DETERIORATION OF OIL CAKES BY FUNGI

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Deterioration of oil cakes by fungi" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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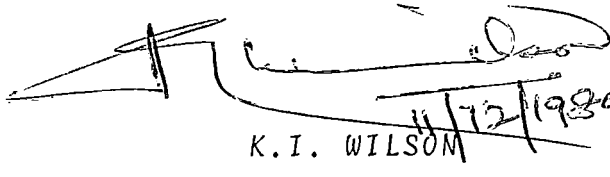

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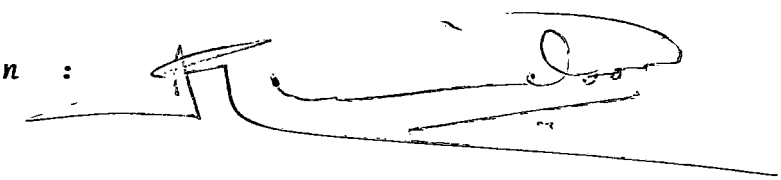

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INTRODUCTION

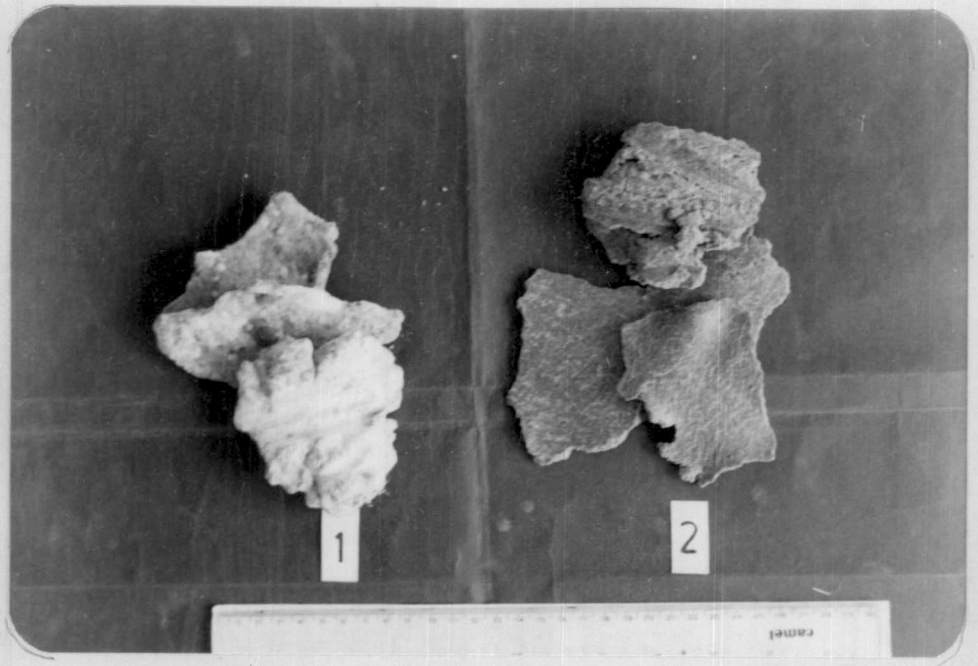
INTRODUCTION

Oil cakes are traditionally and widely used as manure for growing plants and are the major constituents of cattle and poultry feed. These are also used in the manufacture of certain adhesives and paints. Edible oil cakes, especially from groundnut serve as a good source of protein and are utilized for the manufacture of some biscuits and baby foods.

Deterioration by micro-organisms is a serious problem encountered in the storage and transport of oil cakes and fungi are the major group responsible for the spoilage [Philip, 1978; Nusrath and Nahdi, 1983 and Reddy *et al.*, 1986-Plate I]. It has been estimated by Subrahmanyan *et al.* [1967] that at least 10 per cent of coconut products are lost as a result of microbial activity.

Some of the fungi invading oil cakes [Viz., *Aspergillus flavus* and *A. niger*] are known to produce certain harmful toxins called aflatoxins [Gopal *et al.*, 1968; Neelakantan *et al.*, 1981; Niza, 1981 and Balasubrahmanian, 1985] in addition to depletion of nutrients such as carbohydrates, proteins, amino acids and most of the mineral

Plate I. *Deterioration of oil cakes by fungi.*



1. Groundnut oil cake colonised by fungi
2. Control

elements. The aflatoxins are known to be carcinogenic especially causing liver cancer in farm animals [Sastry et al., 1965 and Gupta et al., 1985]. Some of these toxins [M_1 and M_2] are even excreted through milk, causing toxic hazards to human beings [Krishnamachari et al., 1975 and Sreenivasamurthy, 1975].

The fungi associated with oil cakes and the various deleterious effects caused by them have not been studied in detail. In the present investigation, fungi invading coconut, groundnut and sesamum oil cakes collected from the southern, central and northern regions of Kerala during different seasons were isolated, their effect on the nutritive value, aflatoxins if any, produced by them, and the methods for checking/ minimising deterioration during storage and transit were studied and the results are presented in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A large number of fungi are known to cause deterioration and spoilage of oil cakes.

Walker (1906) and Brill et al. (1917) reported the presence of Aspergillus flavus, A. niger, A. glaucus, Rhizopus nigricans and Penicillium citrinum in coconut products. Passmore (1931) isolated A. tamarii, A. chevalieri, Rhizopus nigricans, Scopulariopsis sp., Syncephalastrum racemosum, Penicillium candidoflavus and Mucor racemosus from copra samples collected from London. Aspergillus niger, Aspergillus sp., Penicillium sp., Trichoderma sp., Diplodia sp., and Rhizopus sp., were isolated from copra collected from different sources (Nair, 1968). Paul (1969) isolated nine fungi, namely, A. niger, A. flavus, A. ustus, Rhizopus sp., Penicillium sp., Diplodia sp., Trichoderma viride, Cunninghamella verticilliata and Syncephalastrum racemosum from copra collected from different districts of Kerala.

Philip (1978) conducted preliminary studies on the fungal population of coconut oil cake in Kerala and reported the presence of A. flavus, A. niger, Rhizopus stolonifer and Penicillium citrinum . Ogundero (1980) isolated Acremonium

sp., A. candidus, A. fumigatus, Chaetomium virginicum, Malbranchea sulfurea, Mucor pusillus, Mucor sp., Paecilomyces varioti, Rhizopus homothallicus and Thermoascus aurantia from poultry feeds of which oil cake are the major ingredients.

Niza (1981) isolated A. flavus, A. niger, A. oryzae, A. terreus, A. fumigatus, A. niveus, Mucor hiemalis, Rhizopus stolonifer, Penicillium citrinum, Penicillium citreo-viride, Botryodiplodia theobromae, Ceratostomella adiposum, Fusarium sp., and Colletotrichum sp., from copra. Quantitative assessment of fungi during different months revealed that high population was present during June-July, followed by October-November. Very low population of fungi was recorded during the period from January to April.

Abdel-Fattah et al. (1982) reported that fungi frequently noted in deteriorated animal feeds were A. niger, A. flavus, Mucor racemosus, Alternaria alternata, Rhizopus stolonifer, Penicillium corylophilum and Penicillium notatum. Three genera, namely, Mucor, Rhizopus and Alternaria were moderate in occurrence while Cladosporium, Fusarium and Neurospora were of low occurrence. Nusrath and Nahdi (1983) isolated A. flavus, A. glaucus, A. nidulans, A. niger, A. terreus, Cladosporium herbarum and Penicillium viridicatum from groundnut oil cake and Alternaria

alternata, A. candidus, A. flavus, A. nidulans, A. niger, A. versicolor and Cladosporium herbarum from coconut oil cake. Of these, A. flavus occurred in a higher frequency of 39 and 38 per cent respectively in groundnut and coconut oil cakes.

Bilgrami (1985) isolated A. flavus, A. niger, A. ochraceus, A. sulphureus, Penicillium oxalicum, Penicillium citrinum, Alternaria alternata, Rhizopus stolonifer and Actinomucor repens from copra. Of these, A. flavus and A. niger dominated with higher frequency of 100 and 79 per cent respectively.

Hastino (1986) reported that A. fumigatus, A. flavus, A.niger, A. terreus, A. glaucus and other species of Aspergillus were found in poultry feeds in a frequency of 8.39, 61.15, 5.28, 2,64, 0.96 and 21.58 per cent respectively. Kharchenko (1986) isolated A. flavus, A. fumigatus, A. nidulans, A. niger, Penicillium cyclopium, Penicillium viridicatum, Penicillium citrinum, Fusarium graminearum and Rhizopus nigricans from contaminated feed stuffs. Kulkarni et al. (1986) isolated Aspergillus sp., Penicillium sp., Rhizopus sp., Alternaria sp. and Fusarium sp. from infected copra samples.

Reddy et al. (1986) studied fungal infestation in some feed stuff in Andhra Pradesh (India). They found that the predominent fungi infesting the feeds were A. flavus, A. niger, and species of Fusarium, Penicillium, Rhizopus, Curvularia, Drechslera and Alternaria. Groundnut cake was found to be the most favoured substrate for A. flavus. Romo and Fernandez (1986, a) in studies on the mycoflora of 125 samples of commercial poultry feeds noted Penicillium, Aspergillus and yeast in more than 80 per cent samples, Fusarium, Mucor, Cladosporium and Aureobasidium in nearly 50 per cent of the samples and Circinella and Rhizopus in about 25 per cent of the samples. Sandor (1986), based on mycological examination of 3598 feed samples reported that approximately 65-71 per cent of fungal flora was constituted by Aspergillus, Fusarium and Penicillium. Shrivastava and Rab (1986) reported that Aspergillus, Penicillium, Fusarium, Curvularia and Alternaria were the dominant fungi in contaminated cattle feeds.

Effect of storage conditions on the incidence of fungi in oil cakes.

Heintzeler (1939) conducted a comprehensive survey to understand the relationship of atmospheric humidity with

growth of moulds. She found that the optimum relative humidity (RH) for the growth of Aspergillus niger and A. glaucus in steam sterilized atmosphere was 98 and 90 per cent respectively. The optimum temperature for A. glaucus was 30 °C, while 20 °C, was favourable to Penicillium glaucum, Rhizopus nigricans, Phycomyces niteus and Oidium lactis.

Bonner (1948) conducted in vitro studies on the temperature and humidity requirements of A. niger. The results showed that optimum temperature requirement for growth of A. niger was related to RH. At 95 per cent RH, the temperature requirement was around 40 °C, at 100 per cent RH, the optimum temperature was near 30°C. Bottomley et al. (1950) reported that in the case of stored yellow corn, as the RH of atmosphere was raised from 75 to 100 per cent, the total moisture content increased logarithmically, consequently increasing the internal mould infection and fat acidity and decreasing the viability of the seeds. The composition of microflora varied with moisture, temperature and oxygen concentration in the atmosphere.

Subramanian (1956) investigated the influence of seasonal factors on the deterioration of copra and coconut oil marketed in South India. In all the samples studied, the July and October samples showed higher moisture content and

had more number of fungi than samples collected during other months. Among the fungi, species of Aspergillus caused appreciable deterioration and the most frequent in this group was A.niger.

Qasem and Christensen (1958) reported the influence of moisture content on the deterioration of stored corn by fungi when good quality yellow dent maize inoculated with different species of Aspergillus was stored at moisture contents ranging from 5 to 25 per cent. They found that at 14 per cent moisture A.repens, at 16 per cent A. candidus and at 18 per cent A.flavus were the chief invaders.

Marar and Padmanabhan (1960) studied the effect of moisture content and RH on the deterioration of copra, during different seasons of the year and noted maximum deterioration during June-July, when the RH ranged from 92 to 95 per cent. Rajasekharan et al. (1960) in their studies on the shelf life of coconut oil cake found that when stored at 79.1 and 76 per cent RH, no mould attack was evident.

Subrahmanyan et al. (1965) in their studies on the agencies responsible for the spoilage and destruction of coconut products observed a relationship between moisture content and occurrence of different fungi on copra. At 15 per cent and above A. flavus - oryzae and Rhizopus nigricans

penetrated deep into the kernel and produced heat, gases and rancidity. At 8 to 12 per cent moisture content A. tamarii and A. niger appeared, followed by A. glaucus and Penicillium sp. Frias (1967) found that the degree of invasion of maize by all isolates of A. flavus was proportional to the increase in moisture content and storage periods.

Nair (1968) indicated that deterioration of copra can occur at higher relative humidities even if the initial moisture content was low. He found that copra samples kept at RH 100, 90.2, 85.1 and 79.1 per cent showed fungal growth within 6, 7, 16 and 30 days respectively, whereas no fungal growth was observed in samples kept at 76 per cent RH even after 65 days.

Olutiola (1976) reported that in the case of A. flavus, best growth and sporulation were observed at 35 °C. Omprakash and Siradhana (1978) observed that 35 °C was the optimum temperature for the growth of four species of Aspergillus from maize.

Philip (1978) reported a correlation between RH of the atmosphere and microbial population of copra. As the RH increased, there was an increase in the microbial population of copra. She found that a moisture content of 20 per cent was favourable for the incidence of Mucorales,

whereas 17-13 per cent was highly favourable for the growth of Aspergillus spp.

Storage temperature, RH of the atmosphere and moisture content of copra exerted great influence on the colonization of A. flavus (Niza, 1981). In vitro studies on the incidence of A. flavus revealed that copra kept at a temperature of 30 °C, and 100 per cent RH had maximum infection with good sporulation. Nandi et al. (1982) in their studies on the deterioration of some oil seeds in storage found that seed moisture and fungal infection were higher at 90 per cent RH and 20 °C.

Dange and Patil (1984) observed that groundnut seeds stored at higher RH ranging from 76 to 93 per cent showed high invasion by A. niger, A. flavus and Rhizopus sp., resulting in loss of viability. At 62 per cent RH, the invasion was low and the viability was little affected even after storage for 180 days. Aibara et al. (1985) found that A. parasiticus in shelled peanuts could not grow during storage at 25 °C, if the RH was less than 79 per cent.

Effect of fungi on the oil content of oil cakes and seeds.

Stoke (1928) found that Penicillium sp. caused rancidity of coconut oil. This is due to the presence of methylamyl ketone in higher concentrations resulting in the characteristic odour of the rancid oil.

Horowitz-Viassoma and Livschitz (1936) studied the microbial action on fats and found that numerous fungi of the genera Penicillium, Aspergillus, Sterigmatocystis as well as bacteria are capable of splitting fats and oils. Stansbury (1947) found that in cotton seeds and peanuts stored at room temperature, the increase in free fatty acids was very large in the case of mouldy samples.

In stored soybean, the oil content was not much affected due to fungal invasion (Milner, 1950). In shelled peanuts, Aspergillus tamarii caused a more rapid decline and greater loss of oil than A. glaucus and Penicillium citrinum (Ward and Diener, 1961). Nair (1968) observed reduction in the oil content of copra inoculated with different fungi. A. niger, Aspergillus sp., Trichoderma sp., Penicillium sp., Diplodia sp. and Rhizopus sp. reduced the initial oil content of 62.86 per cent to 47.73, 51.57, 55.63, 55.96, 54.00, 59.70 per cent respectively.

Paul (1969) obtained a progressive fall in the oil content of copra due to fungal infection. A. niger and A. flavus penetrated deep into the tissues and brought about maximum reduction in oil content. Jaganathan (1970) noted wide variation in the oil content of copra samples depending upon the duration of storage and concomitant microbial deterioration.

According to Lalithakumari et al. (1971,a) infection of groundnut seeds by A. flavus, Botryodiplodia sp. and Cladosporium herbarum reduced the oil content appreciably whereas Rhizoctonia bataticola increased the oil content. Helminthosporium tetramera had little effect on the oil content of groundnut. Lalithakumari et al. (1971, b) established that in the case of castor seeds, storage fungi reduced the oil content to very great extent. Alternaria brassicola and Curvularia pallescens reduced the oil content to the extent of 36.5 and 40.5 per cent respectively.

Studies of Paul et al. (1980) and Niza (1981) indicated that maximum reduction in oil content was in copra samples inoculated with A. flavus and A. niger. When copra was inoculated with fungi and bacteria together, the reduction in oil content was greater, which according to them was due to the synergistic action of the two organisms on a common substrate.

According to Sharma (1981), seed-borne fungi caused reduction in the quantity of oil in sesamum seeds. Of the fungi, A. niger and A. tamarii were the most active ones which caused 58.4 and 26.2 per cent loss in the total seed oil. The other fungi viz., Penicillium multicolor, A. flavus, P. citrinum, P. funiculosum, A. versicolor and Cladosporium herbarum caused a reduction of 20.0, 15.9, 11.6, 10.4, 8.9 and 8.8 per cent respectively in the oil content of the seed.

Basha and Pancholy (1986) reported that in peanuts infested with A. flavus and A. parasiticus significant reduction in the oil content was noted after 18 days of incubation. In shelled oil palm kernels, A. flavus caused 2.6 per cent decrease in the oil content after two weeks of inoculation (Airede and Esuruoso, 1987). Significant decrease occurred four and eight weeks after inoculation when the initial oil content of 45.2 per cent was decreased to between 44.2 and 13.4 per cent. A. flavus caused a more rapid decrease and a greater loss of oil than other fungi. A. niger and Fusarium oxysporum were nearly as effective as A. flavus in reducing the oil content of the kernels. According to Ogundero (1987) A. clavatus and A. flavus were able to degrade the feed stuffs, leading to significant loss in oil content.

Saxena (1988) reported significant reduction in the total oil content of sesamum seeds due to infection by Alternaria alternata, A. flavus, A. niger, Chaetomium globosum and Fusarium moniliforme on incubation for 30 days. A. flavus caused maximum loss in total oil content.

Effect of fungi on the nutrient content of oil cakes and seeds.

Studies conducted by Nagel and Semenuik (1947) on some mould induced changes in shelled corn indicated that the protein content decreased due to infection by seed-borne fungi. Farkas and Kiraly (1954) and Shaw and Colotelo (1961) reported that infection processes lead to increase in respiration and consequently rapid utilization of substrate materials like carbohydrates.

Ward and Diener (1961) reported that in peanut, seed components like dry matter, protein, oil, fatty acids, carbohydrates and amino acids were affected due to invasion by Aspergillus spp.

Cherry et al. (1975) found that when A. parasiticus was allowed to grow on peanuts for time intervals ranging

from one to 18 days, the level of buffer soluble proteins decreased rapidly to quantities much lower than those of non-infected seeds, shortly after inoculation. Similarly the levels of insoluble proteins increased to quantities greater than those contained in soluble fractions. The level of free amino acids varied due to the differential utilization of these components by the fungus.

Cherry and Beuchat (1976) reported that infection by Neurospora sitophila and Rhizopus oligosporus on peanuts produced a decline in buffer soluble proteins. Seeds inoculated with R.oligosporus showed a decline in the percentage of protein from 43 to 29.5 within four to seven days. A decline in protein content from 61.0 to 36.5 per cent was noted in N. sitophila infected seeds during the same period. The relative level of total amino acids in various peanut fractions also changed substantially as a result of infection by these two fungi.

Rice grains stored under natural conditions for one year showed heavy infection by fungi and considerable reduction in carbohydrate content. At 14 per cent moisture level the initial carbohydrate content of 66.65 per cent decreased to 56.98 per cent after six months and 48.3 per cent after 12 months; at 17 per cent moisture level the same

was reduced to 29.3 per cent and 17.44 per cent after six and 12 months respectively (Mallick and Nandi, 1979).

Bilgrami et al. (1980) recorded changes in the qualitative and quantitative pattern of amino acids, organic acids, sugars, starch, protein, nitrogen and caloric value of maize seeds due to infection by A. parasiticus. Storage fungi caused significant decrease in the oil and nitrogen contents of coriander seed (Prasad, 1980) and carbohydrates content of wheat seeds (Ghosh and Nandi, 1986).

Sinha and Chauhan (1981) found that in gram seed (Cicer arietinum) inoculated with A. flavus, the constituents of the cotyledons, viz., proteins, oil, crude fibre, carbohydrates and ash decreased with increasing incubation period. Maximum losses were observed for carbohydrates and proteins. In the case of carbohydrates, the percentage decrease over control was 11.5, 22.71 and 40.40 and in proteins 8.41, 15.52 and 28.86 respectively after seven, 15 and 21 days of incubation .

Sinha and Roy (1981) studied the effect of RH in bringing about changes in the seed constituents of mung (Vigna radiata) inoculated with A.flavus. Remarkable depletion in protein and starch contents were seen at 75 and

96 per cent RH, whereas at lower levels (33 and 55 per cent) the changes were insignificant. The content of soluble nitrogen exhibited fluctuating trend at these relative humidities at different periods of incubation.

Vijayakumari and Karan (1981) found a continuous loss in protein content of cowpea seeds inoculated with A.flavus. On the 7th and 10th days of incubation, the initial protein content of 25.86 decreased to 21.95 and 15.81 per cent respectively. Bilgrami (1985) during his investigation on the nutrient contents of copra recorded reduction of 73.95 and 65.1 per cent in total sugars, 74.68 and 67.09 per cent in reducing sugars and 53.22 and 93.55 per cent in ascorbic acid due to infection with toxigenic and non-toxigenic strains of A.flavus.

