of the isolated fungi showed any sign of sporulation on the carbon source (sorbose). A previous report by Ogundero (1978) showed that out of several thermophilic fungi supplied with sorbose, only Chrysosporium thermophilum showed appreciable growth. Previous workers have reported the toxic nature of this carbon source to fungi (Oso, 1974). It is known to interfere with a respiratory pathway so that only those fungi which are not dependent on this pathway can utilize it (Ogundero, 1978). Utilization of cellulose by fungi is of paramount importance for the colonization and deterioration of plant products by storage moulds. For example, the penetration of cocoa beans by fungi is thought to be initiated by A. fumigatus because of its ability to produce cellulases

which could attack cellulose and pentosans in the seed coat (Chatt, 1953). This paves way for other species to enter the beans (Dade, 1929), or even assists them in doing so (Bunting, 1931). The present studies showed that all the fungal isolates utilized carboxymethyl cellulose (soluble cellulose) for mycelial growth and sporulation (Table 5). Unlike the other isolates, Rhizomucor pusillus and Rhizopus arrhizus recorded no growth on native cellulose (filter papers). Similar results were obtained by Somkuti et al. (1969) and Rosenberg (1978) while working with Mucor pusillus. The current view is that the degradation of native cellulose and consequently its utilization involves two types of enzymes designated C_1 and C_x (Mandels and Reese, 1965; Mandels and Steinberg, 1976). The C_1 enzymes act on the crystalline parts of the cellulose chain thus loosening the micro-fibrils in such a way that subsequent action by C_x enzymes becomes possible. The C_x enzymes were found to be an induced complex of enzymes hydrolysing the Beta 1-4 glucosidic bonds in the cellulose molecule. The C_x enzymes have the ability to degrade cellulose derivatives such as soluble carboxymethyl cellulose (CMC) and celluloses

that have been modified by grinding with concentrated acid or alkali. However, for crystalline cellulose to be hydrolysed both C_1 and C_x enzymes must be present. The failure of Rhizomucor pusillus and Rhizopus arrhizus to utilize native cellulose (filter papers) for growth and their ability to utilize CMC indicate the presence of C_x enzymes and lack of C_1 enzymes. The role of other microbial enzymes in the break-down of all types of organic food components had also been reported (Adler-Nissen, 1987; Park et al; 1987; Morihara, 1987).

Animal feeds and feed concentrates also contain several nitrogen sources (Oyenuga, 1968). Utilization of seveal nitrogen sources both organic and inorganic by fungi for growth and sporulation has also been reported by several workers (Sorenson and Hesseltine, 1966; Ruperez and Leal, 1979; Ogundero, 1981C). Results obtained from the present study showed that the fungal isolates utilized all the organic and inorganic nitrogen sources supplied. The highly abundant growth and sporulation of all the fungal isolates on casein hydrolysate was due to the constituent amino acids. According to Nolan (1970), the amino-acid contents of casein hydrolysate includes: glutamic acid, valine, threonine, arginine, aspartic acid, glycine, leucine, isoleucine, lysine, methoinine, phenylanine, histidine and tyrosine each of which provides a good source of

nitrogen for fungal growth. The good growth recorded for the fungal isolates on inorganic nitrogen sources is an indication that these nitrogen sources can effectively substitute amino acids as nitrogen sources for growth.

The growth and sporulation of all the isolated fungi on the feed infusion medium is an indication that the latter contained nutrients which were available, to all the fungal isolates. However, the growth and sporulation recorded on the medium was comparatively less than what was obtained when casein hydrolysate, starch or other good nitrogen or carbon source were supplied. This could be due to the dilute nature of the feed infusion medium since most of the solid components of the substrate were filtered out with the muslin cloth at preparation. Fungistatic substances added to poultry feeds by feed compounders could also retard fungal growth.

The screening of all the isolated fungi for aflatoxin production implicated only Aspergillus flavus and A. oryzae. This confirmed previous reports that some strains of A. flavus are major aflatoxin producers (Kulik and Holaday, 1966; Taber and Schroeder, 1967; Niles, 1978). The production of aflatoxins by A.

oryzae in the two types of modified Czapek-Dox medium is not significantly different (at $p = 0.1$) from the amounts produced by *A. flavus*. This observation is not consistent with the report by Schroeder (1969) that *A. oryzae* strains produced small quantities of aflatoxin B_1 or none at all. The difference in the two reports could be explained by the variability of results usually obtained when working with cultures of the *A. flavus* group (Schroeder, 1969). Several strains of *A. flavus* are however generally known to produce both aflatoxins B_1 and B_2 (Wicklow and Shortwell, 1983; Doner *et al*; 1984). The attack and growth of *A. flavus* group on almost all crops particularly when the latter are ripe or senescent should therefore be considered with a view to control contamination with toxin metabolites of fungi.