Analysis of peanut seeds, infected with A. flavus and A.parasiticus showed a decrease in oil, iodine value, soluble carbohydrate and protein contents. The soluble carbohydrate content of the seeds drastically decreased with increasing period of incubation. Rapid decline of about 80 per cent occurred six days after inoculation and more than 90 per cent of the carbohydrates disappeared by 18 days (Basha and Pancholy, 1986). Degradation of starch, total sugars and protein in cereals due to invasion by fungi like species of

Aspergillus, Penicillium and Rhizopus has been reported by Prasad and Pathak (1986). The depletion in starch content ranged from 0.95 to 10.77 per cent and the content of total sugars and proteins ranged from 0.10 to 0.79 per cent and 0.88 to 2.83 per cent respectively.

Seed-borne fungi such as A. flavus, A. niger, Penicillium chrysogenum , P. janthinellum, P.varioti, Syncephalastrum racemosum and Fusarium oxysporum depleted the proteins and total sugars in oil palm kernel (Airede and Esuruoso, 1987).A.flavus reduced the protein content from 22.9 per cent to between 5.1 and 15.6 per cent in eight weeks. All the fungi caused signicant reduction in the sugar content. Of these, A. niger caused a very rapid depletion. Ogundero (1987) reported that A. flavus and A.clavatus degraded poultry feed samples leading to significant loss in the protein and crude fibre contents. A.clavatus caused a greater loss in crude fibre content than A. flavus.

Prasad et al. (1988) studied the physicochemical changes in the nutrient content of coriander seed due to storage fungi. They found that maximum per cent loss of lipid was by Alternaria tenuis, Curvularia lunata and Cladosporium oxysporum, protein by Aspergillus flavus and Curvularia pallescens and starch by A. flavus.

Production of aflatoxin by fungi.

Investigations of Blount (1961) on the cause of "Turkey 'X' disease", led to the discovery that strains of the fungus, A. flavus present in groundnut meal used as feed material were responsible for producing the toxic factor which was named as 'Aflatoxin'. The extraction of the toxic principle present in groundnut meal infected by A. flavus was carried out by Sargeant et al.(1961). They reported that this extract produced mortality in the young ones of ducks and turkey.

Forgacs and Carll (1962) reviewed the work on mycotoxins and reported many pathologial cases of animals due to ingestion of feed contaminated by fungi including Aspergillus spp. Thin layer chromatographic (TLC) separation of aflatoxins from mouldy groundnut were carried out by Nesbitt et al. (1962).

Carnagham et al. (1963) reported that relative fluorescence intensity of four aflatoxin compounds viz., B_1, B_2, G_1 and G_2 were,0.5, 4, 2.5 and 6.5 and their LD 50 value for four day old ducklings were 18, 85, 39 and 173 µg respectively.

All the strains of A. flavus do not possess the capacity of elaborating aflatoxin (Austwick and Ayerst, 1963; Codner et al., 1963; Diener and Davis, 1969; and Detroy et al., 1971).

Aflatoxicoses in Murrah buffaloes characterised by loss of appetite, diarrhoea, dullness, ascites, emaciation and icterus due to intake of aflatoxin contaminated groundnut cake has been reported by Sastry et al. (1965).

According to Schindler et al. (1967) the maximum growth of A. flavus occurred at 29 and 35 °C and the ratio of production of aflatoxin B_1 and G_1 varied with temperature. Also, they found that aflatoxin production by A. flavus was related to the growth rate of the organism. Gopal et al. (1968) reported aflatoxin in groundnut cake at a level of 1.1 to 25 ppm.

Goldblatt(1969) found that high carbohydrate substrates generally supported luxurious growth of A. flavus and helped to produce larger quantities of aflatoxin. Labadan (1969) while studying the effect of mould in the feed value of copra observed that coconut meat formed a good substrate for the production of aflatoxin. Schroeder (1969) observed that members of A. flavus group were the principal producers of

aflatoxin. A temperature ranging from 25 to 30°C and 80 to 100 per cent RH were found to be the most favourable conditions for toxin production in storage.

Elnur and Ibrahim (1970), Lafort and Lafort (1970), Hara *et al.* (1974), Halloin (1975) and Ilag (1975) studied the production of aflatoxins by strains of *A. flavus* in animal feeds, stored corn and cotton seeds. Lalithakumari *et al.* (1970) found that all the species of *Aspergillus* isolated from stored groundnut pods and kernels caused failure of seed germination and reduction in seedling vigour, but only *A. flavus* and *A. tamarii* produced aflatoxin. Oke (1970) analysed the toxic chemicals in Nigerian groundnuts and recorded an aflatoxin content from 0.1 to 0.3 ppm.

Baur and Armstrong (1971), Arseculeratne and De Silva (1972), Jones (1972) and Samarjeewa (1972) evaluated the aflatoxin content in coconut products by different techniques. They noted that some of the samples of copra, cakes and coconut oil had medium to high aflatoxin levels. Samarjeewa and Arseculeratne (1973) assayed the aflatoxin in coconut products and found that in artificially inoculated coconut meal, aflatoxin B_1 content ranged from 1000-1500 ppm and naturally contaminated copra meal contained 0.09 to 0.14 ppm.

Studies of Lillehoj et al. (1975) revealed that in a stored sample of white maize, 80 per cent of the kernel contained A. flavus and the sample recorded 0.4 ppm aflatoxin B_1. Strzeleckl and Gasiorowska (1975) noted that aflatoxin was present in 12.7 per cent of the cattle, sheep, poultry and pig feed samples tested and the concentration was high in pig feeds as compared to others.

Brodnik and Klemenc (1976) found that maize seeds inoculated with four isolates of A. flavus produced 420, 280, 210 and 500 ppb of aflatoxin. The heavy mortality and reduction in egg production in some poultry farms of Periyar district were investigated by Neelakantan et al. (1978). They found that all the samples of poultry feeds analysed were positive for A. flavus spores and the levels of aflatoxin were as high as 1000 ppb. Philip (1978) studied the production of mycotoxins in deteriorated copra samples and reported aflatoxin B_1 upto two ppm. Thompson and Mehdy (1978) reported that 70 per cent of the isolates of A. flavus from pistachio nuts produced aflatoxin B_1.

Misra et al. (1980) conducted a survey on aflatoxin contamination in some food commodities and reported that, of the 415 samples collected, 127 contained aflatoxins ranging

in concentration from eight to 1850 ppb. Average concentrations (ppb) of aflatoxin B_1 were 61.78 in maize, 43 in wheat flour, 24.66 in suji, 13 in sorghum and four in rice.

Studies of Neelakantan et al. (1981) on the aflatoxin contents of certain feed materials, revealed that groundnut cake samples were severely contaminated with aflatoxin. Of the 134 samples analysed, 28 were positive for aflatoxin and 15 of them contained both aflatoxins B_1 and B_2. Similarly in the case of gingelly and coconut oil cakes, four out of 83 and three out of 96 samples respectively were positive for aflatoxin.

Based on investigations on the presence of aflatoxin in different types of oil cakes, Niza (1981) reported that groundnut oil cake had a high concentration of aflatoxin B_1 (0.408 ppm), followed by coconut oil cake (0.333 ppm). Gingelly and neem cake contained only very low concentration (less than 0.1 ppm) of aflatoxin B_1.

Buchanan and Lewis (1984) reported that the seed components such as proteins and carbohydrates not only served as nutrient source for fungi during their invasion but were also involved in aflatoxin biosynthesis.

Krishnakumari et al. (1984) observed aflatoxin contamination in a very high percentage of the samples of coconut oil (100 per cent) followed by copra (76.92 per cent). Further, the occurrence of aflatoxin B_1 in these samples was more frequent than that of B_2 , G_1 and G_2 . The concentration of aflatoxins in copra varied from 20-500, 20-40, 40-100 and 40-100 ppb of B_1 , B_2 , G_1 and G_2 respectively.

Results of the survey conducted by Reddy et al. (1984) indicated that, among the contaminated poultry feed ingredients, groundnut cake had the maximum aflatoxin content of 587 µg/kg. Storage of the samples for a period of two months significantly increased the aflatoxin content. Singh et al. (1984) while investigating the occurrence of aflatoxin B_1 in animal and poultry feeds observed that groundnut oil cake had the highest percentage (73.33) of contaminated samples and 45.5 per cent of these had one to 30 ppb of aflatoxin B_1.

Aibara et al. (1985) reported that aflatoxin was present in raw shelled groundnut inoculated with A. parasiticus and incubated at 100 per cent RH and 25°C for two months. However, growth of aflatoxigenic fungi and aflatoxin production were not observed after storage for the same period below 79 per cent RH at 25°C.

Balasubramanian (1985) studied the presence of aflatoxin B_1 in animal feeds and detected the toxin in 66 per cent of the samples analysed. Of the 13 feed ingredients analysed only groundnut oil cake contained aflatoxin B_1 to the extent of 0.33 - 26.7 mg/kg.

Bilgrami (1985) studied aflatoxin production by different isolates of Aspergillus spp. and found that, out of 568 isolates of A. flavus screened, 280 were toxigenic and produced different fractions of aflatoxins in varying concentrations (0.5-15 ppm). However, six isolates were capable of producing all the four aflatoxins viz., B_1 , B_2 , G_1 and G_2 . Other mycotoxin producing fungi also showed good potentiality for aflatoxin production. Out of the 23 isolates of A. niger screened in liquid medium, nine produced aflatoxin in low concentrations of upto 0.2 ppm only. In natural contamination five out of 19 samples of copra contained 20-2840 ppb of aflatoxin B_1 .

Chiou et al. (1985) reported that groundnut samples inoculated with A. parasiticus and incubated at 28°C, and at 100 per cent RH for three weeks, produced 1.5 to 159.1 µg/g aflatoxin. Kaya et al. (1985) detected aflatoxin B_1 by TLC method in 17 out of 76 feed samples analysed upto a mean

level of 12.28 ppb and B_2 at a mean level of 8.5 ppb. Highest level was in broiler feed (34.8 ppb) followed by soybean oil meal (20 ppb), livestock feed (15.2 ppb) and cotton seed oil meal (15 ppb). Natour et al. (1985) reported the presence of aflatoxins in 63.9 per cent of poultry feed samples analysed, of which aflatoxin B_1 (6-201 ppb) was detected in 44.8 per cent samples, aflatoxin B_2 (8-335 ppb) in 32 per cent samples and aflatoxin G_1 and G_2 (12-200 ppb) in 19 and 17.2 per cent samples respectively.

Kulkarni et al. (1986) reported the presence of aflatoxin B_1 upto 14.28 mg/kg of deteriorated copra. Studies conducted by Romo and Fernandez (1986, b) revealed that, of the 125 isolates of A.flavus from commercial mixed feeds, 45 produced aflatoxins in Crushed moist wheat medium, whereas only 16 showed specific fluorescence in Aflatoxin producing ability medium (APAM). In both media mainly aflatoxin B_1 and B_2 were detected and with an average concentration of 4294 µg/kg in Crushed moist wheat medium and 877 µg/kg in APAM. One isolate produced very little quantity of aflatoxin G_1.

Romo et al. (1986) noted that the minimal moisture content of the substrate for growth of A. parasiticus was 16.51 $\pm$ 0.45 per cent. Very low amounts of aflatoxins were produced in two days with an initial moisture content of 17

per cent. Significant amounts of aflatoxins were detected when the initial moisture content was 18 per cent or higher.

Rosiles (1986) analysed aflatoxin B_1 in livestock feeds and detected its presence in 46 out of 290 samples. A. flavus isolated from wheat grains with a moisture percentage of 18.9, produced aflatoxin B_1 ranging from 0.2 to 0.62 mg/50 ml of the basal medium.

Sinha et al. (1988) studied the aflatoxin production by isolates of A. flavus from mustard seeds. They found that out of the 181 isolates screened, five produced aflatoxin B_1, B_2, G_1 and G_2. Of the 273 seed samples analysed, 33 contained aflatoxin B_1, the concentration of which ranged from eight to 640 µg/kg.

Control of fungi causing deterioration of oil cakes.

Various investigators have suggested different methods for minimising the deterioration of stored products. Fishlock (1928) obtained promising results with sulphur dioxide fumigation of copra at the rate of 1/2 lb/cu.ft. for a period of 36 hrs.

Microban, a propionic acid salt has been reported as an effective deterrant of mould growth in bread at the rate of three oz/100 Ib flour (Anon, 1940). According to Miller (1940) propionic acid was about twice as effective as the salt solutions for preventing mould growth in cheese. Hendriz and Vleeschauwar (1953) reported that sodium propionate inhibited the development of cheese moulds under controlled conditions.

Aycock (1955) observed that dipping sweet potato tubers in 2.5 per cent borax solution or soaking for ten minutes in one per cent solution reduced soft rot caused by Rhizopus sp.

Marar and Padmanabhan (1960) found that copra stored in alkathene lined gunny bags remained in good condition for six months.

Schroeder (1964) reported that treatment of rough rice with 5000 ppm of sodium propionate increased the keeping quality. Studies conducted by Subrahmanyan et al. (1967) indicated the usefulness of chemicals like calcium hydroxide, sodium chloride, mineral acids, ammonia, ammonia compounds, benzoate, hypochlorite, sorbic acid and different solvents like hydrocarbons and terpenes for the control of fungal infection in copra. Most of the chemicals were effective upto 48 hours.

Nambiar et al. (1972) observed that well dried arecanuts stored in polythene lined gunny bags were free from microbial infection. Bland (1977) reported that propionic acid absorbed in a particular carrier material was useful as a preservative for various agricultural products and their by-products including animal feeds.

Philip (1978) studied the effectiveness of a number of chemicals having bacteriostatic and fungistatic properties as well as food preservatives for preventing microbial deterioration of copra during drying and storage. Only three chemicals viz., streptocycline (500 ppm and 1000 ppm), sodium carbonate and acetic acid (two to four per cent) were found effective in preventing deterioration during storage upto sixty days.

Paster (1979) reported calcium propionate as a common fungistatic chemical used in feed materials in Israel mainly because of its less corrosive nature. He found that the number of fungal colonies in the untreated feed was higher than in those treated with calcium propionate (0.5 per cent, w/w) or propionic acid (0.3 per cent, w/w).

Rath and Mohanty (1979) obtained satisfactory control of Macrophomina phaseolina in stored garlic, by dipping

bulbs in two per cent boric acid for five minutes immediately after harvest.

A variety of chemicals were tried by Sreemulanathan et al. (1980) during drying of coconut to find out a suitable treatment to provide complete protection even under rainy conditions. These included calcium hydroxide, pentachlorophenol, potassium metabisulphite, sodium benzoate, sodium carbonate, sodium chloride, sodium hydroxide, sorbic acid, dilute sulphuric acid and acetic acid. Some of these chemicals could delay the onset of infection by one to three days.

Niza (1981) reported that treatment of copra with chemicals viz., propionic acid (two to three per cent) sodium benzoate (two to three per cent) and acetic acid (three per cent) were effective in inhibiting fungal growth upto 60 days of storage.

Vanselow et al. (1985) studied the effect of calcium propionate in preserving poultry feed and reported that the chemical when used at 0.3 to 0.6 per cent concentration depressed the total fungal population in the feed.

MATERIALS AND METHODS

MATERIALS AND METHODS

Oil cakes of coconut, groundnut and seasmum were collected from oil mills at Trivandrum, Quilon and Alleppey (Southern region) Trichur and Palghat (Central region), Cannanore and Kasaragod (Northern region) districts of Kerala state during three periods, namely, February-March, June-July and October-November. The samples (1000 g each) were brought to laboratory and stored in gunny bags at room temperature.

1. Quantitative and qualitative estimation of fungi in oil cakes

The fungal population in different samples of oil cakes was estimated two times.

i) Immediately after collection

ii) After storage for one month

Estimation of fungal population was carried out using Peptone Dextrose Agar with Rose-bengal and Streptomycin (Timonin, 1940). Five hundred milligrams of powdered oil cake

was transferred into 250 ml conical flask containing 100 ml sterile, distilled water and thoroughly shaken for 30 minutes in a mechanical shaker. Serial dilutions were made by transferring one ml aliquot to 99 ml of sterile distilled water blank. The flask containing the suspension was shaken for five minutes, before taking further samples for serial dilution. The final dilution used for the estimation of fungal population was one in ten thousand (10^{-4}). One ml of this diluted suspension was pipetted out into sterile petri dish and about 20 ml of melted medium cooled to 45 to 50 °C was added and allowed to solidify. Five replications were maintained for each sample of oil cake. The plates were then incubated at room temperature (28$\pm$ 2 °C). Fungal count was taken at three, seven and 12 days after plating. The number of fungal propagules was expressed in millions per gram of dry sample.

The fungal colonies were transferred to Potato Dextrose Agar (PDA) slants. The cultures were purified by hyphal tip and single spore isolation method and maintained on PDA slants. Pure cultures of the fungi were sent to Commonwealth Mycological Institute, U.K. for specific identification.

2. Effect of storage conditions on the growth of fungi on oil cakes

2.1. Temperature

Pieces of oil cakes, weighing approximately 100 g with moisture content of 7-8 per cent were placed in desiccators with a thin layer of water at the bottom and stored in B.O.D. incubators for a period of 60 days at different temperatures, 25 (±1), 27(±1), 29(±1), 32(±1) and 35 (±1) °C. Four replications were maintained for each treatment. The extent of fungal growth was recorded at seven, 15,30,45 and 60 days interval.

2.2 Relative Humidity

Pieces of oil cakes, weighing approximately 100 g with moisture content of 7-8 per cent were kept at room temperature (28±2°C) in desiccators maintained at 75.6, 82.9, 88.5, 92.9, 96.1 and 100 per cent relative humidity, using different concentrations of sulphuric acid in water (Anon, 1983,a). Four replications were maintained for each treatment. The extent of fungal growth was recorded at seven, 15, 30, 45 and 60 days interval.

3. Effect of mycoflora on oil and other nutrient content of oil cakes

Pieces of oil cakes (100 g each) were taken and surface sterilized by swabing with a piece of cotton dipped in rectified spirit. These were inoculated with eight to ten day old culture of different fungi singly and in combinations, and incubated at room temperature (28±2°C) and over 95 per cent RH in a desiccator. In the case of combinations of fungi, only those present in all the three oil cakes were used. Three replications were maintained for each treatment and control. The treatments are listed below:

A. Coconut oil cake

Sl. No.	Treatment
1	1 - *Aspergillus flavus* Link
2	2 - *Aspergillus niger* van Tieghem
3	3 - *Aspergillus terreus* Thom
4	4 - *Rhizopus stolonifer* (Fr.) Lind
5	5 - *Penicillium pinophilum* Hedgcock
6	6 - *Curvularia clavata* Jain
7	7 - *Pestalotiopsis palmarum* (Cooke) Steyaert

8 — 8 - *Bipolaris hawaiiensis* (M.B.Ellis) Uchida and Aragaki

9 — 1, 2 - *A. flavus* + *A. niger*

10 — 1, 3 - *A. flavus* + *A. terreus*

11 — 1, 4 - *A. flavus* + *R. stolonifer*

12 — 1, 5 - *A. flavus* + *P. pinophilum*

13 — 2, 3 - *A. niger* + *A. terreus*

14 — 2, 4 - *A. niger* + *R. stolonifer*

15 — 2, 5 - *A. niger* + *P. pinophilum*

16 — 3, 4 - *A. terreus* + *R. stolonifer*

17 — 3, 5 - *A. terreus* + *P. pinophilum*

18 — 4, 5 - *R. stolonifer* + *P. pinophilum*

19 — 1,2,3 - *A. flavus* + *A. niger* + *A. terreus*

20 — 1,2,4 - *A. flavus* + *A. niger* + *R. stolonifer*

21 — 1,2,5 - *A. flavus* + *A. niger* + *P. pinophilum*

22 — 1,3,4 - *A. flavus* + *A. terreus* + *R. stolonifer*

23 — 1,3,5 - *A. flavus* + *A. terreus* + *P. pinophilum*

24 — 1,4,5 - *A. flavus* + *R. stolonifer* + *P. pinophilum*

25 — 2,3,4 - *A. niger* + *A. terreus* + *R. stolonifer*

26 2,3,5 - A. niger + A. terreus + P. pinophilum

27 2,4,5 - A. niger + R. stolonifer + P. pinophilum

28 3,4,5 - A. terreus + R. stolonifer + P. pinophilum

29 1,2,3,4- A. flavus + A. niger + A. terreus + R. stolonifer

30 1,2,3,5- A. flavus + A. niger + A.terreus + P. pinophilum

31 1,2,4,5- A. flavus + A. niger + R.stolonifer + P. pinophilum

32 1,3,4,5- A. flavus + A. terreus + R. stolonifer + P. pinophilum

33 2,3,4,5- A. niger + A. terreus + R. stolonifer + P. pinophilum

34 1,2,3,4,5- A. flavus + A. niger + A.terreus + R. stolonifer + P.pinophilum

35 Co.o - Control

B. Groundnut oil cake

Sl.No.	Treatment
1	1 - *Aspergillus flavus* Link
2	2 - *Aspergillus niger* van Tieghem
3	3 - *Aspergillus terreus* Thom
4	4 - *Rhizopus stolonifer* (Fr.) Lind
5	5 - *Penicillium pinophilum* Hedgcock
6	1,2 - *A. flavus* + *A. niger*
7	1,3 - *A. flavus* + *A. terreus*
8	1,4 - *A. flavus* + *R. stolonifer*
9	1,5 - *A. flavus* + *P. pinophilum*
10	2,3 - *A. niger* + *A. terreus*
11	2,4 - *A. niger* + *R. stolonifer*
12	2,5 - *A. niger* + *P. pinophilum*
13	3,4 - *A. terreus* + *R. stolonifer*
14	3,5 - *A. terreus* + *P. pinophilum*
15	4,5 - *R. stolonifer* + *P. pinophilum*
16	1,2,3 - *A. flavus* + *A. niger* + *A. terreus*
17	1,2,4 - *A. flavus* + *A. niger* + *R. stolonifer*
18	1,2,5 - *A. flavus* + *A. niger* + *P. pinophilum*
19	1,3,4 - *A. flavus* + *A. terreus* + *R. stolonifer*
20	1,3,5 - *A. flavus* + *A. terreus* + *P. pinophilum*

21 1,4,5 - A. flavus + R. stolonifer + P. pinophilum

22 2,3,4 - A. niger + A. terreus + R. stolonifer

23 2,3,5 - A. niger + A. terreus + P. pinophilum

24 2,4,5 - A. niger + R. stolonifer + P. pinophilum

25 3,4,5 - A. terreus + R. stolonifer + P. pinophilum

26 1,2,3,4 - A. flavus + A. niger + A. terreus + R. stolonifer

27 1,2,3,5 - A. flavus + A. niger + A. terreus + P. pinophilum

28 1,2,4,5 - A. flavus + A. niger + R. stolonifer + P. pinophilum

29 1,3,4,5 - A. flavus + A. terreus + R. stolonifer + P. pinophilum

30 2,3,4,5 - A. niger + A. terreus + R. stolonifer P. pinophilum

31 1,2,3,4,5- A. flavus + A. niger + A. terreus + R. stolonifer + P. pinophilum

32 G.o - Control

C . Sesamum oil cake

Sl.No.	Treatment
1	1 - *Aspergillus flavus* Link
2	2 - *Aspergillus niger* van Tieghem
3	3 - *Aspergillus terreus* Thom
4	4 - *Rhizopus oryzae* Went & Prinsen Geerligs
5	5 - *Penicillium pinophilum* Hedgcock
6	6 - *Fusarium pallidoroseum* (Cooke) Sacc.
7	7 - *Curvularia clavata* Jain
8	1,2 - *A. flavus* + *A. niger*
9	1,3 - *A. flavus* + *A. terreus*
10	1,4 - *A. flavus* + *R. oryzae*
11	1,5 - *A. flavus* + *P. pinophilum*
12	2,3 - *A. niger* + *A. terreus*
13	2,4 - *A. niger* + *R. oryzae*
14	2,5 - *A. niger* + *P. pinophilum*
15	3,4 - *A. terreus* + *R. oryzae*
16	3,5 - *A. terreus* + *P. pinophilum*
17	4,5 - *R. oryzae* + *P. pinophilum*
18	1,2,3 - *A. flavus* + *A. niger* + *A. terreus*
19	1,2,4 - *A. flavus* + *A. niger* + *R. oryzae*
20	1,2,5 - *A. flavus* + *A. niger* + *P. pinophilum*

21	1,3,4	-	*A. flavus* + *A. terreus* + *R. oryzae*
22	1,3,5	-	*A. flavus* + *A. terreus* + *P. pinophilum*
23	1,4,5	-	*A. flavus* + *R. oryzae* + *P. pinophilum*
24	2,3,4	-	*A. niger* + *A. terreus* + *R. oryzae*
25	2,3,5	-	*A. niger* + *A. terreus* + *P. pinophilum*
26	2,4,5	-	*A. niger* + *R. oryzae* + *P. pinophilum*
27	3,4,5	-	*A. terreus* + *R. oryzae* + *P. pinophilum*
28	1,2,3,4	-	*A. flavus* + *A. niger* + *A. terreus* + *R. oryzae*
29	1,2,3,5	-	*A. flavus* + *A. niger* + *A. terreus* + *P. pinophilum*
30	1,2,4,5	-	*A. flavus* + *A. niger* + *R. oryzae* + *P. pinophilum*
31	1,3,4,5	-	*A. flavus* + *A. terreus* + *R. oryzae* + *P. pinophilum*
32	2,3,4,5	-	*A. niger* + *A. terreus* + *R. oryzae* + *P. pinophilum*
33	1,2,3,4,5	-	*A. flavus* + *A. niger* + *A. terreus* + *R. oryzae* + *P. pinophilum*
34	S.o.	-	Control

The following nutrients were estimated

3.1. Oil

The oil content was estimated by the hot percolation method (A.O.A.C. 1960). Ten gram oven dried sample was taken in a thimble and the oil extracted with petroleum ether (B.P. 40-60°C) in a soxhlet extractor. The solvent was distilled off, dried in a desiccator and the weight of oil was recorded. The percentage of oil in the sample was calculated.

3.2. Total carbohydrates

The total carbohydrate content was estimated following the procedure of Aminoff et al (1970).

One gram fat free sample was taken and digested with 10 ml concentrated hydrochloric acid and 50 ml water for two hours. This was cooled and neutralised with sodium hydroxide solution and finally with solid sodium carbonate. The solution was filtered and made upto 250 ml with distilled water. The filtrate was taken in a burette and titrated

against 5o ml of hot Felhing solution prepared by mixing five ml each of Felhing solution A and B along with 40 ml of distilled water. The result was expressed as percentage of sucrose per gram of oil cake on moisture free basis.

3.3. Crude protein

The total nitrogen content of the oil cakes was determined by Microkjeldahl method (A.O.A.C., 1960). The protein content was calculated by multiplying the nitrogen content by 6.25.

3.4. Crude fibre

The crude fibre content was estimated by A.O.A.C. (1960) method. Five gram defatted sample was taken in a 250 ml beaker marked at 100 ml level, 100 ml of 1.25 per cent sulphuric acid was added and boiled for half an hour. The level was maintained by adding hot water. The residue was filtered through muslin cloth, washed with hot water till it became acid free. This was then transferred to the beaker and boiled for half an hour after adding 100 ml of 1.25 per cent sodium hydroxide. The contents were filtered through muslin

cloth, washed the residue free from alkali using hot water, one per cent hydrochloric acid and hot water again. The residue was finally washed with 95 per cent alcohol, transferred to a previously weighed silica dish and dried at 100°C, till the weight was constant. This was ignited in a muffle furnace at 600°C for three hours, cooled in a desiccator and the weight of ash was noted. The loss in weight due to ignition was equal to crude fibre and was expressed as percentage.

3.5. **Ash**

The ash content of the sample was determined as per A.O.A.C. (1960). Two grams of oil cake was taken in a silica crucible, weighed and ignited in a muffle furnance at 600°C for 4 hours till ash was left behind. This was cooled in a desiccator and the final weight was noted. The difference in weight gave the ash content of the sample and was expressed in percentage.

3.6. Minerals

Triple acid extract

Five hundred milligrams of dried and powdered sample was digested by heating with ten ml triple acid mixture (Concentrated Nitric, Perchloric and Sulphuric acids mixed in the ratio of 9:3:1) in a boiling tube. The digestion was continued till the solution became clear. After cooling, the contents were diluted to 100 ml in a volumetric flask, using distilled water.

3.6.a. Phosphorus

The total phosphorus content was estimated by the Chlorostannous reduced molybdo phosphoric blue colour method in Hydrochloric acid system (Jackson, 1973). One half ml of the triple acid extract was taken in a 50 ml volumetric flask. Two drops of 2,4 dinitrophenol and then liquor ammonia (drop by drop) were added till the colour changed to yellow. The solution was decolourised by adding drops of 4 N Hydrochloric acid. Ten ml chloromolybdic acid solution was added to this and the volume was made up with distilled water

upto a little below the 50 ml mark of the flask. One ml of chlorostannous acid reductant was added, mixed thoroughly and the volume made upto 50 ml with distilled water. The colour intensity was read after five minutes in a Klett Summerson Photoelectric Colorimeter, using red filter (660 nm). The phosphorus content (ppm) in the solution read from a standard curve, was expressed as percentage.

3.6.b. Potassium

Five ml of triple acid extract was diluted to 50 ml in a volumetric flask and potassium was estimated by means of a EEL Flame photometer (Jackson, 1973). The per cent potassium in the sample was calculated by means of a standard curve.

3.6.c. Calcium, Magnesium, Copper and Iron

These elements were estimated from the triple acid extract by means of an Atomic Absorption Spectrophotometer, P.E.3030 (Perkin Elmer).

3.7. Total free amino nitrogen

The free amino nitrogen was estimated by the method of Moore and Stein (1958). The amino nitrogen was extracted in 80 per cent ethanol. Fifteen ml of 80 per cent ethanol was added to 500 mg of powdered fat-free sample, centrifuged and the supernatant was used for estimation.

One ml of ninhydrin reagent was added to two ml of the ethanol extract in a test tube and boiled for 15 minutes in a water bath. After cooling, the contents were transferred to a standard flask and made upto 50 ml with distilled water. The absorbance was read at 550 nm in a Spectrophotometer (Spectronic 2000). The total free amino nitrogen (mg/g of oil cake) was computed from a standard curve prepared with different concentrations of leucine.

4. Estimation of aflatoxins

4.1. Aflatoxins in culture medium

The following isolates of Aspergillus flavus and A. niger obtained from the oil cakes collected from different regions during different periods of the year were tested for their ability to produce aflatoxin in culture medium.

Aspergillus flavus		*Aspergillus niger*	
Isolate number	Code	Isolate number	Code
1	Co II a	1	Co III a
2	Co III a	2	Co I b
3	Co I b	3	Co II b
4	Co II b	4	Co III b
5	Co III b	5	Co I c
6	Co I c	6	Co II c
7	Co II c	7	Co III c
8	Co III c	8	G II a
9	G II a	9	G II b
10	G II b	10	G II c
11	G II c	11	S I a
12	S I a	12	S II a
13	S II a	13	S III a
14	S III a	14	S I b
15	S I b	15	S II b
16	S II b	16	S III b
17	S III b	17	S I c
18	S I c	18	S II c
19	S II c	19	S III c
20	S III c		

Co - Coconut G - Groundnut S - Sesamum

I- Southern region
II- Central region
III- Northern region

a - February-March
b - June-July
c - October-November

Aflatoxins produced in culture medium were estimated by the method of Diener and Davis (1966). The isolates of A. flavus and A. niger were inoculated into 250 ml conical flasks each containing 100 ml of sterile SMKY liquid medium. After incubation for seven days at room temperature, chloroform was sprayed over the culture and the culture filtrate was taken in a 250 ml conical flask. Aflatoxins in the culture filtrate were extracted by vigourously refluxing with chloroform (2:1 v/v) for one hour. The chloroform layer was separated into a 100 ml beaker using a separating funnel and evaporated in a hot water bath. The residue was dissolved in one ml of chloroform and assayed for aflatoxins B_1, B_2 and G_2 by means of Thin layer chromatography (TLC).

4.2. Aflatoxins in oil cakes

Coconut, groundnut and sesamum oil cakes were artificially inoculated with toxigenic isolates of A. flavus and A. niger and incubated for two weeks at room temperature and above 95 per cent RH. Aflatoxins were estimated following the procedure of Pons et al. (1971).

Fat in the sample was removed by hot percolation method (A.O.A.C.,1960). Twenty five grams of defatted sample was then transferred to a 250 ml conical flask containing 125 ml of 70 per cent acetone and kept in a mechanical shaker for

30 minutes. The material was filtered through Whatman No.I filter paper. Seventy five ml of the filtrate was taken in a 250 ml beaker and the level was marked. Ten ml of two per cent lead acetate was added to this to remove proteins and carbohydrates which are likely to interfere with aflatoxin estimation. Thirty ml of distilled water was then added and the beaker was placed in a hot water bath till the contents evaporated upto the marked level. After cooling to room temperature the contents were filtered through Whatman No.I filter paper into a separating funnel and extracted in three changes of 25 ml chloroform. Two columns were separated in the separating funnel and the bottom layer of chloroform containing aflatoxins was allowed to run off through a bed of anhydrous sodium sulphate in a conical flask, to remove traces of water. The solvent was allowed to evaporate completely in a hot water bath and the residue resuspended in one ml of chloroform and used for TLC.

With the help of a micropipette, 50 µl of the test materials were spotted on TLC plates (coated with silica gel G, 0.5 mm thickness). Three µl of standard aflatoxins B_1, B_2 and G_2 obtained from Sigma Chemical Company, U.S.A. were also spotted on the plate.

The plates were placed in the solvent chamber containing chloroform and methanol (98:2 v/v) was allowed to

run till the solvent reached 3/4 height of the plate. The plates were then air dried and observed under UV light (320-360 nm) in a dark room. The characteristic fluorescent spots at the same Rf value as that of the standard toxins under UV excitation were marked by means of a sharp needle.

The silica gel covering each spot was scraped carefully with a blade and collected individually in clean, dry centrifuge tubes and five ml of methanol was added to each. The tubes were centrifuged at 3000 rpm for five minutes. The methanol layer was decanted and used for spectrophotometry (Anon, 1983,b). The aflatoxin content was determined by recording the absorbance in a Spectrophotometer (Spectronic 2000) at 360 nm (for B_1) and 362 nm (for B_2 and G_2). The amount of aflatoxin present in 50 µl of the chloroform extract was computed from a standard curve. The aflatoxin content (µg/kg or ppb) of the orginal sample was calculated by using the following formula:

$$\frac{S \times V \times 5}{A \times W} \times 1000$$

where

W = Weight in gm of original sample

V = µl of sample extract prepared for TLC

A = µl of sample extract spotted

S = µl of aflatoxin calculated from the standard curve

5. Control of deterioration of oil cakes by fungi

5.1. Using chemicals

The following chemicals were tested to study their ability to control deterioration of oil cakes by fungi.

Chemicals	Concentration (per cent)	
1. Boric acid	1.5,	2.0
2. Calcium propionate	0.3,	0.6
3. Sodium chloride	3.0,	4.5
4. Sodium carbonate	2.0,	3.0
5. Sodium bicarbonate	2.0,	4.0

The chemicals were finely powdered and mixed with equal quantity of talc. Two hundred grams of the oil cake was thoroughly mixed with the chemical and stored in small gunny bag (15 x 15 cm) under laboratory conditions . Five replications were kept for each treatment. Observations on the growth of fungi and their population were taken upto six months.

5.2. Using different containers

The following containers were tested for their efficiency to prevent deterioration of oil cakes during storage.

1. Gunny bag
2. Polythene bag
3. Polythene lined gunny bag
4. High Density Poly Ethylene (HDPE) woven bag

Two hundred grams each of the oil cakes were stored in the laboratory in these containers (15 x 15 cm) for a period of six months during May to November 1987 and November 1987 to May 1988. Five replications were kept for each treatment. Observations on the growth of fungi and their population were taken upto six months.

6. Statistical analysis

Data relating to different experiments were analysed by applying appropriate statistical methods (Panse and Sukhatme, 1967).

7. Composition of culture media used

Peptone Dextrose Agar with Rose-bengal and Streptomycin

Peptone	- 5.00 g
Dextrose	- 10.00 g
Potassium dihydrogen phosphate	- 1.00 g
Magnesium sulphate	- 0.50 g
Agar agar	- 15.00 g
Rose-bengal	- 1 part in 30,000 part of the medium
Streptomycin	- 30 mg
Distilled water	- 1000 ml

SMKY liquid medium (Diener and Davis, 1966)

Sucrose	- 200 g
Magnesium sulphate	- 0.5 g
Potassium nitrate	- 3.0 g
Yeast extract	- 7.0 g
Distilled water	- 1000 ml

RESULTS

RESULTS

1.1. Fungi isolasted from oil cakes

The following fungi were isolated from the oil cakes (Table 1):

A. Coconut oil cake

Acremonium implicatum (Gilman and Abbot) W. Gams, Aspergillus aculeatus Iizuka, Aspergillus caesiellus Saito, Aspergillus flavus Link, Aspergillus fumigatus Fres, Aspergillus niger van Tieghem, Aspergillus terreus Thom, Aspergillus versicolor (Vuill) Tiraboschi, Bipolaris hawaiiensis (M.B.Ellis) Uchida and Aragaki, Curvularia clavata Jain, Monascus ruber van Tieghem, Penicillium aurantiogriseum Dierckx, Penicillium pinophilum Hedgcock, Pestalotiopsis palmarum (Cooke) Steyaert, Rhizomucor pusillus (Lindst) and Rhizopus stolonifer (Fr.) Lind.

B. Groundnut oil cake

Aspergillus flavus Link, Aspergillus niger van Tieghem, Aspergillus terreus Thom, Aspergillus versicolor (Vuill) Tiraboschi, Gliocladium sp., Penicillium pinophilum Hedgcock, Rhizopus oryzae Went and Prinsen Geerligs and Rhizopus stolonifer (Fr.) Lind.

Table 1. Fungi isolated from different oil cakes.

Sl. No.	Fungi*	Herb. IMI No.	Per cent occurrence in Coconut oil cake	Groundnut oil cake	Sesamum oil cake
1	Acremonium implicatum (Gilman and Abbott) W. Gams	322712	11.11	-	-
2	Aspergillus aculeatus Iizuka	322724	33.33	-	-
3	Aspergillus caesiellus Saito	322717	11.11	-	-
4	Aspergillus candidus Link	322715	-	-	50.00
5	Aspergillus flavus Link	322718	88.89	100.00	100.00
6	Aspergillus fumigatus Fres.	322725	11.11	-	22.22
7	Aspergillus niger van Tieghem	322722	77.78	100.00	100.00
8	Aspergillus tamarii Kita	322721	-	-	16.67
9	Aspergillus terreus Thom	322726	55.56	66.67	55.56
10	Aspergillus versicolor (Vuill.) Tiraboschi	322723	11.11	33.33	-
11	Bipolaris hawaiiensis (M.B.Ellis) Uchida and Aragaki (=Cochliobolus hawaiiensis Alcorn)	322708	5.56	-	-
12	Curvularia clavata Jain	322711	38.89	-	22.22
13	Eurotium chevalieri Margin (=Aspergillus chevalieri Thom & Church)	322720	-	-	11.11
14	Fusarium pallidoroseum (Cooke) Sacc.	322709	-	-	11.11
15	Gliocladium sp.	322729	-	16.67	-
16	Monascus ruber van Tieghem	322713	22.22	-	22.22
17	Penicillium aurantiogriseum Dierckx	322719	38.89	-	-
18	Penicillium pinophilum Hedgcock	322716	27.78	66.67	44.44
19	Pestalotiopsis palmarum (Cooke) Steyaert	322710	22.22	-	11.11
20	Rhizomucor pusillus (Lindt) Schipper	322727	16.67	-	-
21	Rhizopus oryzae Went and Prinsen Geerligs	322728	-	11.11	50.00
22	Rhizopus stolonifer (Fr.) Lind	322714	50.00	50.00	-

* Specific identification was provided by the CMI, England.

C. Sesamum oil cake

Aspergillus candidus Link, Aspergillus flavus Link, Aspergillus fumigatus Fres, Aspergillus niger van Tieghem, Aspergillus tamarii Kita, Aspergillus terreus Thom, Curvularia clavata Jain, Eurotium chevalieri Margin, Fusarium pallidoroseum (Cooke) Sacc, Monascus ruber van Tieghem, Penicillium pinophilum Hedgcock, Pestalotiopsis palmarum (Cooke) Steyaert and Rhizopus oryzae Went and Prinsen Geerligs.

Aspergillus flavus and A. niger were obtained from all the samples of groundnut and sesamum oil cakes. In coconut oil cake, these two fungi were present in 88.89 and 77.78 per cent of the samples (Table 1). A. terreus was isolated from 66.67 per cent of groundnut and 55.56 per cent of coconut and sesamum oil cake samples. A. candidus was obtained from sesamum oil cake only in 50 per cent samples. A. aculeatus and A. versicolor were present in 33.33 per cent of coconut and groundnut oil cake samples. Twenty two per cent of the samples of sesamum cake yielded A. fumigatus.

Penicillium pinophilum was isolated from 66.67 per cent of groundnut, 44.44 per cent of sesamum and 27.78 per cent of coconut oil cakes. P. aurantiogriseum was present in 38.89 per cent of the coconut oil cake samples.