Apart from the fungus, the most important factors affecting the development of aflatoxin contamination are: substrate, temperature, pH, moisture conditions and time. In the present study, the amounts of aflatoxin B_1 in the culture filtrates of the two aflatoxin producing fungal species increased after inoculation until the 8^{th} day when the peak was

recorded. During the incubation period the pH of the culture filtrates fell gradually and was lowest on the 8th day which incidentally recorded the peak toxin production and accumulation. The toxin production curve obtained is similar to microbial growth curve (Cooper, 1971). According to Ogundero (1978), the initial lowering of the pH of fungal culture medium could be due to the formation of organic acids or absorption of cations as a result of the fungal metabolic activitites. The effects of incubation temperature and pH of the medium on aflatoxin B_1 production is similar to their effects on fungal growth (see Figs. 1, 2, 5 and 6). Similar results on the effect of temperature on the production and accumulation of the toxins in stored field crops has been reported (Schroeder, 1969). Since aflatoxins are products of metabolic activities in fungi, their production and accumulation therefore are affected by the environmental conditions that affect fungal growth. Ogundero (1987) reported that the singular limiting factor for aflatoxin contamination of Nigerian groundnuts, apart from the moisture contents is temperature. The substrate upon which a toxigenic

fungus is growing affects the production and accumulation of mycotoxins. Broadbent et al. (1963), Taber and Schroeder (1967) and Halliday (1969) have reported that groundnut in particular is a very good substrate for aflatoxin production by A. flavus group. The production and accumulation of aflatoxins in corn has also been reported (Broadbent, 1969b; Opadokun et al., 1979; Lillehoj et al., 1980). Other agricultural products that had been found to be suitable for aflatoxin production by toxin producing fungi include cotton seed, rice, Brazil nuts, coconut, oats, pinto bean and wheat (Schroeder, 1969). In the present work, corn, groundnut cake and palm kernel meals were the only feed concentrates that contained detectable amounts of aflatoxins at time of collection. Detection of the toxins in wheat offals and dried brewers grains after adjusting the moisture content to 35% (w/v) and inoculated with the toxigenic fungal isolates implied that if conditions are favourable, the two feed concentrates could be good substrates for toxin production and accumulation. All the remaining feed concentrates analysed: fish, blood, oyster shell and bone meals appeared to be unsuitable substrates for

aflatoxin production and accumulation. Similarly, lack of aflatoxins in the poultry droppings inoculated with the toxin producing fungi and in uninoculated samples even when favourable conditions of temperature and moisture were provided suggested the unsuitability of the product for aflatoxin production and accumulation.

The 'storage fungi' like other microorganisms are able to attack and utilize the nutrients available in stored agricultural products for their cellular growth. This utilization of nutrients in stored products is possible because of the numerous extracellular enzymes produced and released into the substrates by the invading fungi. Starch, cellulose, hemicellulose, lignocellulose, lignin, pectin, proteins, lipids, fats and oils are some of the major classes into which plant components are grouped. Lignified cellulose or lignocellulose is much more resistant to decay than the other plant components while pure, isolated lignin has not been shown to be degraded significantly by any organism (Rosenberg, 1978). Food is usually stored in plant tissues in form of starch. The production of enzymes which are capable of breaking down such storage products are of prime importance in fungi that obtain

nutrients from plant materials. Amylases are responsible for the breakdown of starchy materials to maltose. Amylases are classified into two groups. The & - amylases or endoglycosidases hydrolyse starch chains at points far from chain ends to form short polysaccharide chains and B-amylases which have an exo-action, cutting off chain ends to two sugar units usually maltose (Bernfeld, 1951). Results of the present work showed that peak amylase production was attained on the 4th day by Aspergillus niger and Rhizopus arrhizus; on the 5th day by Rhizomucor pusillus and on the 6th day by A. flavus, A. fumigatus and A. oryzae. These results are consistent with previous reports that the rates of amylase production are different for most fungi. Chapamn et al. (1975) reported that maximum amylase activity was attained on the 4th day for Papulaspora thermophila on starch - yeast-extract medium and after 8days for the same organism on a yeast extract medium. Ogundero (1979) recorded highest amylase production for Humicola lanuginosa and H. insolens on the 4th and 6th days of incubation respectively in an oat meal chaff medium. Oso (1979) also reported that Talaromyces emersonii had

peak amylase activity at different days when incubated at different temperatures.

All the fungal isolates used for the present studies had peak amylase activity on media with acidic pH (5-6). The peak amylase activity was also recorded at temperatures between 45° and 50° C for all the test fungi. Similar findings on the effects of pH and temperature on amylase activity of some fungal species has also been reported (Ogundero, 1979; Oso, 1979).

Results obtained in the present work also showed that amylases were produced by all the isolated fungi when grown in media with different types of carbon sources although the largest amounts of amylases were recorded when the medium contained starch. Adams (1976) reported that amylase production could be induced in fungi when the growth medium contained either glucose, fructose or lactose as sole carbon source but then the amount of amylases produced was usually less than when starch was used.

Cellulases are responsible for the breakdown of cellulose by moulds and other microorganisms. Reports of several workers have shown that the production of extracellular cellulases by fungi is common but not

universal (Fergus, 1969; Rosenberg, 1978; Ogundero, 1978). Cellulases are designated C_1 and C_x as previously indicated. Only the C_x is necessary for the degradation of CMC while both C_1 and C_x must be present before native cellulose is degraded (Mandels and Reese, 1965). Factors such as time, pH, temperature and the type of substrate have been reported to affect cellulase synthesis and activity. The peak of cellulase production in this study was recorded on the 5^{th} day for Aspergillus fumigatus and A. niger; on the 6^{th} day for A. flavus, A. oryzae and Rhizopus arrhizus and on the 8^{th} day for Rhizomucor pusillus. The results also showed that there were differences in the amounts of cellulase activity recorded for the various fungi. This variation in the peak production time and amounts of cellulase activity in different fungi has been reported (Ogundero, 1978; 1979).

All the fungal isolates used for the present studies had peak amylase activity on acidic media with pH (5-6). The peak amylase activity was also recorded at temperatures between 45° and 50° C for all the test fungi. Similar findings on the effects of pH and

temperature on amylase activity of some fungal species has also been reported (Ogundero, 1979; Oso, 1979).

Peak cellulase activity of the culture filtrates of all the isolated fungi was recorded at pH 5-6. Robertson and Koehn (1978) reported that Poronia punctata cellulase assays on carboxymethyl cellulose (CMC) gave major and minor peaks of activity at pH 4.8 and 5.6 respectively. In the same experiment, a side peak of cellulase activity also occurred at pH 4.4. Reports by other workers also showed that peak cellulase activity was obtained for several fungi at pH values under 7 (Oso, 1978, Ogundero, 1979; Ogundero and Adebajo, 1987). According to Fergus (1969), the pH for mycelial growth of Humicola insolens, Mucor meihei and Torula thermophila were the same as those at which peak cellulase activity was attained. Results of the present study also showed that A. niger, Rhizomucor pusillus and Rhizopus arrhizus had optimal mycelial growth and peak cellulase activity at the same pH level (pH 6). The remaining fungal isolates : A. flavus, A. fumigatus and A. oryzae had only a slight difference of 0.5 in the two pH values. For all the fungal isolates, the optimum pH for both mycelial growth and cellulase

activity were however under pH 7. Mandels and Reese (1965) reported that cellulases are induced enzymes which are produced only on cellulose or related substances. The results of the present studies showed that while the highest amounts of cellulases were produced on medium with CMC and on feed infusion medium which is also rich in cellulose, other media without cellulose component also supported the synthesis of cellulases in some of the fungal organisms. Aspergillus flavus, A. fumigatus, A. niger and A. oryzae produced varying quantities of cellulases on media with glucose, dextrin, starch, pectin, CMC and feed infusion medium. Rhizomucor pusillus and Rhizopus arrhizus on the other hand produced cellulases only on media with pectin, CMC and feed infusion medium. Ogundero (1978) also reported the production of cellulases by fungi on carbon sources other than cellulose. From the present results and those previously reported it can be suggested that while cellulases could be induced enzymes in some fungi, they could both be constitutive and induced enzymes in others. In both cases, largest synthesis of cellulases is obtained in cellulose containing medium.