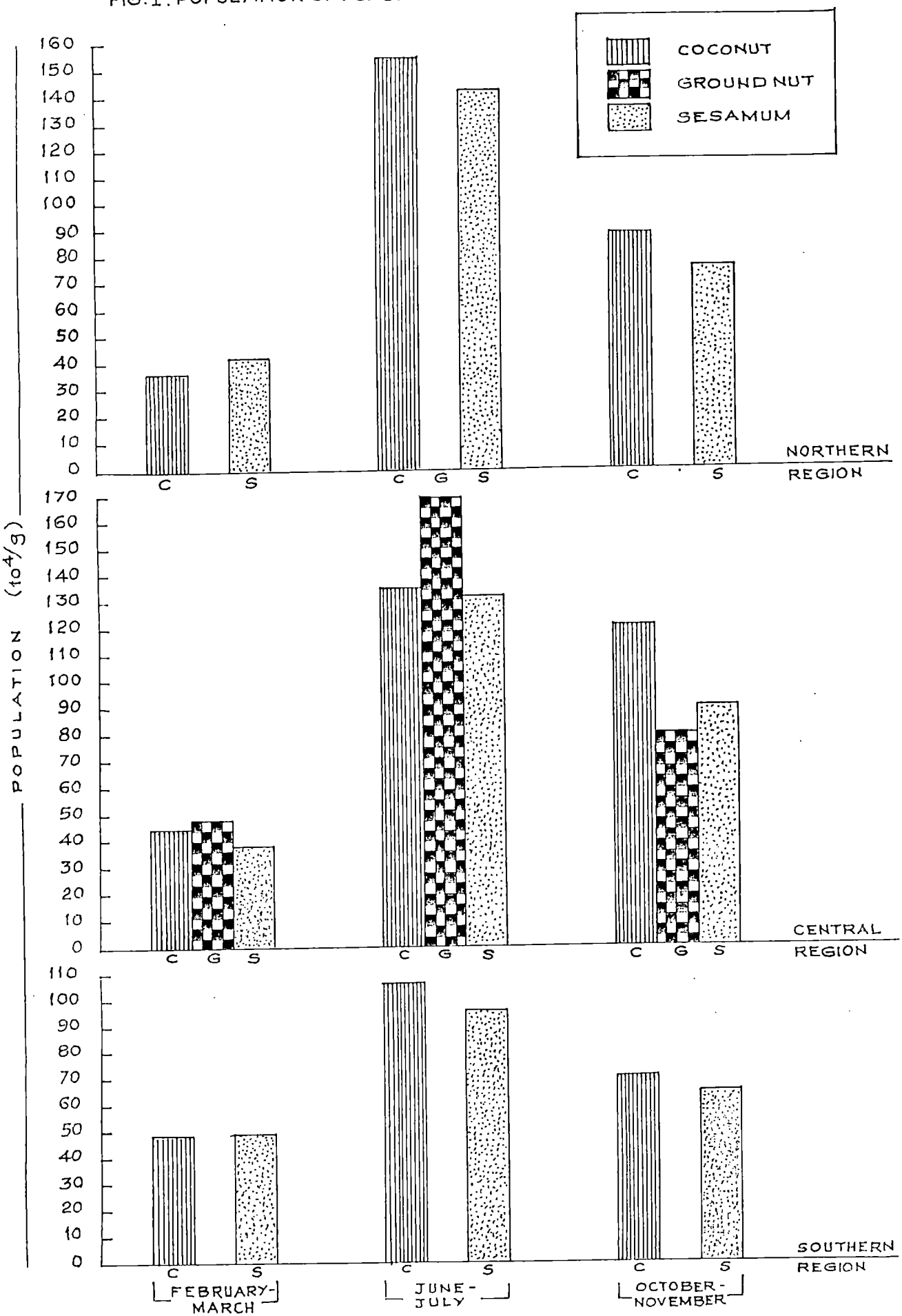
Table 2. Population of fungal propagules in oil cakes.

Region	Period	Population of fungal propagules (10^4/g)								
		Coconut			Goundnut			Sesamum		
		I	II	Mean	I	II	Mean	I	II	Mean
Southern	February- March	10.48	88.00	49.24	-	-	-	7.60	90.13	48.87
	June-July	28.40	184.40	106.40	-	-	-	24.67	168.00	96.34
	October-November	17.00	103.30	71.12	-	-	-	15.60	114.10	64.85
	Mean	18.63	125.23		-	-	-	15.96	124.08	
Central	February-March	12.13	77.87	45.00	4.90	90.50	47.70	15.47	61.20	38.34
	June-July	59.73	209.33	134.53	54.75	283.50	169.13	30.13	233.47	131.80
	October-November	48.40	193.33	120.87	22.70	136.60	79.65	22.27	159.73	91.00
	Mean	40.09	160.18		27.45	170.20		22.62	151.47	
Northern	February-March	14.16	57.68	35.92	-	-	-	21.67	62.67	42.17
	June-July	64.40	243.60	154.00	-	-	-	57.84	228.88	143.36
	October-November	20.25	156.90	88.58	-	-	-	41.47	111.20	76.34
	Mean	32.94	152.73		-	-	-	40.33	134.25	

I - Isolated immediately after collection of samples.
II - Isolated after storage for one month.

CD at (0.05) level.
Between oil cakes - 18.550
Between regions - 8.098
Between periods - 8.098
Between isolations- 6.612

FIG. 1. POPULATION OF FUNGAL PROPAGULES IN OIL CAKES.



Rhizopus stolonifer was isolated from 50 per cent of coconut and groundnut oil cakes, while R. oryzae was present in 50 per cent of sesamum oil cake samples only. Curvularia clavata was isolated from 38.89 per cent of coconut and 22.22 per cent of sesamum oil cakes respectively.

Monascus ruber was obtained from 22.22 per cent of coconut and sesamum oil cakes. Pestalotiopsis palmarum was isolated from 22.22 per cent of coconut cake samples. All the other fungi were present in less than 20 per cent of the oil cake samples tested.

1.2. Quantitative estimation of fungal propagules in oil cakes

Wide variation was noticed in the population of fungal propagules present in the oil cakes collected from different regions during different periods of the year (Table 2 and Fig.1). The variations in population between different regions, periods and the interaction between oil cakes, regions and periods were found to be significant (Appendix I and II). Maximum population of fungi was recorded in coconut oil cake. Highest population was recorded during June-July in all the oil cakes. Samples collected from the central and northern regions had highest population of fungi (Fig.2).

FIG. 2. POPULATION OF FUNGI IN OIL CAKES FROM DIFFERENT REGIONS DURING DIFFERENT PERIODS.

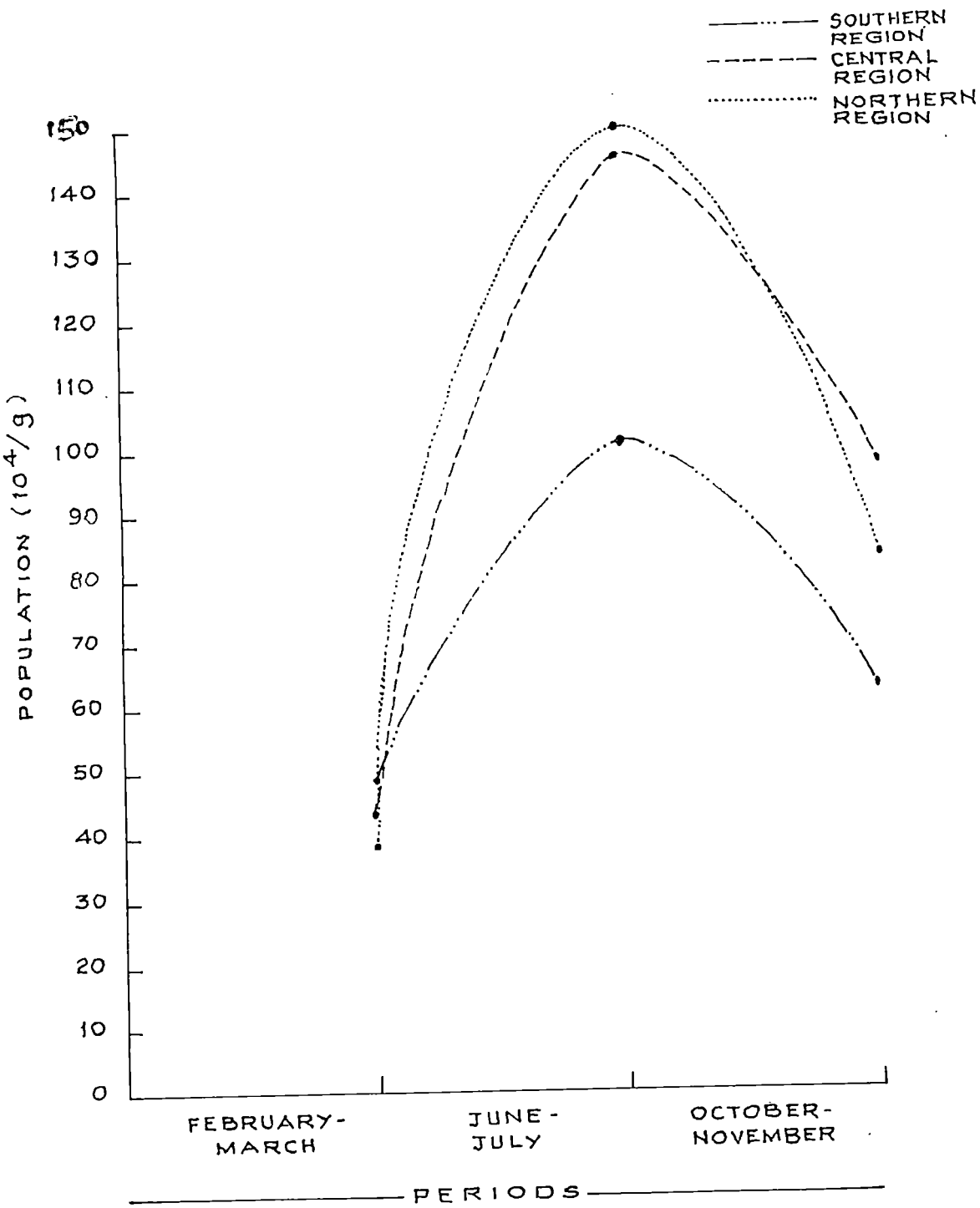


Table 3. Effect of weather parameters on the fungal population of oil cakes.

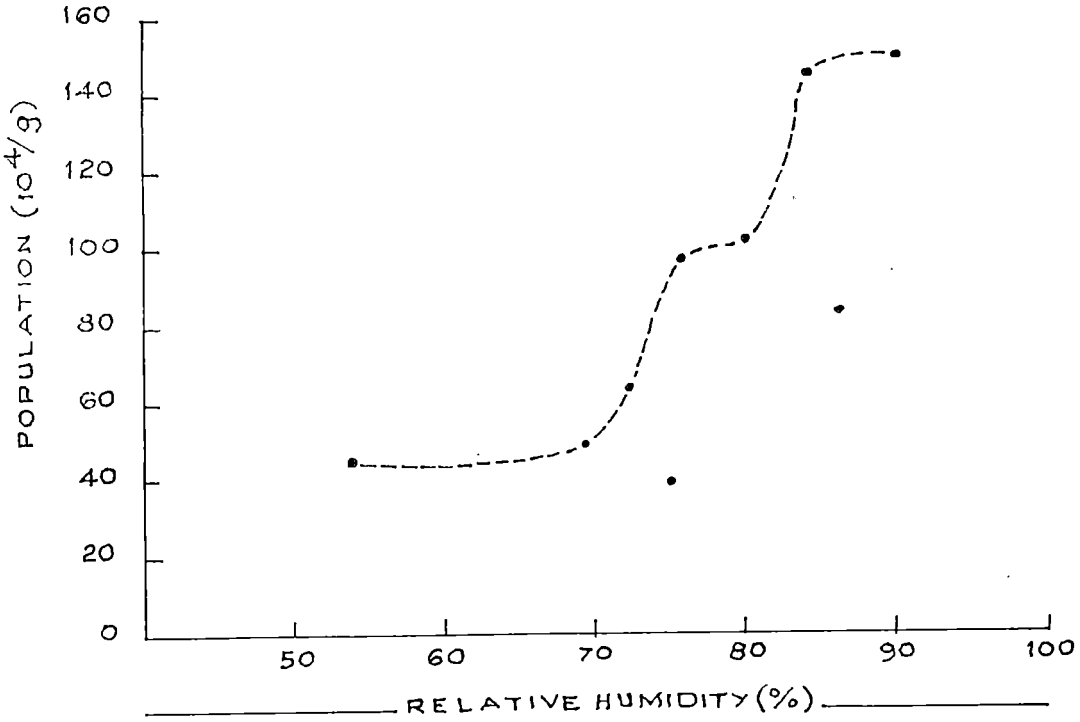
Region	Period	Fungal population (10^4/g)	Temperature(°C)		Relative humidity (%)	Number of rainy days	Total rainfall (mm)
			Maximum	Minimum			
Southern	February-March	49.05	33.89	22.71	69.47	0.50	2.40
	June-July	101.37	30.70	24.21	80.18	15.50	199.30
	October-November	62.50	31.14	22.00	71.85	9.00	131.80
Central	February-March	43.68	35.70	22.30	53.50	0.00	0.00
	June-July	145.15	30.50	23.60	83.50	19.00	587.10
	October-November	97.17	31.50	22.45	75.50	10.00	298.75
Northern	February-March	39.05	33.73	21.53	74.88	0.00	0.00
	June-July	148.68	30.35	24.53	90.04	23.00	743.55
	October-November	82.46	31.20	22.97	85.77	8.00	128.05

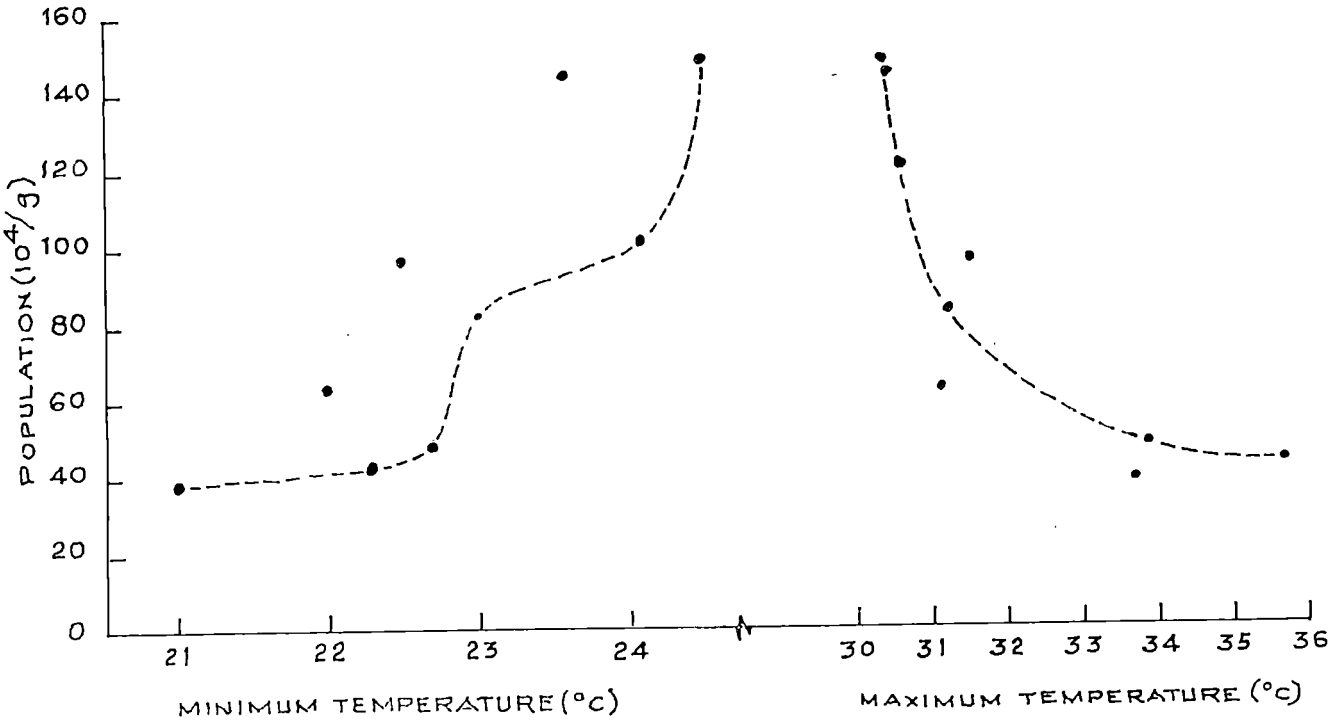
Table 4. Correlation coefficient studies

Sl. No.	Relation between	Coefficient of correlation
1	Fungal population x Maximum temperature	+0.4202*
2	Fungal population x Minimum temperature	+0.5367*
3	Fungal population x Relative humidity	+0.5706*
4	Fungal population x Number of rainy days	+0.6510*
5	Fungal population x Total rainfall	+0.6879*

*Significant at 0.01 level

FIG. 3. EFFECT OF WEATHER PARAMETERS ON THE POPULATION OF FUNGI.





1.3. Effect of weather parameters on the fungal population of oil cakes

The data regarding effect of weather parameters on the population of fungi in different oil cakes during different periods of the year are presented in table(3) and Fig.(3). The population of fungi in oil cakes was maximum at low daily average temperature and high relative humidity in all the three regions. This coincided with highest number of rainy days and maximum rainfall received during the period. Significant and positive correlations were obtained between fungal population and weather elements (Table 4). Maximum correlation was noticed between fungal population and total rainfall.

2. Effect of storage conditions on the growth of fungi on oil cakes

2.1. Temperature

The effect of temperature on the growth of fungi on oil cakes is presented in Table (5). There was no fungus growth in any of the oil cakes for 15 days, at all the temperatures tested. At 25 °C and 35 °C, there was no visible growth of fungi on the oil cakes upto 30 days of incubation.

Table 5. Effect of temperature on the growth of fungi on oil cakes

Temperature (°C)	Oil cake	Fungal growth after (days)				
		7	15	30	45	60
25	C	-	-	-	+	+
	G	-	-	-	+	+
	S	-	-	-	+	++
27	C	-	-	-	+	++
	G	-	-	+	+	++
	S	-	-	+	++	++
29	C	-	-	-	+	+
	G	-	-	+	++	+++
	S	-	-	+	++	++
32	C	-	-	-	+	++
	G	-	-	-	++	++
	S	-	-	+	+	++
35	C	-	-	-	+	++
	G	-	-	-	+	+
	S	-	-	-	+	+

C - Coconut G - Groundnut S - Sesamum

\- No visible growth
\+ 25 per cent area covered
++ 50 per cent area covered
+++ More than 50 per cent area covered

Table 6. Effect of humidity on the growth of fungi on oil cakes

Per cent humidity	Oil cake	Fungal growth after (days)				
		7	15	30	45	60
75.6	C	-	-	-	-	-
	G	-	-	+	++	++
	S	-	-	-	-	-
82.9	C	-	-	-	-	-
	G	-	+	++	++	+++
	S	-	-	-	-	-
88.5	C	-	+	++	+++	+++
	G	-	++	++	+++	+++
	S	-	-	+	++	+++
92.9	C	+	++	+++	+++	+++
	G	+	++	+++	+++	+++
	S	-	++	+++	+++	+++
96.1	C	++	+++	+++	+++	+++
	G	+	++	+++	+++	+++
	S	+	+++	+++	+++	+++
100	C	++	+++	+++	+++	+++
	G	++	+++	+++	+++	+++
	S	++	+++	+++	+++	+++

C - Coconut G - Groundnut S - Sesamum

- No visible growth
+ 25 per cent area covered
++ 50 per cent area covered
+++ More than 50 per cent area covered

Fungal growth was visible on groundnut and sesamum oil cakes kept at 27 and 29°C when observations were taken at 30 days, whereas at 32°C growth was visible on sesamum oil cake only. Mycelial growth was visible on all the oil cakes, at all temperatures tested at 45 and 60 days incubation. Good mycelial growth was noticed in all the samples kept at 27, 29 and 32°C during the period.

2.2.Relative humidity

As the Relative humidity (RH) increased, the growth of fungi on oil cakes also increased. At 75.6 and 82.9 per cent RH, no fungus growth was visible on coconut and sesamum oil cakes upto 60 days. Maximum fungus growth in the oil cakes was noticed at 100 per cent RH followed by 96.1 and 92.9 in the descending order (Table 6,Plates II,III,IV).

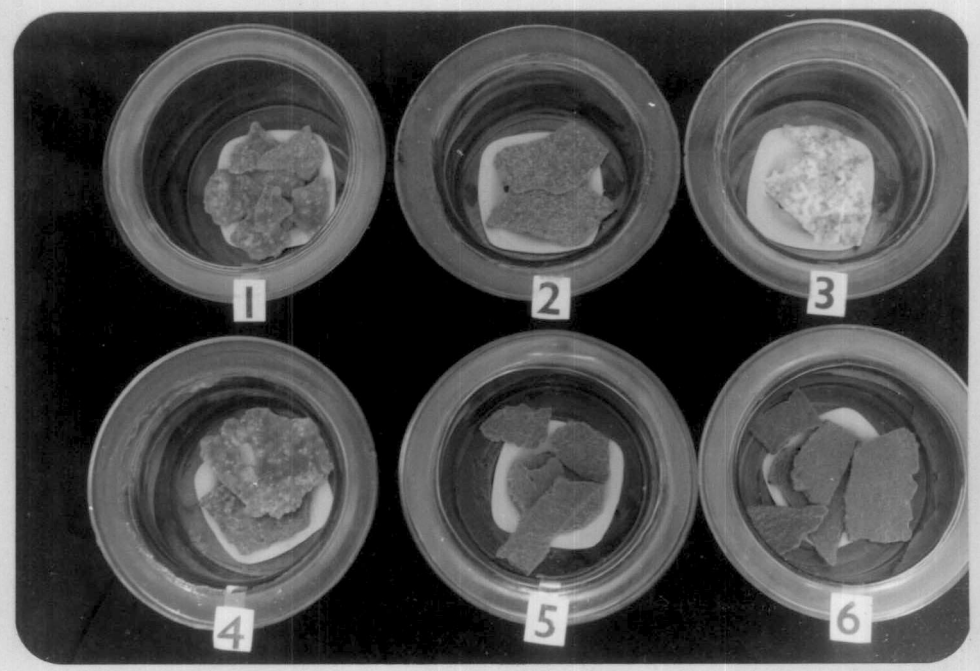
3.1.Effect of fungi on the oil content of oil cakes

The oil content was found to be considerably reduced due to the growth of all the fungi tested individually and in combination (Table 7). Maximum reduction in oil content of coconut oil cake was noticed due to the growth of Pestalotiopsis palmarum (78.43 per cent). Bipolaris hawaiiensis also caused almost similar per cent reduction in

Plate II. Coconut oil cake stored in desiccators at different relative humidities.

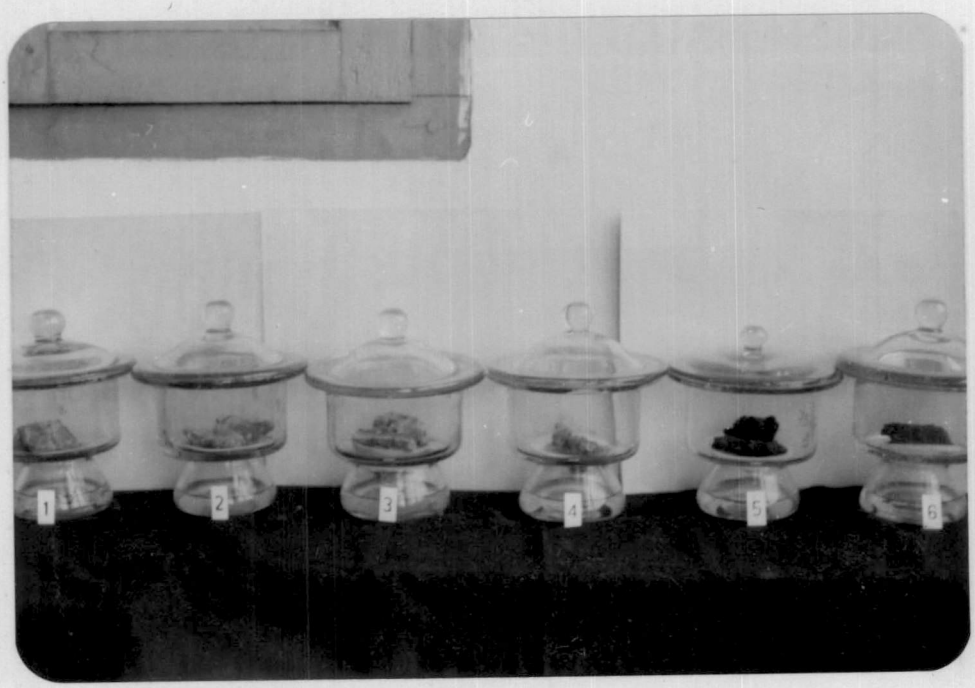
1. 100%
2. 96.1%
3. 92.9%
4. 88.5%
5. 82.9%
6. 75.6%

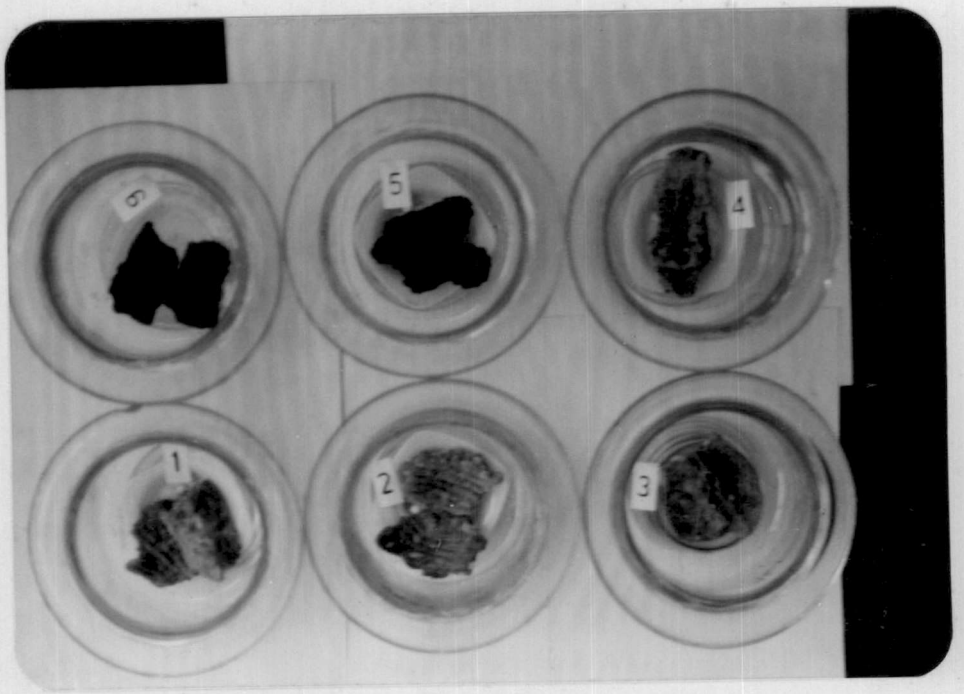
Plate III. Groundnut oil cake stored in desiccators at different relative humidities.



1. 100%
2. 96.1%
3. 92.9%
4. 88.5%
5. 82.9%
6. 75.6%

Plate IV. *Sesamum oil cake stored in desiccators at different relative humidities.*





1. 100%
2. 96.1%
3. 92.9%
4. 88.5%
5. 82.9%
6. 75.6%

Table 7. Effect of fungi on the oil content of oil cakes.

Sl. No.	Treatment*	Per cent oil content		
		Coconut	Groundnut	Sesamum
1	1	4.46 (-60.25)	5.09 (-59.25)	1.70 (-85.00)
2	2	4.15 (-63.01)	3.18 (-74.54)	3.27 (-71.14)
3	3	4.00 (-64.35)	3.02 (-75.82)	2.00 (-82.35)
4	4	3.49 (-68.90)	2.32 (-81.43)	5.37 (-52.60)
5	5	4.22 (-62.39)	3.50 (-71.98)	3.00 (-73.52)
6	6	3.53 (-68.54)	- -	1.67 (-85.26)
7	7	2.42 (-78.43)	- -	3.57 (-68.49)
8	8	2.46 (-78.08)	- -	- -
9	1,2	3.36 (-70.05)	4.51 (-63.89)	3.01 (-73.43)
10	1,3	6.17 (-45.01)	3.43 (-72.54)	2.30 (-79.70)
11	1,4	5.16 (-54.01)	5.47 (-56.21)	4.13 (-63.55)
12	1,5	5.78 (-48.49)	4.83 (-61.33)	3.07 (-72.90)
13	2,3	7.38 (-34.23)	2.66 (-78.70)	4.13 (-63.55)
14	2,4	2.12 (-81.11)	3.39 (-72.86)	4.48 (-60.46)
15	2,5	1.76 (-84.31)	3.53 (-71.74)	1.37 (-87.91)
16	3,4	3.35 (-70.14)	4.59 (-63.17)	4.05 (-64.25)
17	3,5	7.32 (-34.76)	2.99 (-76.06)	5.43 (-52.07)

(Contd...)

Sl. No.	Treatment*	Per cent oil content		
		Coconut	Groundnut	Sesamum
18	4,5	4.23 (-62.30)	3.87 (-69.02)	2.40 (-78.82)
19	1,2,3	4.15 (-63.01)	1.56 (-87.51)	4.00 (-64.70)
20	1,2,4	4.00 (-64.35)	2.36 (-81.11)	3.05 (-73.08)
21	1,2,5	1.72 (-84.67)	3.50 (-71.98)	2.43 (-78.55)
22	1,3,4	1.97 (-82.44)	3.26 (-73.90)	4.00 (-64.70)
23	1,3,5	3.53 (-68.54)	4.03 (-67.73)	2.33 (-79.44)
24	1,4,5	4.05 (-63.90)	2.33 (-81.35)	2.50 (-77.94)
25	2,3,4	5.69 (-49.29)	3.06 (-75.50)	4.00 (-64.70)
26	2,3,5	5.46 (-51.34)	3.27 (-73.82)	3.07 (-72.90)
27	2,4,5	7.62 (-32.09)	4.00 (-67.97)	3.33 (-70.61)
28	3,4,5	3.11 (-72.28)	3.50 (-71.98)	4.00 (-64.70)
29	1,2,3,4	2.06 (-81.64)	5.90 (-52.76)	3.07 (-72.90)
30	1,2,3,5	4.32 (-61.50)	4.83 (-61.57)	4.07 (-64.08)
31	1,2,4,5	4.67 (-58.38)	3.90 (-68.78)	2.80 (-75.29)
32	1,3,4,5	4.72 (-57.93)	1.87 (-85.03)	2.50 (-77.94)
33	2,3,4,5	6.17 (-45.01)	3.40 (-72.78)	2.45 (-78.38)
34	1,2,3,4,5	6.77 (-39.66)	3.43 (-72.54)	2.22 (-80.41)
35	Control	11.22	12.49	11.33
	CD (0.05)	1.557	2.393	0.944

Figures in parentheses are per cent decrease over control.

*Details given in "Materials and Methods".

oil (78.08). In groundnut oil cake, maxium reduction (81.43 per cent) was caused by Rhizopus stolonifer. In sesamum oil cake Fusarium pallidoroseum caused maximum reduction (85.26 per cent) in oil content. A. flavus caused almost similar reduction in oil (85.00 per cent).

In the case of combination of fungi, maximum reduction of oil in coconut oil cake (84.67 per cent) was noticed with A. flavus + A. niger + P. pinophilum. Combination of A. niger and P. pinophilum also showed almost similar reduction in oil content (84.31 per cent). In groundnut oil cake, the maximum reduction in oil (87.51 per cent) was due to the combined effect of A. flavus, A. niger and A. terreus whereas in sesamum oil cake maximum reduction was caused by A. niger + P. pinophilum (87.91 per cent).

Statistical analysis of the data revealed that all the fungi individually and in combination caused significant reduction in the oil content of coconut, groundnut and sesamum oil cakes.

3.2. Effect of fungi on the nutrient content of oil cakes

A . Coconut oil cake

There was considerable reduction in most of the chemical constituents of coconut oil cake due to invasion by

Table 8. Effect of fungi on the nutrient content of coconut oil cake.

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
1	1	20.19 (-52.56)	22.05 (-8.35)	5.18 (-59.63)	6.45 (+9.14)	0.34 (-59.04)	0.75 (-53.42)	0.26 (-31.58)	248.23 (-85.38)	71.70 (-69.95)	376.37 (-49.42)	2.143 (-84.08)
2	2	9.81 (-76.95)	20.28 (-15.71)	10.62 (-17.23)	5.90 (-0.17)	0.31 (-62.65)	0.68 (-57.76)	0.24 (-36.84)	292.20 (-82.79)	33.27 (-86.06)	558.30 (-24.97)	5.121 (-62.02)
3	3	21.45 (-49.60)	19.53 (-18.83)	6.35 (-50.51)	5.08 (-14.04)	0.28 (-66.27)	1.08 (-32.92)	0.25 (-34.21)	210.83 (-87.59)	85.17 (-64.31)	388.27 (-47.82)	3.081 (-77.21)
4	4	17.43 (-59.05)	21.42 (-10.97)	6.41 (-50.04)	5.68 (-3.89)	0.29 (-65.06)	0.47 (-70.81)	0.24 (-36.84)	216.67 (-87.24)	75.40 (-68.40)	644.40 (-13.40)	2.634 (-80.45)
5	5	16.50 (-61.23)	18.53 (-22.98)	6.50 (-49.34)	4.67 (-20.98)	0.31 (-62.65)	0.68 (-57.76)	0.21 (-44.74)	199.67 (-88.24)	79.67 (-66.61)	522.00 (-29.85)	3.090 (-77.12)
6	6	11.24 (-73.59)	18.81 (-21.82)	13.66 (+6.47)	6.83 (+15.57)	0.59 (-28.92)	0.96 (-40.37)	0.25 (-34.21)	250.67 (-85.24)	40.33 (-83.10)	504.47 (-32.20)	1.261 (-90.59)
7	7	16.70 (-60.76)	20.51 (-14.75)	10.54 (-17.85)	5.07 (-14.21)	0.59 (-28.92)	0.82 (-49.07)	0.24 (-36.84)	263.90 (-84.46)	76.00 (-68.15)	523.43 (-29.65)	2.500 (-81.49)
8	8	14.29 (-66.42)	20.80 (-13.55)	11.31 (-11.85)	5.02 (-15.06)	0.39 (-53.01)	0.70 (-56.52)	0.25 (-34.21)	322.73 (-81.00)	64.93 (-72.79)	382.50 (-48.59)	3.122 (-76.83)

(Figures in parentheses are per cent increase or decrease over control)

1. *Aspergillus flavus*
2. *A. niger*
3. *A. terreus*
4. *Rhizopus stolonifer*
5. *Penicillium pinophilum*
6. *Curvularia clavata*
7. *Pestalotiopsis palmarum*
8. *Bipolaris hawaiiensis*

(contd...)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
9	1,2	20.22 (-52.49)	20.85 (-13.34)	11.30 (-11.93)	5.89 (-0.34)	0.29 (-65.06)	0.84 (-47.83)	0.24 (-36.84)	215.93 (-87.29)	30.60 (-87.18)	430.40 (-42.16)	2.048 (-84.82)
10	1,3	19.00 (-55.36)	21.31 (-11.43)	11.35 (-11.54)	5.14 (-13.03)	0.30 (-63.86)	1.01 (-37.27)	0.22 (-42.11)	215.07 (-87.34)	66.77 (-72.02)	368.20 (-50.22)	3.231 (-76.01)
11	1,4	18.57 (-56.37)	16.99 (-29.38)	12.43 (-3.12)	6.01 (+1.69)	0.47 (-43.37)	1.01 (-37.27)	0.26 (-31.58)	401.00 (-76.39)	96.03 (-59.76)	618.47 (-16.88)	1.696 (-87.41)
12	1,5	12.86 (-69.78)	21.68 (-9.89)	12.04 (-6.16)	6.56 (+11.00)	0.33 (-60.24)	0.83 (-48.45)	0.20 (-47.37)	283.43 (-83.31)	44.83 (-81.21)	428.07 (-42.47)	4.212 (-68.76)
13	2,3	11.52 (-72.93)	19.87 (-17.41)	10.05 (-21.67)	4.86 (-17.77)	0.39 (-53.01)	0.68 (-57.76)	0.24 (-36.84)	278.73 (-83.59)	90.50 (-62.08)	654.10 (-12.09)	2.344 (-82.60)
14	2,4	12.23 (-71.26)	21.05 (-12.51)	13.72 (+6.94)	6.80 (+15.06)	0.26 (-68.67)	0.10 (-93.79)	0.15 (-60.53)	166.37 (-90.20)	16.67 (-93.01)	493.80 (-33.64)	3.331 (-75.27)
15	2,5	13.94 (-67.25)	19.26 (-19.95)	10.08 (-21.43)	5.71 (-3.38)	0.54 (-34.94)	0.98 (-39.13)	0.27 (-28.95)	419.93 (-75.27)	108.83 (-54.39)	639.53 (-14.05)	3.116 (-76.90)
16	3,4	17.58 (-58.69)	18.55 (-22.90)	6.58 (-48.71)	5.17 (-12.52)	0.46 (-44.58)	0.89 (-44.72)	0.22 (-42.11)	211.03 (-87.57)	68.07 (-71.47)	513.73 (-30.96)	2.108 (-84.38)
17	3,5	22.43 (-47.30)	18.21 (-24.31)	11.67 (-9.04)	5.13 (-13.20)	0.25 (-69.88)	0.83 (-48.45)	0.25 (-34.21)	220.17 (-87.04)	110.83 (-53.56)	364.30 (-51.04)	2.007 (-85.12)
18	4,5	16.33 (-61.63)	19.03 (-20.91)	9.46 (-26.27)	7.48 (+26.57)	0.32 (-61.45)	0.61 (-62.11)	0.25 (-34.21)	203.43 (-88.02)	69.33 (-70.95)	588.67 (-20.89)	2.104 (-84.38)

(Figures in parentheses are per cent increase or decrease over control

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

(Contd.....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
19	1,2,3	14.49 (-65.95)	19.30 (-19.78)	11.51 (-10.29)	6.19 (+4.74)	0.23 (-72.29)	0.82 (-49.07)	0.25 (-34.21)	26.07 (-84.51)	27.07 (-88.66)	468.73 (-37.00)	1.002 (-92.52)
20	1,2,4	20.10 (-52.77)	21.02 (-12.64)	11.41 (-11.07)	5.04 (-14.72)	0.48 (-42.17)	0.79 (-50.93)	0.26 (-31.58)	259.70 (-84.71)	76.53 (-67.93)	447.00 (-39.93)	2.307 (-82.90)
21	1,2,5	12.41 (-70.84)	21.64 (-10.06)	12.28 (-4.29)	4.17 (-29.44)	0.40 (-51.81)	0.64 (-60.25)	0.22 (-42.11)	184.77 (-89.12)	28.40 (-88.10)	536.53 (-27.89)	1.531 (-88.60)
22	1,3,4	10.08 (-76.32)	21.86 (-9.14)	14.10 (+9.90)	6.10 (+3.21)	0.59 (-28.92)	0.95 (-40.99)	0.27 (-28.95)	218.60 (-87.13)	47.33 (-80.17)	603.77 (-18.86)	3.118 (-76.90)
23	1,3,5	12.25 (-71.22)	20.21 (-16.00)	10.23 (-20.27)	5.70 (-3.55)	0.43 (-48.19)	0.97 (-39.75)	0.25 (-34.21)	384.40 (-77.37)	58.73 (-75.39)	422.17 (-43.26)	5.058 (-62.54)
24	1,4,5	17.35 (-59.23)	18.12 (-24.69)	7.67 (-40.22)	5.08 (-14.04)	0.38 (-54.22)	0.88 (-45.34)	0.20 (-47.37)	243.60 (-85.66)	96.27 (-59.66)	571.90 (-23.14)	4.104 (-69.57)
25	2,3,4	11.31 (-73.43)	22.24 (-7.56)	12.67 (-1.25)	5.49 (-7.11)	0.54 (-34.94)	1.01 (-37.27)	0.20 (-47.37)	242.80 (-85.70)	58.60 (-75.44)	541.50 (-27.22)	5.062 (-62.47)
26	2,3,5	18.79 (-55.85)	21.63 (-10.10)	9.19 (-28.37)	4.75 (-19.63)	0.38 (-54.22)	0.67 (-58.39)	0.26 (-31.58)	245.73 (-85.53)	45.97 (-80.74)	568.13 (-23.65)	2.831 (-78.97)
27	2,4,5	19.31 (-54.63)	20.38 (-15.30)	12.43 (-3.12)	7.15 (+20.98)	0.41 (-50.60)	0.41 (-74.53)	0.27 (-28.95)	451.97 (-73.39)	41.40 (-82.65)	393.87 (-47.07)	3.045 (-77.42)
28	3,4,5	12.89 (-69.71)	20.48 (-14.88)	9.45 (-26.34)	5.11 (-13.54)	0.30 (-63.86)	0.77 (-52.17)	0.23 (-39.47)	287.80 (-83.05)	90.87 (-61.92)	291.93 (-60.77)	2.813 (-79.12)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

(Contd.....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
29	1,2,3,4	14.81 (-65.20)	21.65 (-10.02)	12.15 (-5.30)	7.37 (+24.70)	0.49 (-40.96)	0.96 (-40.37)	0.26 (-31.58)	278.27 (-83.62)	42.07 (-82.37)	471.03 (-36.70)	10.272 (-23.90)
30	1,2,3,5	18.93 (-55.52)	18.33 (-23.82)	7.77 (-39.44)	7.01 (+18.61)	0.33 (-60.24)	0.67 (-58.39)	0.22 (-42.11)	236.00 (-86.10)	46.97 (-80.32)	439.33 (-40.96)	4.397 (-67.43)
31	1,2,4,5	15.06 (-64.21)	20.42 (-15.13)	8.67 (-32.42)	5.13 (-13.20)	0.40 (-51.81)	0.73 (-54.66)	0.20 (-47.37)	289.67 (-82.94)	63.73 (-73.29)	462.50 (-37.84)	4.916 (-63.58)
32	1,3,4,5	12.42 (-70.82)	19.33 (-19.66)	6.30 (-50.90)	6.13 (+3.72)	0.51 (-38.55)	0.82 (-49.07)	0.20 (-47.37)	243.33 (-85.67)	59.60 (-75.02)	555.67 (-25.32)	4.216 (-68.76)
33	2,3,4,5	12.18 (-71.38)	21.18 (-11.97)	8.80 (-31.41)	5.58 (-5.58)	0.58 (-30.12)	0.88 (-45.34)	0.21 (-44.74)	261.83 (-84.58)	68.63 (-71.24)	603.53 (-18.89)	6.553 (-51.44)
34	1,2,3,4,5	12.54 (-70.54)	20.03 (-4.28)	6.50 (-49.34)	5.27 (-10.83)	0.30 (-63.86)	0.64 (-60.25)	0.22 (-42.11)	219.93 (-87.05)	95.67 (-59.91)	437.87 (-41.15)	4.621 (-65.72)
35	Control	42.56	24.06	12.83	5.91	0.83	1.61	0.38	1698.33	238.63	744.07	13.510
	CD (0.05)	1.416	1.785	2.311	2.805	0.048	0.091	0.023	10.427	7.732	40.687	0.8341