Cellulase activity of the culture filtrates in the present work was found to increase with temperature up to 45° C after which there was a drop in activity. Robertson and Koehn (1978) reported that optimal temperature activities for Poronia punctata cellulases was at 55° C with CMC as substrate and 45° C with filter paper and microcrystalline cellulose (MCC) as substrates.

Observations in the present work indicated that proteases were present in the culture filtrates of all the fungal isolates. The peak protease production and activity by the isolated fungi was recorded at different days for the different fungal species. It was recorded for Rhizopus arrhizus on the 5th day and for A. flavus, A. niger and A. oryzae on the 6th day. Aspergillus fumigatus and Rhizomucor pusillus had peak protease production on the 7th and 8th days of incubation respectively. Factors such as pH of the medium and temperature of incubation were found to affect the protease activity. The peak protease activity was recorded for all the isolated fungi at pH 5-6. Optimum temperature for protease activity was 40° C for all the fungal species except Rhizopus

arrhizus which recorded peak protease activity at 30° C. Somkuti and Babel (1967) and Ogundero (1983) recorded similar results for the proteases of Mucor pusillus and Thermoascus aurantiacus respectively. They found that pH and temperature greatly affected the synthesis and activity of proteases of the fungi.

Reports on the hydrolyses of natural and synthetic glycerides to free fatty acids by extracellular lipases of fungi has been made (Somkuti and Babel, 1968; Ogundero, 1980a). Results of the present work showed that lipase activity was recorded in culture filtrates of all the isolated fungi. The production and activity of the lipases were found to be affected by the period of incubation, pH and incubation temperature. The peak lipase production was recorded for the fungal isolates between 5 and 7 days of incubation. Optimum pH for the lipase activity was recorded in the acidic medium for all the fungi, being pH 5 for Aspergillus niger and pH 6 for the remaining fungal isolates. A temperature range of between 40° and 45° C was found to be optimum for lipase activity of all the test fungal organisms. These results are similar to those previously reported (Somkuti and Babel, 1968; Ogundero, 1980a).

In view of the adverse consequences resulting from fungal infestation and growth on stored agricultural products particularly feeds and feed concentrates, concerted efforts are being made by several workers to ameliorate the situation. These control activities involve mainly the prevention of infection in stored products and the regulation of fungal growth in already infected produce.

Perhaps the most feasible and practically attainable method for the control of fungal infestation, growth and other deteriorative activities in stored agricultural products particularly feeds and feed concentrates is the regulation of moisture content levels in such produce. Koehler (1938) and Tuite and Christensen (1955, 1957) found that various microflora developed on corn, wheat and barley stored at various moisture contents of 13% and above. The present studies showed that with the exception of some samples of dried brewers grains, fish, oyster shell and bone meal, all other feed concentrates and compound feeds collected had moisture contents above 13%. This suggests that most of the samples were predisposed to microbial deterioration.

The conditions under which the bagged poultry feeds were stored in some feed depots from where some samples analysed were taken took no cognizance of the effect of humid atmosphere on the moisture content of produce. The storage condition in some poultry houses was even worse with the feeds being kept in partially opened sheds which could allow inflow of rain water during wet season. The negligence of poultry attendants at not properly sealing the mouths of bags with left-over feeds is likely to worsen the problem of moisture absorption by feeds. Furthermore, the stuffy nature of several poultry houses resulting from the decomposing poultry droppings and litter on the floor makes fungal contamination of feeds kept in such places inevitable.

Bearing in mind the conditions under which feeds are prepared by feed compounders, stored and handled by retailers and in the poultry farms, the following suggestions based on the results of the present work and those previously reported can be made:

Livestock feed compounders and millers should always use feed inputs with low moisture contents (less than 12%) and with little or 'safe' microbial

infestations. To make this possible, each feed mill should have a microbiological and quality control unit. The establishment of Feed Quality Advisory Group by the Federal and State Governments is also recommended for the same purpose.

Water-proof polythene bags or multiwall paper bags should be used for packing feeds. Both the feed inputs and finished product should always be kept under dry conditions to reduce absorption of moisture by produce.

In the poultry farms, feeds should also be stored in dry places and moistened litter should be removed regularly to reduce fungal infestation and growth.

The foregoing reports and suggestions not withstanding, further work on the role of microorganisms particularly fungi on feeds and feed inputs is still necessary. This could bring better understanding of the conflicting interpretations currently being placed upon the role of toxigenic and zoopathogenic fungi associated with feeds and other stored agricultural products. The need for more concerted efforts at controlling these obnoxious fungi and their deteriorating activities could even become more urgent and mandatory.

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