(Figures in parentheses are per cent increase or decrease over control)

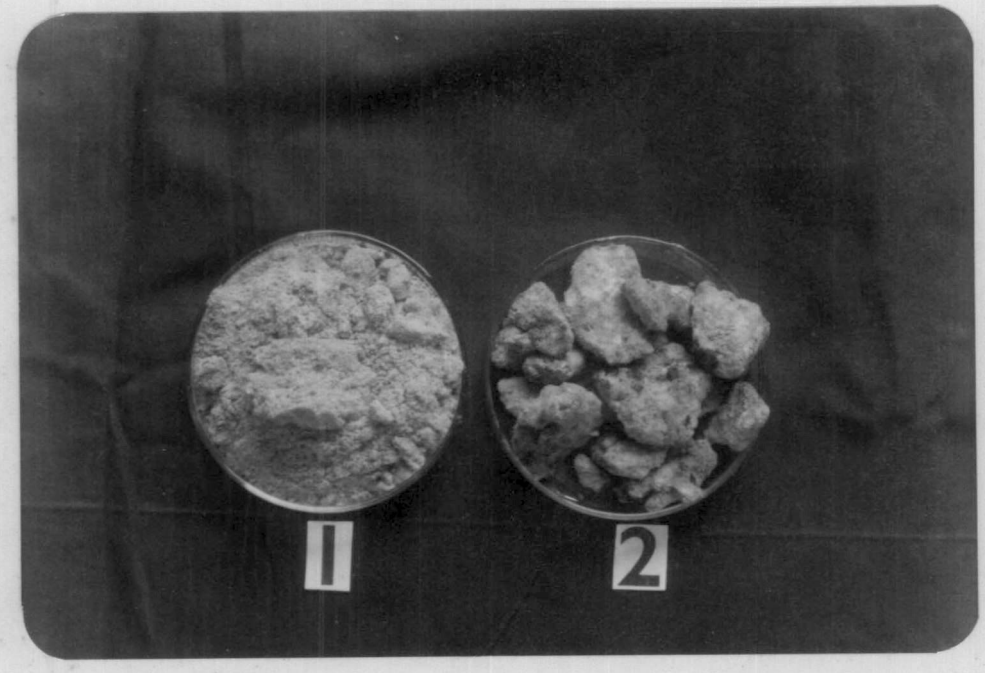
1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

Plate VII. *Deterioration of coconut oil cake by*

a) *Penicillium pinophilum*

1. Control 2. Inoculated

b) *A. flavus*, *A. niger*, *A. terreus* *R. stoloniger* and *P. pinophilum*



1. Control 2. Inoculated

Plate VI. *Deterioration of coconut oil cake by*

a) *Aspergillus terreus*

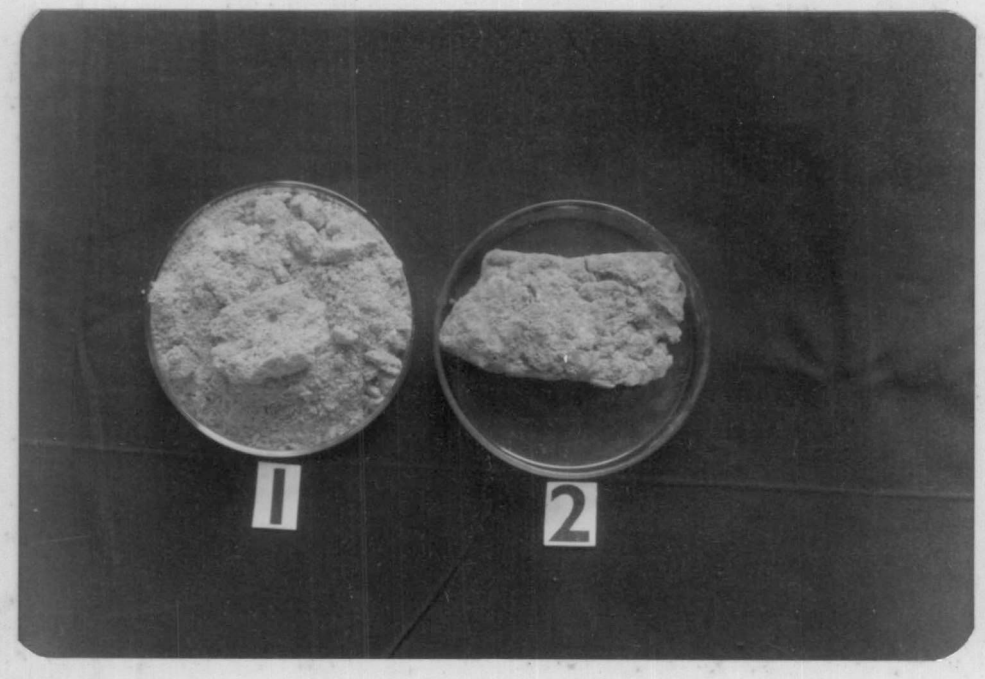
1. Control 2. Inoculated

b) *Rhizopus stolonifer*

1. Control 2. Inoculated

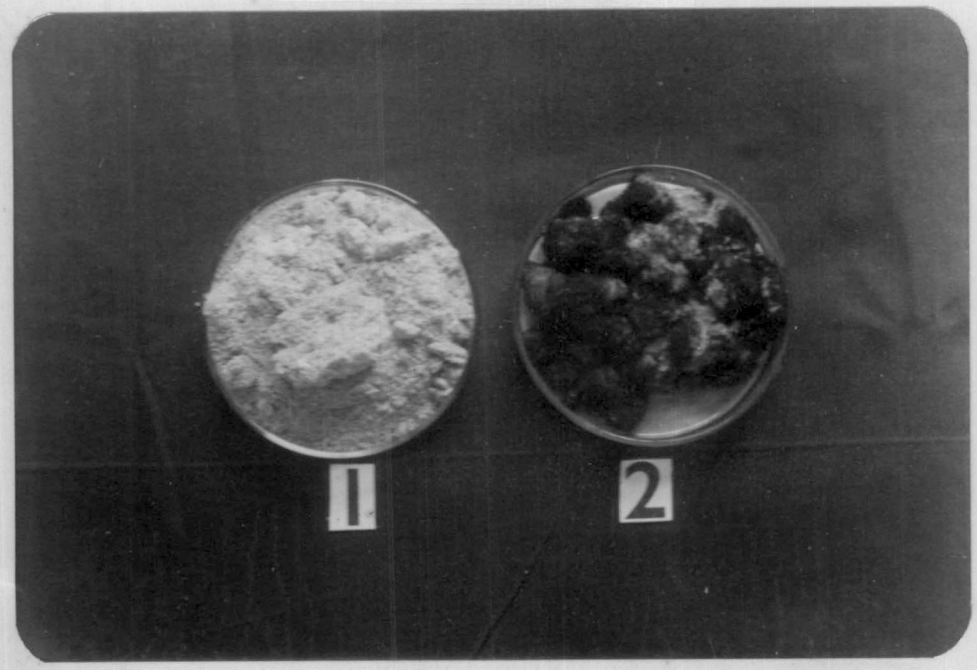
Plate V. Deterioration of coconut oil cake by

a) *Aspergillus flavus*



1. Control 2. Inoculated

b) *Aspergillus niger*



1. Control 2. Inoculated

fungi (Table 8). Significant reduction was noticed in nutrients like total carbohydrates, crude protein, and total free amino nitrogen and minerals like phosphorus, potassium, calcium, magnesium, copper and iron.

Maximum reduction in carbohydrate (76.95 per cent) was noticed due to the growth of Aspergillus niger. Combined growth of A. flavus and Rhizopus stolonifer caused highest reduction (29.38 per cent) in crude protein. A. flavus, A. niger and A. terreus together caused 92.52 and 72.29 per cent reduction respectively in total free amino nitrogen and phosphorus. Growth of A. niger and R. stolonifer together caused maximum reduction in potassium (93.79 per cent), magnesium (60.53 per cent), calcium (90.20 per cent) and copper (93.01 per cent). Reduction in iron content upto 60.77 per cent was noticed due to the combined growth of A. terreus, R. stolonifer and Penicillium pinophilum. (plates v, vi and vii)

B. Groundnut oil cake

Growth of fungi caused considerable reduction in most of the chemical constituents of groundnut oil cake (Table 9). Significant reduction was noticed in the case of total carbohydrates, crude protein and total free amino nitrogen and minerals like phosphorus, magnesium, calcium and copper.

Table 9. Effect of fungi on the nutrient content of groundnut oil cake.

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
1	1	13.68 (-45.02)	34.97 (-30.60)	6.41 (-58.13)	10.79 (+74.03)	0.53 (-27.40)	0.77 (-46.15)	0.38 (-39.68)	837.20 (-58.96)	41.07 (-95.10)	1153.07 (+49.56)	3.006 (-87.22)
2	2	12.52 (-49.64)	23.66 (-53.05)	5.53 (-63.88)	8.02 (+29.35)	0.57 (-21.92)	0.31 (-78.32)	0.40 (-36.51)	912.73 (-55.26)	49.80 (-94.06)	1002.33 (+30.00)	3.843 (-83.65)
3	3	21.92 (-11.90)	46.37 (-7.98)	5.49 (-64.14)	9.05 (+45.97)	0.44 (-39.73)	0.08 (-94.41)	0.46 (-26.98)	817.13 (-59.94)	61.00 (-92.72)	893.10 (+15.84)	7.972 (-66.07)
4	4	11.56 (-53.54)	16.11 (-68.03)	4.11 (-73.15)	8.57 (+38.23)	0.28 (-61.64)	0.43 (-69.93)	0.40 (-36.51)	820.93 (-59.76)	31.33 (-96.26)	486.93 (-36.84)	5.996 (-74.50)
5	5	22.52 (-9.49)	37.94 (-24.71)	5.17 (-66.23)	3.42 (-44.84)	0.30 (-58.90)	0.51 (-64.34)	0.41 (-34.92)	1050.40 (-48.51)	398.00 (-52.52)	802.13 (+4.04)	4.965 (-78.88)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

(contd.....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
6	1,2	23.36 (-6.11)	36.82 (-26.93)	3.37 (-77.99)	8.73 (+40.81)	0.62 (-15.07)	0.57 (-60.14)	0.45 (-28.57)	1004.87 (-50.74)	89.67 (-89.30)	946.40 (+22.75)	5.249 (-77.69)
7	1,3	17.28 (-30.55)	34.12 (-32.29)	5.30 (-65.38)	11.24 (+81.29)	0.52 (-28.77)	0.11 (-92.31)	0.48 (-23.81)	1014.73 (-50.26)	40.97 (-95.11)	773.63 (+0.34)	5.775 (-75.43)
8	1,4	21.87 (-12.10)	44.61 (-11.47)	7.65 (-50.03)	6.59 (+6.29)	0.28 (-61.64)	0.21 (-85.31)	0.41 (-34.92)	811.17 (-60.24)	33.87 (-95.96)	663.90 (+13.89)	4.048 (-82.79)
9	1,5	14.26 (-42.68)	36.09 (-28.38)	2.09 (-86.35)	5.35 (-13.71)	0.25 (-65.75)	0.45 (-68.53)	0.40 (-36.51)	628.23 (-69.20)	40.06 (-95.22)	895.87 (+16.20)	5.393 (-77.05)
10	2,3	23.13 (-7.03)	40.79 (-19.05)	6.19 (-59.57)	11.97 (+93.06)	0.56 (-23.29)	0.16 (-88.81)	0.41 (-34.92)	828.17 (-59.40)	33.33 (-96.02)	906.60 (+17.59)	7.009 (-70.20)
11	2,4	11.11 (-55.35)	46.27 (-8.18)	13.46 (-12.08)	6.10 (-1.61)	0.51 (-30.14)	0.51 (-64.34)	0.40 (-36.51)	842.33 (-58.71)	40.00 (-95.23)	694.47 (-9.93)	7.293 (-68.97)
12	2,5	15.26 (-38.67)	26.78 (-46.85)	6.65 (-56.56)	3.74 (-39.68)	0.46 (-36.99)	0.50 (-65.03)	0.37 (-41.27)	716.17 (-64.89)	47.50 (-94.33)	985.13 (+27.77)	4.067 (-82.71)
13	3,4	18.05 (-27.45)	18.17 (-63.94)	5.96 (-61.07)	4.97 (-19.84)	0.54 (-26.03)	0.39 (-72.73)	0.37 (-41.27)	838.37 (-58.90)	36.13 (-95.69)	930.30 (+20.66)	5.144 (-78.11)
14	3,5	18.05 (-27.45)	40.89 (-18.85)	1.14 (-92.55)	6.30 (+1.61)	0.42 (-42.47)	0.38 (-73.43)	0.44 (-30.16)	1216.33 (-40.38)	60.33 (-92.80)	853.17 (+10.66)	4.243 (-81.94)
15	4,5	14.33 (-42.40)	20.66 (-59.00)	5.22 (-65.90)	4.14 (-33.23)	0.44 (-39.73)	0.46 (-67.83)	0.39 (-38.10)	956.17 (-53.13)	43.00 (-94.87)	911.50 (+18.22)	5.773 (-75.43)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

(Contd....)

Sl. No.	Treatment	Total carbo-hydrate (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
16	1,2,3	13.42 (-46.02)	45.12 (-10.46)	3.16 (-79.36)	6.50 (+4.84)	0.52 (-28.77)	0.54 (-62.24)	0.40 (-36.51)	954.00 (-53.24)	40.33 (-95.19)	861.03 (+11.68)	6.010 (-74.41)
17	1,2,4	14.97 (-39.83)	26.13 (-48.14)	4.01 (-73.81)	11.40 (+83.87)	0.62 (-15.07)	0.70 (-51.05)	0.50 (-20.63)	931.23 (-54.35)	45.33 (-94.59)	687.83 (-10.79)	2.911 (-87.60)
18	1,2,5	10.80 (-56.59)	28.35 (-43.74)	2.52 (-83.54)	9.47 (+52.74)	0.26 (-64.36)	0.61 (-57.34)	0.41 (-34.92)	931.00 (-54.36)	100.67 (-87.99)	845.33 (+9.64)	5.042 (-78.54)
19	1,3,4	22.73 (-8.64)	42.42 (-15.82)	7.51 (-50.95)	8.40 (+35.48)	0.54 (-26.03)	0.47 (-67.13)	0.43 (-31.75)	902.93 (-55.74)	39.53 (-95.28)	808.83 (+4.91)	9.892 (-57.91)
20	1,3,5	17.07 (-31.39)	34.66 (-31.22)	4.29 (-71.98)	7.56 (+21.94)	0.47 (-35.62)	0.32 (-77.62)	0.45 (-28.75)	1080.33 (-47.04)	63.00 (-92.48)	813.40 (+5.50)	5.244 (-77.69)
21	1,4,5	20.25 (-18.61)	34.87 (-30.80)	4.46 (-70.87)	5.83 (-5.97)	0.29 (-60.27)	0.46 (-67.83)	0.42 (-33.33)	736.00 (-63.92)	36.33 (-95.67)	713.97 (-7.40)	11.252 (-52.12)
22	2,3,4	18.36 (-26.21)	22.63 (-55.09)	6.46 (-57.81)	6.09 (-1.77)	0.53 (-27.40)	0.58 (-59.44)	0.38 (-39.68)	979.60 (-51.98)	41.20 (-95.08)	1016.33 (+31.82)	4.440 (-81.09)
23	2,3,5	22.74 (-8.60)	31.03 (-38.42)	6.49 (-57.61)	4.66 (-24.84)	0.46 (-36.99)	0.32 (-77.62)	0.40 (-36.51)	741.77 (-63.64)	27.33 (-96.74)	687.43 (-10.84)	7.155 (-69.56)
24	2,4,5	14.52 (-41.64)	37.13 (-26.31)	4.06 (-73.48)	4.21 (-32.10)	0.44 (-39.73)	0.62 (-56.64)	0.37 (-41.27)	896.00 (-56.08)	41.33 (-95.07)	796.07 (+3.25)	5.965 (-74.63)
25	3,4,5	16.51 (-33.64)	21.23 (-57.87)	5.33 (-65.19)	4.33 (-30.16)	0.30 (-58.90)	0.48 (-66.43)	0.42 (-33.33)	664.00 (-67.45)	71.40 (-91.48)	893.20 (+15.85)	4.979 (-78.84)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

(Contd.....)

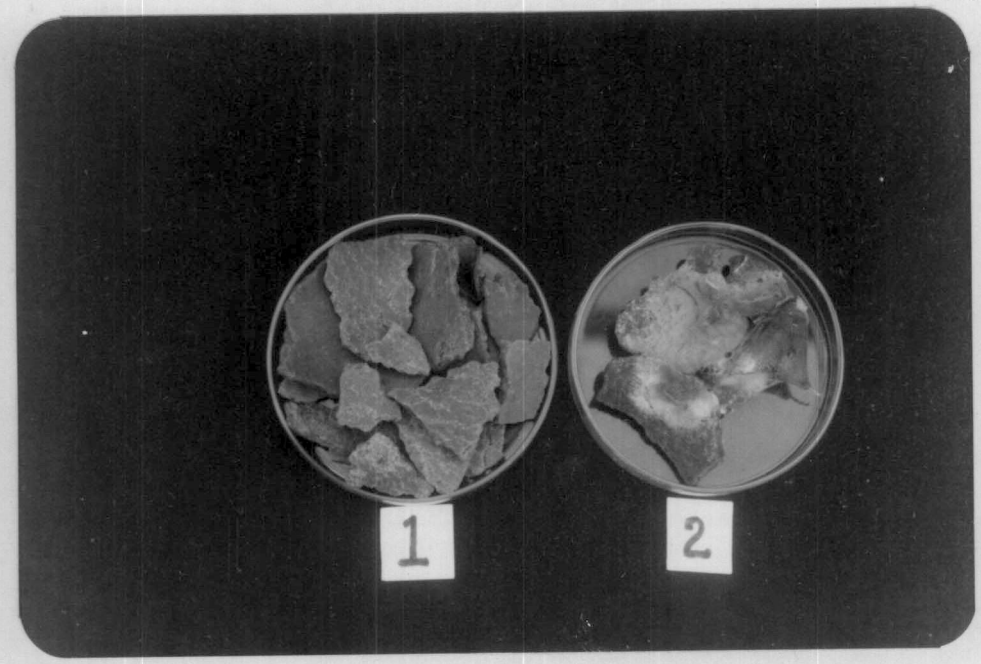
Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (ppm)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
26	1,2,3,4	22.37 (-10.09)	43.71 (-13.26)	8.09 (-47.16)	2.16 (-65.16)	0.37 (-49.32)	0.41 (-71.33)	0.41 (-34.92)	906.57 (-55.56)	43.80 (-94.77)	595.17 (-22.81)	4.140 (-82.37)
27	1,2,3,5	11.44 (-54.02)	37.43 (-25.72)	3.29 (-78.51)	5.85 (-5.65)	0.45 (-38.36)	0.55 (-61.54)	0.39 (-38.10)	913.17 (-55.24)	30.83 (-96.32)	793.00 (+2.85)	4.935 (-79.01)
28	1,2,4,5	16.14 (-35.13)	29.33 (-41.79)	3.87 (-74.72)	2.78 (-55.16)	0.28 (-61.64)	0.89 (-37.76)	0.41 (-34.92)	617.17 (-69.75)	27.17 (-96.76)	694.70 (-9.90)	6.183 (-73.69)
29	1,3,4,5	20.85 (-16.20)	36.46 (-27.64)	1.51 (-90.14)	6.24 (+0.65)	0.55 (-24.66)	0.47 (-67.13)	0.39 (-38.10)	918.77 (-54.96)	40.33 (-95.19)	793.00 (+2.85)	5.785 (-75.39)
30	2,3,4,5	13.65 (-45.14)	22.46 (-55.43)	4.25 (-72.24)	9.97 (+60.81)	0.42 (-42.47)	0.56 (-60.84)	0.37 (-41.27)	792.83 (-61.14)	43.33 (-94.83)	909.20 (+17.92)	4.067 (-82.71)
31	1,2,3,4,5	15.09 (-39.35)	36.60 (-27.37)	1.63 (-89.35)	5.57 (-10.16)	0.53 (-27.40)	0.33 (-76.92)	0.32 (-49.21)	907.07 (-55.54)	24.17 (-97.12)	613.83 (-20.39)	3.033 (-87.09)
32	Control	24.88	50.39	15.31	6.20	0.73	1.43	0.63	2040.00	838.20	771.00	23.504
	CD (0.05)	1.389	4.560	2.511	2.835	0.036	0.129	0.015	73.420	27.846	104.781	0.9967

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. stolonifer
5. P. pinophilum

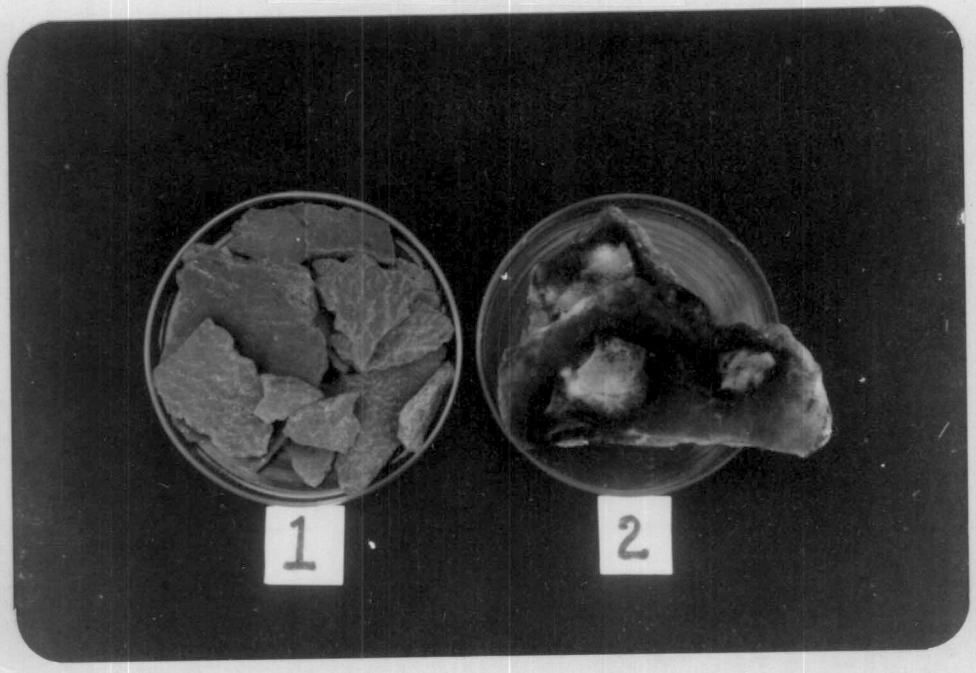
Plate VIII. Deterioration of groundnut oil cake by

a) *Aspergillus flavus*



1. Control 2. Inoculated

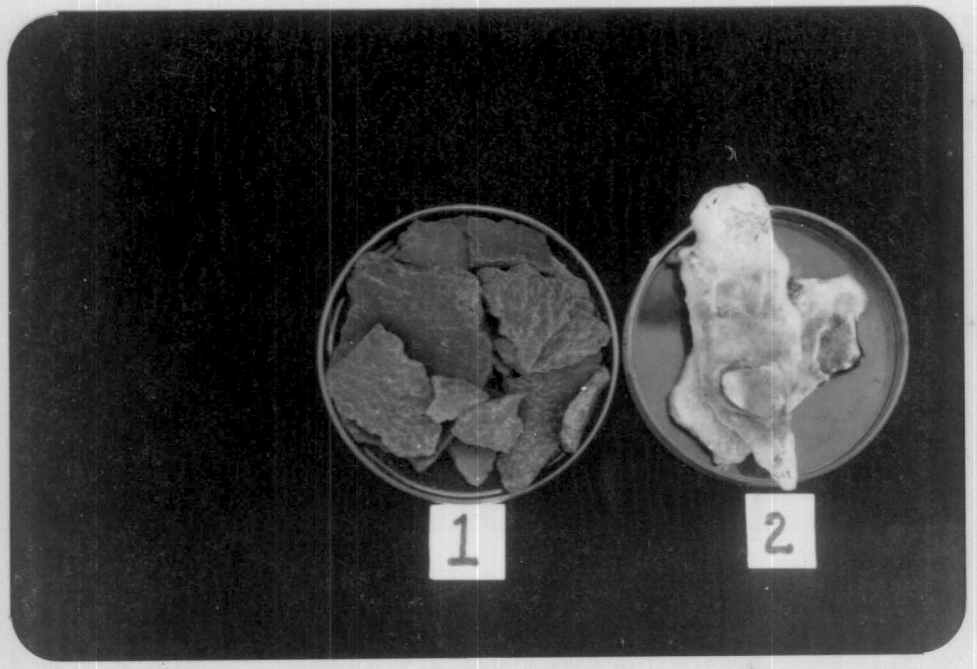
b) *Aspergillus niger*



1. Control 2. Inoculated

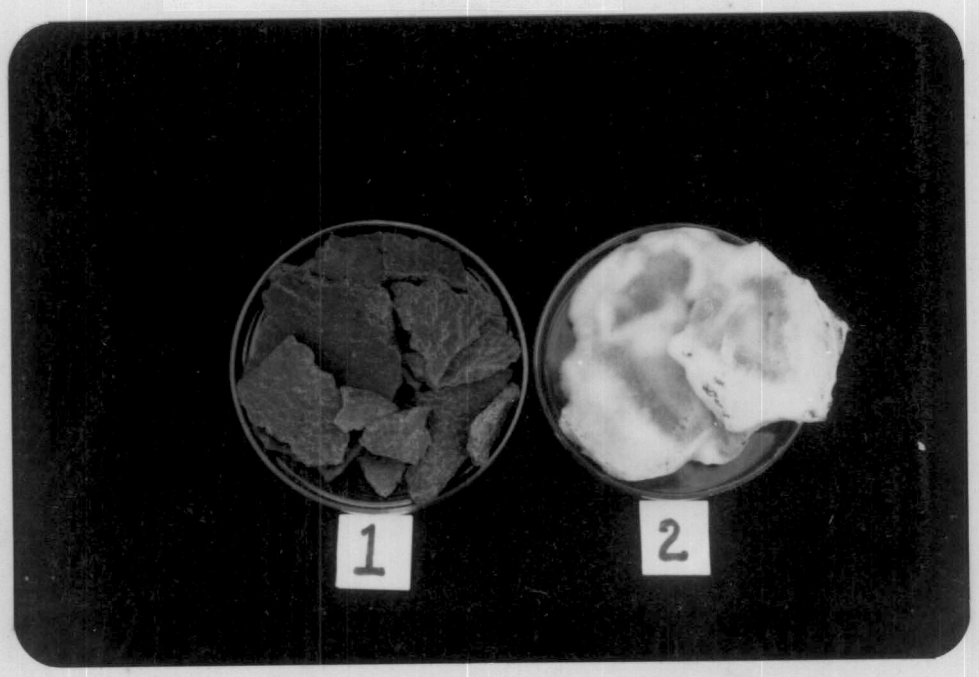
Plate IX. *Deterioration of groundnut oil cake by*

a) *Aspergillus terreus*



1. Control 2. Inoculated

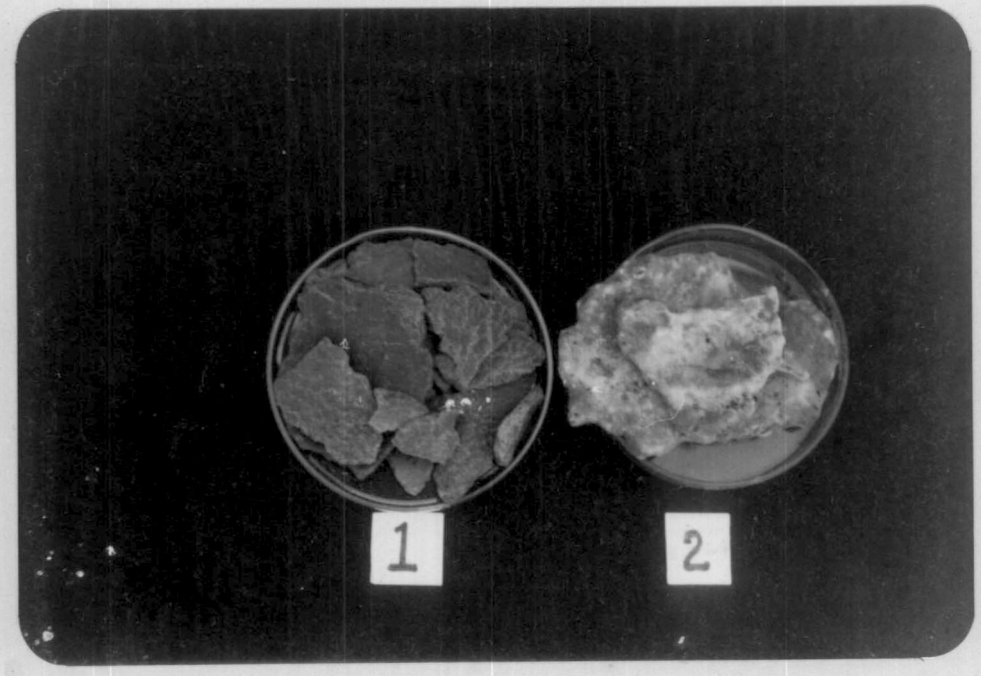
b) *Rhizopus stolonifer*



1. Control 2. Inoculated

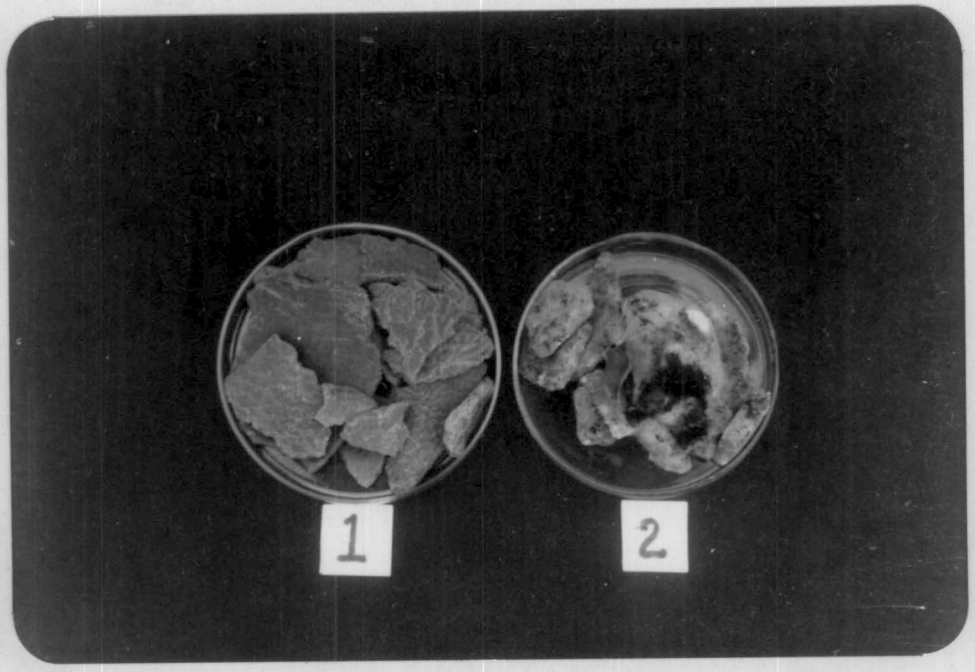
Plate X. Deterioration of groundnut oil cake by

a) *Penicillium pinophilum*



1. Control 2. Inoculated

b) *A. flavus*, *A. niger*, *A. terreus*
R. stolonifer and *P. pinophilum*



1. Control 2. Inoculated

Growth of A. flavus, A. niger and P. pinophilum together caused maximum reduction in total carbohydrate (56.59 per cent). Maximum reduction in crude protein (68.03 per cent) was noticed due to the growth of R. stolonifer. A. flavus, A. niger and R. stolonifer together caused 87.60 per cent reduction in total free amino nitrogen. Reduction in phosphorus content upto 65.75 per cent was observed due to the combined growth of A. flavus and P. pinophilum. A. terreus caused maximum reduction in potassium (94.41 per cent). Magnesium was reduced upto 49.21 per cent by the growth of all the five fungi together. Combined growth of A. flavus, A. niger, R. stolonifer and P. pinophilum caused maximum depletion in calcium (69.75 per cent). All the five fungi tested together caused maximum reduction (97.12 per cent) in copper (Plates, VIII, IX, X).

C. Sesamum oil cake

Considerable reduction in some of the chemical constituents of sesamum oil cake was caused due to the growth of fungi (Table 10). Significant reduction was noticed in nutrients like total carbohydrates, crude protein and total free amino nitrogen and minerals like phosphorus, potassium, magnesium, calcium and copper.

Table 10. Effect of fungi on the nutrient content of sesamum oil cake.

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
1	1	16.92 (-30.88)	32.67 (-18.83)	2.80 (-60.40)	14.53 (+17.18)	1.04 (-25.18)	0.95 (-29.10)	0.47 (-53.47)	1.08 (-47.32)	43.37 (-86.20)	1044.00 (-11.03)	7.355 (-59.41)
2	2	14.29 (-41.63)	33.55 (-16.65)	7.68 (+8.63)	12.79 (+3.15)	1.01 (-27.34)	0.83 (-38.06)	0.39 (-61.39)	1.07 (-47.80)	46.00 (-85.36)	1035.90 (-11.72)	12.811 (-29.26)
3	3	12.38 (-49.43)	33.86 (-15.88)	3.07 (-56.58)	12.36 (-0.32)	1.02 (-26.62)	0.74 (-44.78)	0.40 (-60.40)	1.28 (-37.56)	42.73 (-86.40)	1470.40 (+25.31)	5.059 (-72.06)
4	4	15.32 (-37.42)	31.21 (-22.46)	8.71 (+22.20)	9.17 (-26.05)	0.98 (-29.50)	0.52 (-61.19)	0.67 (-33.66)	1.10 (-46.34)	40.43 (-87.13)	1046.10 (-10.85)	6.270 (-65.37)
5	5	12.18 (-50.25)	28.19 (-29.96)	8.02 (+13.44)	10.99 (-11.37)	0.40 (-71.22)	0.53 (-60.45)	0.60 (-40.59)	1.25 (-39.02)	46.13 (-85.32)	1097.60 (-6.46)	8.370 (-53.78)
6	6	13.85 (-43.42)	34.06 (-15.38)	10.69 (+51.20)	12.29 (-0.89)	0.88 (-36.69)	0.60 (-55.22)	0.82 (-18.81)	1.21 (-40.98)	69.40 (-77.91)	1538.40 (+31.10)	11.239 (-37.93)
7	7	11.29 (-53.88)	37.06 (-7.93)	8.47 (+19.80)	13.80 (+11.29)	0.84 (-39.57)	0.67 (-50.00)	0.67 (-33.66)	1.18 (-42.44)	55.77 (-82.25)	1374.37 (+17.12)	11.472 (-36.66)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. oryzae
5. P. pinophilum
6. Fusarium pallidoroseum
7. Curvularia clavata

(Contd....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
8	1,2	12.83 (-47.59)	28.39 (-29.47)	1.40 (-80.20)	15.01 (+21.05)	0.71 (-48.92)	0.30 (-77.61)	0.76 (-24.75)	1.09 (-46.83)	42.20 (-86.57)	1656.57 (+41.17)	10.125 (-44.12)
9	1,3	10.04 (-58.99)	29.06 (-27.80)	9.48 (+34.09)	13.35 (+7.66)	0.99 (-28.78)	0.10 (-92.54)	0.81 (-19.80)	1.03 (-49.76)	40.23 (-87.19)	1538.97 (+31.15)	15.412 (-14.91)
10	1,4	14.75 (-39.75)	32.23 (-19.93)	8.53 (+20.65)	13.21 (+6.53)	0.64 (-53.96)	0.08 (-94.03)	0.68 (-32.67)	1.34 (-34.63)	45.87 (-85.40)	1700.43 (+44.91)	11.851 (-34.56)
11	1,5	14.33 (-41.46)	31.74 (-21.14)	8.23 (+16.41)	10.37 (-16.37)	1.00 (-28.06)	0.97 (-27.61)	0.78 (-22.77)	1.46 (-28.78)	52.73 (-83.22)	1010.23 (-13.91)	6.490 (-64.16)
12	2,3	11.91 (-51.35)	32.99 (-18.04)	9.47 (+33.95)	14.47 (+16.69)	0.34 (-75.54)	0.38 (-71.64)	0.37 (-63.37)	0.28 (-86.34)	68.10 (-78.32)	1030.67 (-12.17)	7.461 (-58.80)
13	2,4	12.61 (-48.49)	27.72 (-31.13)	9.50 (+34.37)	15.03 (+21.21)	0.81 (-41.73)	0.46 (-65.67)	0.65 (-35.64)	1.26 (-38.54)	37.23 (-88.15)	1693.07 (+44.28)	14.119 (-22.03)
14	2,5	14.80 (-39.54)	31.72 (-21.19)	11.26 (+59.26)	10.78 (-13.06)	0.76 (-45.32)	0.45 (-66.42)	0.77 (-23.76)	1.43 (-30.24)	66.60 (-78.80)	1574.83 (+34.21)	10.516 (-41.96)
15	3,4	10.71 (-56.25)	32.48 (-19.30)	5.40 (-23.62)	12.37 (-0.24)	0.66 (-52.52)	0.61 (-54.48)	0.75 (-25.74)	1.37 (-33.17)	56.00 (-82.18)	1619.13 (+37.98)	5.373 (-70.34)
16	3,5	13.10 (-46.49)	28.81 (-28.42)	8.04 (+13.72)	12.61 (+1.69)	0.65 (-53.24)	1.03 (-23.13)	0.78 (-22.77)	1.19 (-41.75)	104.13 (-66.86)	1399.23 (+19.24)	6.132 (-66.15)
17	4,5	8.59 (-64.91)	31.72 (-21.19)	9.82 (+38.90)	11.85 (-4.44)	0.81 (-41.73)	0.46 (-65.67)	0.61 (-39.60)	1.15 (-43.90)	41.80 (-86.70)	1556.17 (+32.62)	6.430 (-64.49)

(Figures in parentheses are per cent increase or decrease over control

1. A. flavus
2. A. niger
3. A. terreus
4. R. oryzae
5. P. pinophilum

(Contd....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
18	1,2,3	10.92 (-55.39)	33.48 (-16.82)	9.12 (+29.00)	12.21 (-1.53)	0.82 (-41.01)	0.42 (-68.66)	0.58 (-42.57)	1.15 (-43.90)	59.37 (-81.10)	1423.53 (+21.31)	9.839 (-45.66)
19	1,2,4	12.25 (-49.96)	28.71 (-28.67)	8.45 (+19.52)	10.78 (-13.06)	0.81 (-41.73)	0.46 (-65.67)	0.90 (-10.89)	1.02 (-50.24)	96.03 (-69.43)	1319.20 (+12.42)	10.032 (-44.61)
20	1,2,5	9.69 (-60.42)	30.24 (-24.87)	5.92 (-16.27)	11.83 (-4.60)	0.95 (-31.65)	1.03 (-23.13)	0.83 (-17.82)	1.13 (-44.88)	95.23 (-69.69)	993.37 (-15.34)	6.108 (-66.26)
21	1,3,4	12.33 (-49.63)	31.63 (-21.42)	5.33 (-24.61)	11.92 (-3.87)	0.99 (-28.78)	0.15 (-88.81)	0.58 (-42.57)	0.89 (-56.59)	92.30 (-70.62)	1169.63 (-0.32)	8.073 (-55.44)
22	1,3,5	11.46 (-53.19)	31.42 (-21.94)	5.65 (-20.08)	11.35 (-8.47)	0.96 (-30.94)	0.26 (-80.60)	0.61 (-39.60)	0.96 (-53.17)	49.70 (-84.18)	1245.10 (+6.11)	7.080 (-60.90)
23	1,4,5	13.92 (-43.14)	31.53 (-21.66)	7.15 (+1.13)	12.76 (+2.90)	0.59 (-57.55)	0.09 (-93.28)	0.60 (-40.59)	1.23 (-40.00)	102.77 (-67.29)	1330.23 (+13.36)	9.978 (-44.89)
24	2,3,4	11.72 (-52.12)	34.30 (-14.78)	8.50 (+20.23)	12.47 (+0.56)	0.55 (-60.43)	0.47 (-64.93)	0.62 (-38.61)	1.01 (-50.73)	97.37 (-69.01)	1031.50 (-12.10)	5.863 (-67.64)
25	2,3,5	8.58 (-64.95)	30.48 (-24.27)	11.37 (+60.82)	14.01 (+12.98)	0.56 (-59.71)	0.43 (-67.91)	0.62 (-38.61)	0.85 (-58.54)	85.07 (-72.92)	975.97 (-16.83)	7.289 (-59.74)
26	2,4,5	12.22 (-50.08)	28.03 (-30.36)	12.10 (+71.15)	14.42 (+16.29)	0.74 (-46.76)	0.49 (-63.43)	0.73 (-27.72)	0.89 (-56.59)	91.43 (-70.90)	1214.37 (+3.49)	11.048 (-38.98)
27	3,4,5	11.71 (-52.17)	30.33 (-24.65)	10.17 (+43.85)	13.20 (+6.45)	0.64 (-53.96)	0.54 (-59.70)	0.70 (-30.69)	0.85 (-58.54)	90.10 (-71.32)	1220.53 (+4.01)	7.189 (-60.29)

(Figures in parentheses are per cent increase or decrease over control)

1. A. flavus
2. A. niger
3. A. terreus
4. R. oryzae
5. P. pinophilum

(Contd.....)

Sl. No.	Treatment	Total carbo-hydrates (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Copper (ppm)	Iron (ppm)	Total free amino nitrogen (mg/g)
28	1,2,3,4	7.77 (-68.26)	35.30 (-12.30)	6.98 (-1.27)	13.75 (+10.89)	0.58 (-58.27)	0.37 (-72.39)	0.64 (-36.63)	0.89 (-56.29)	65.13 (-79.27)	1145.67 (-2.37)	7.935 (-56.21)
29	1,2,3,5	10.52 (-57.03)	35.58 (-11.60)	6.17 (-12.73)	12.27 (-1.05)	0.53 (-61.87)	0.31 (-74.10)	0.49 (-51.49)	0.99 (-51.71)	83.07 (-73.56)	1218.40 (+3.83)	6.546 (-63.88)
30	1,2,4,5	11.83 (-51.67)	29.96 (-25.67)	9.70 (+37.20)	13.06 (+5.32)	0.49 (-64.75)	0.47 (-64.93)	0.61 (-39.60)	0.96 (-53.17)	49.57 (-84.22)	1121.03 (-4.47)	9.140 (-49.53)
31	1,3,4,5	10.96 (-55.23)	32.63 (-18.93)	9.05 (+28.01)	13.17 (+6.21)	0.56 (-59.71)	0.28 (-79.10)	0.60 (-40.59)	0.80 (-60.98)	43.00 (-86.31)	1068.00 (-8.98)	8.298 (-54.17)
32	2,3,4,5	10.40 (-57.52)	33.93 (-15.70)	9.65 (+36.49)	14.27 (+15.08)	0.78 (-43.88)	0.24 (-82.09)	0.59 (-41.58)	0.63 (-69.27)	40.70 (-87.05)	1005.73 (-14.29)	6.249 (-65.49)
33	1,2,3,4,5	7.68 (-68.63)	30.91 (-23.20)	10.80 (+52.76)	15.12 (+21.94)	0.61 (-56.12)	0.13 (-90.30)	0.57 (-43.56)	0.52 (-74.63)	54.33 (-82.71)	1148.93 (-2.09)	7.252 (-59.96)
34	Control	24.48	40.25	7.07	12.40	1.39	1.34	1.01	2.05	314.17	1173.43	18.111
	CD (0.05)	1.729	2.836	1.976	2.581	0.078	0.091	0.095	0.213	5.241	162.336	0.8507

(Figures in parentheses are per cent increase or decrease over control)

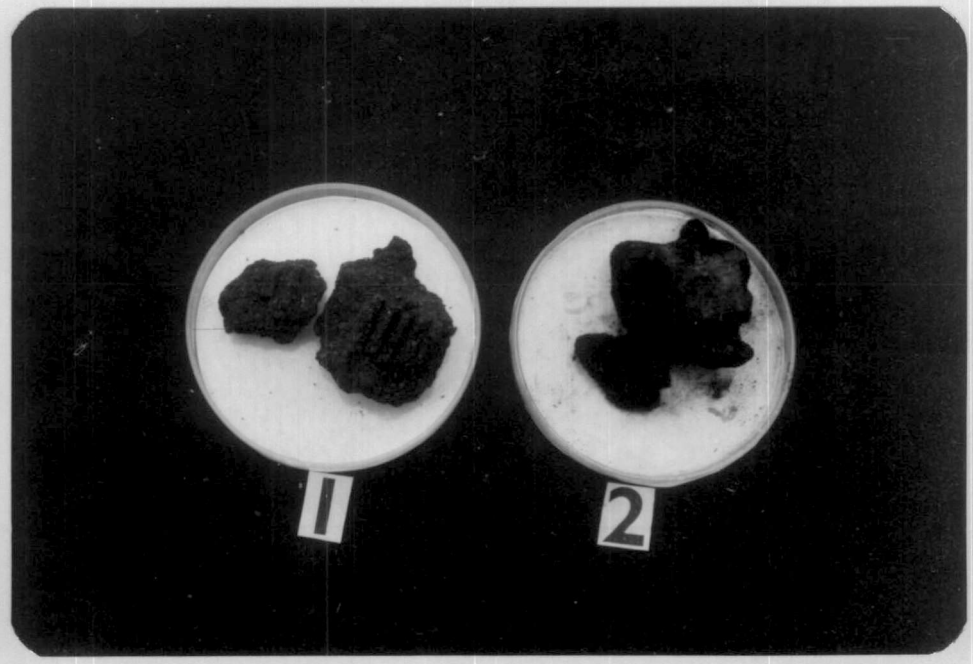
1. A. flavus
2. A. niger
3. A. terreus
4. R. oryzae
5. P. pinophilum

Plate XI. Deterioration of sesamum oil cake by

a) *Aspergillus flavus*

1. Control 2. Inoculated

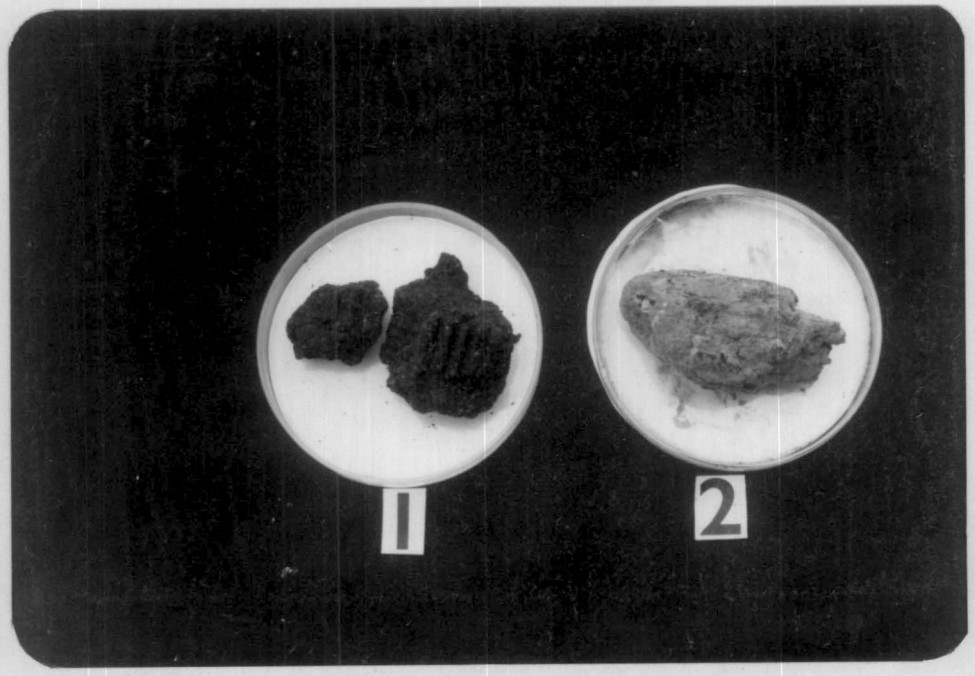
b) *Aspergillus niger*



1. Control 2. Inoculated

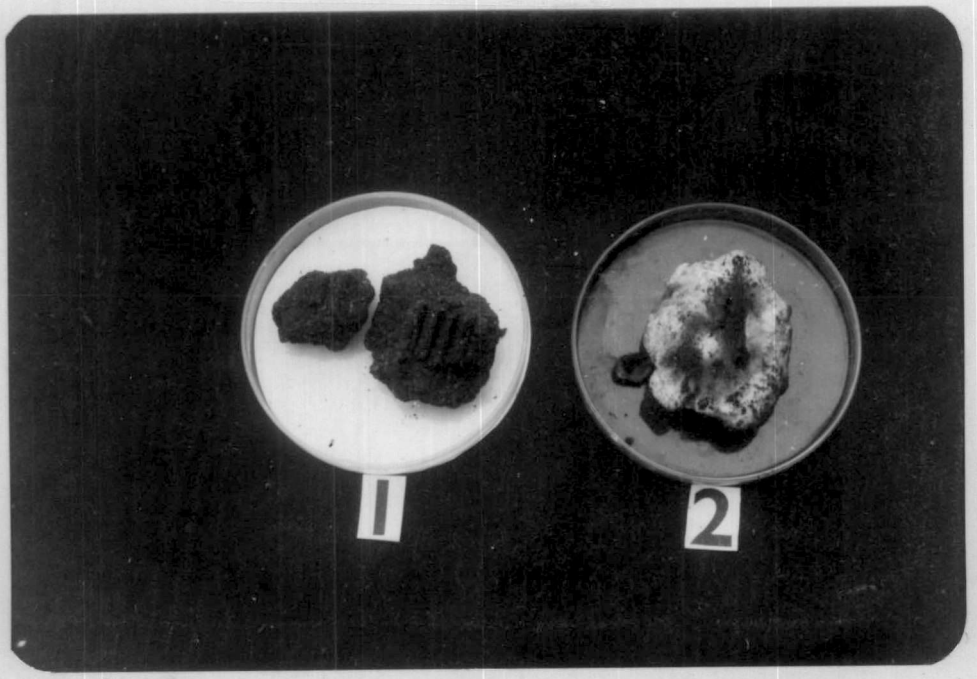
Plate XII. Deterioration of sesamum oil cake by

a) *Aspergillus terreus*



1. Control 2. Inoculated

b) *Rhizopus oryzae*



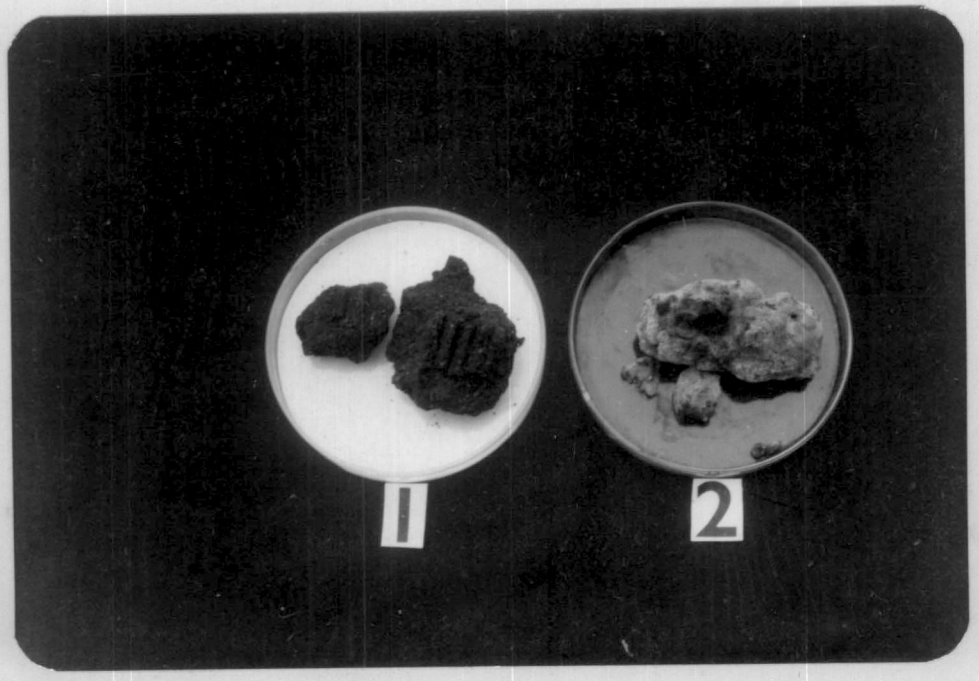
1. Control 2. Inoculated

Plate XIII. Deterioration of sesamum oil cake by

a) *Penicillium pinophilum*

1. Control 2. Inoculated

b) *A. flavus*, *A. niger*, *A. terreus*, *R. oryzae* and *P. pinophilum*



1. Control 2. Inoculated

Table 12. Production of aflatoxins by isolates of Aspergillus flavus in culture medium.

Sl. No.	Isolate		Quantity of aflatoxin (ppb)		
	Number	Code	B_1	B_2	G_2
1	2	Co III a	413	302	151
2	4	Co II b	214	200	100
3	5	Co III b	231	1040	85
4	6	Co I c	242	-	-
5	7	Co II c	1210	-	-
6	8	Co IIIc	434	215	-
7	9	G II a	185	467	63
8	10	G II b	960	344	51
9	11	G II c	410	235	145
10	14	S III a	195	-	-
11	15	S I b	208	408	43
12	16	S II b	307	940	68
13	17	S III b	185	213	-
14	20	S III c	502	-	-

Co - Coconut oil cake
G - Groundnut oil cake
S - Sesamum oil cake

I - Southern region
II - Central region
III - Northern region

a - February - March
b - June - July
c - October - November

Reduction in total carbohydrate contents upto 68.63 per cent was noticed due to the growth of A. flavus, A. niger, A. terreus, R. oryzae and P. pinophilum together. Combined growth of A. niger and R. oryzae caused maximum reduction (31.13 per cent) in crude proteins. Reduction in the total free amino nitrogen upto 72.06 per cent was noticed due to the growth of A. terreus. Phosphorus was reduced upto 75.54 per cent by the combined growth of A. niger and A. terreus. A. flavus and R. oryzae together caused maximum reduction in potassium (94.03 per cent). Combined growth of A. niger and A. terreus caused maximum reduction in magnesium (63.37 per cent) and calcium (86.34 per cent). A. niger and R. oryzae together caused 88.15 per cent reduction in copper (Plates XI, XII, XIII).

In the case of crude fibre, ash and iron, eventhough there was decrease in most of the treatments, increase was noticed in few cases. The crude fibre content was decreased upto 92.55, 80.20 and 59.63 per cent in groundnut, sesamum and coconut oil cakes. Ash content was reduced to the extent of 65.16, 29.44 and 26.05 per cent in groundnut, coconut and sesamum oil cakes. There was 36.84 and 16.83 per cent reduction in iron, in groundnut and sesamum oil cakes.

4. Estimation of aflatoxin

4.1. In culture medium

Out of the 20 isolates of Aspergillus flavus screened, 14 (70 per cent) were found to produce aflatoxins in culture medium (Table 11). Out of 14 toxigenic isolates, eight numbers (57.14 per cent) produced B_1, B_2 and G_2, two (14.29 per cent) produced B_1 and B_2 and four (28.57 per cent) produced B_1 alone in the culture medium. Out of the 19 isolates of A. niger screened, eight (42.10 per cent) produced afatoxin B_1 alone in the culture medium.

Out of the six isolates of A. flavus from coconut oil cake, three produced aflatoxin B_1, B_2 and G_2, one isolate produced B_1 and B_2 and two isolates produced B_1 alone in the culture medium. All the isolates of A. flavus obtained from groundnut oil cake produced B_1, B_2 and G_2. Among the five isolates of A. flavus from sesamum oil cake, two produced B_1, B_2 and G_2, one produced B_1 and B_2 and two isolates had B_1 alone. Maximum quantity of aflatoxin B_1 (1210 ppb), B_2 (1040 ppb) and G_2 (151 ppb) were produced by isolates 7 (Co II c), 5 (Co III b) and 2 (Co III a) from coconut oil cake (Table 12 and Fig.4).

Table 11. Production of aflatoxins by species of *Aspergillus* isolated from oil cakes.

Sl. No.	Fungus	Number of isolates	Number of toxigenic isolates	Percentage of toxigenic isolates	Aflatoxins produced
1	*Aspergillus flavus*	20	14	70.00	
			8	57.14	B_1, B_2 and G_2
			2	14.29	B_1 and B_2
			4	28.57	B_1
2	*Aspergillus niger*	19	8	42.10	B_1

Table 12. Production of aflatoxins by isolates of Aspergillus flavus in culture medium.

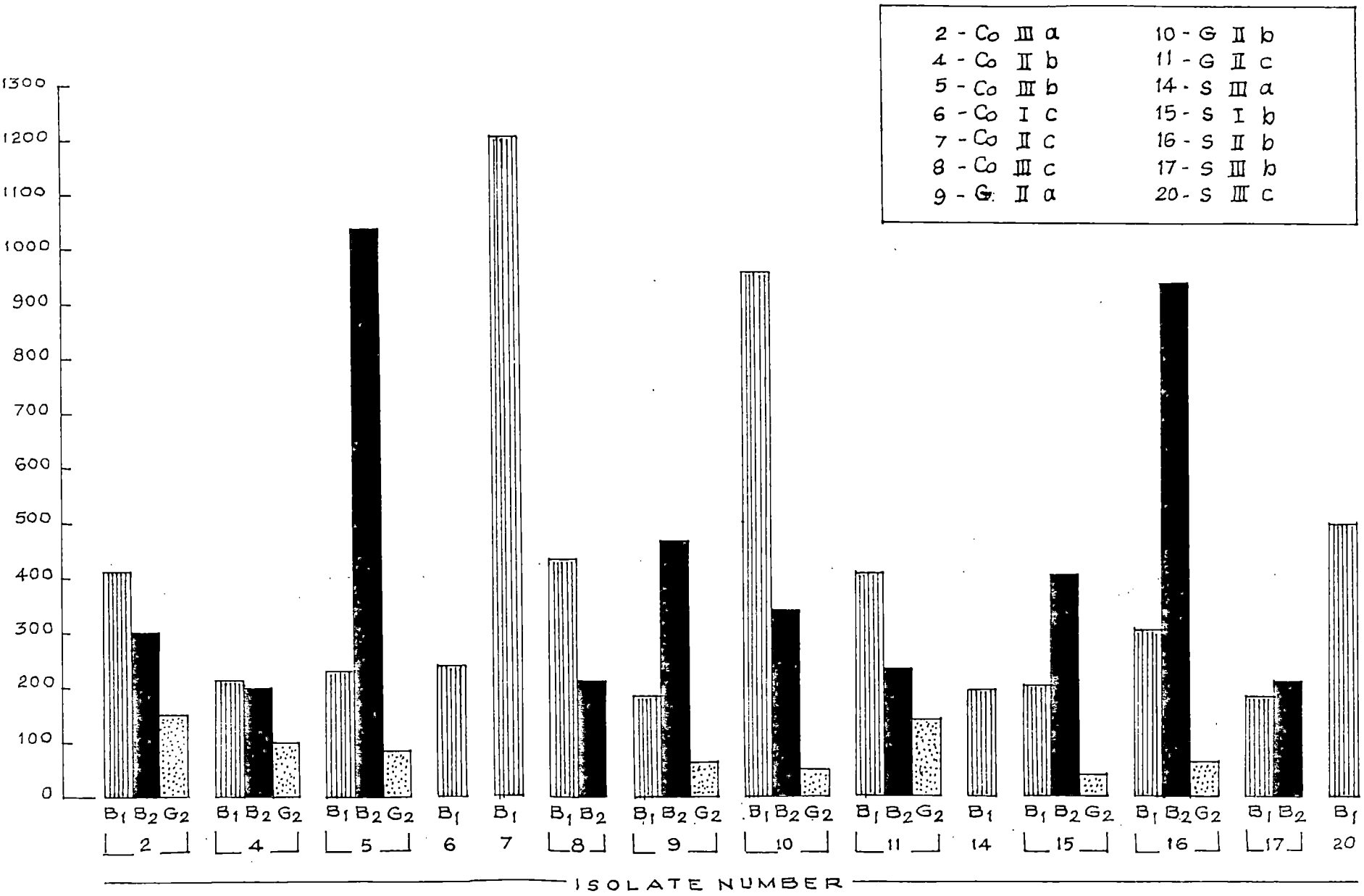
Sl. No.	Isolate		Quantity of aflatoxin (ppb)		
	Number	Code	B_1	B_2	G_2
1	2	Co III a	413	302	151
2	4	Co II b	214	200	100
3	5	Co III b	231	1040	85
4	6	Co I c	242	-	-
5	7	Co II c	1210	-	-
6	8	Co IIIc	434	215	-
7	9	G II a	185	467	63
8	10	G II b	960	344	51
9	11	G II c	410	235	145
10	14	S III a	195	-	-
11	15	S I b	208	408	43
12	16	S II b	307	940	68
13	17	S III b	185	213	-
14	20	S III c	502	-	-

Co - Coconut oil cake
G - Groundnut oil cake
S - Sesamum oil cake

I - Southern region
II - Central region
III - Northern region

a - February - March
b - June - July
c - October - November

FIG. 4. PRODUCTION OF AFLATOXINS BY ISOLATES OF *Aspergillus flavus* IN CULTURE MEDIUM.



Out of the eight toxigenic isolates of A. niger, four from coconut oil cake, one from groundnut oil cake and three from sesamum oil cake produced aflatoxin B_1 in culture medium. Maximum quantity of B_1 (222 ppb) was produced by isolate 12 (S II a) from sesamum oil cake (Table 13 and Fig.5).

4. 2. **In oil cakes**

The toxigenic isolates A. flavus when grown on coconut oil cake produced aflatoxin B_1 (660 to 1517 ppb), B_2 (315 to 1092 ppb) and G_2 (98 to 272 ppb). Isolates 7 (Co II c), 5 (Co III b) and 2 (Co III a) produced maximum quantity of B_1, B_2 and G_2 respectively (Plate, XIV). Isolates of A. niger produced only B_1 (95 to 419 ppb) with the maximum quantity by isolate 1 (Co III a) (Table 14, Fig.6 and Plate XV).

In groundnut oil cake, the isolates of A. flavus produced B_1 (255 to 1210 ppb), B_2 (310 to 1011 ppb) and G_2 (96 to 211 ppb). The maximum quantity of B_1, B_2 and G_2 was produced by isolates 10 (G II b), 9 (G II a) and 11 (G II c) respectively. Only B_1 (238 ppb) was produced by A. niger isolate 9 (G II b) (Table 15 and Fig.7).

Isolates of A. flavus produced B_1 (285 to 948 ppb), B_2 (278 to 577 ppb) and G_2 (76 to 110 ppb) in sesamum oil cake.

Table 13. Production of aflatoxin by isolates of *Aspergillus niger* in culture medium.

Sl. No.	Isolate		Quantity of aflatoxin B_1 (ppb)
	Number	Code	
1	1	Co III a	210
2	2	Co I b	170
3	4	Co III b	101
4	6	Co II c	122
5	9	G II b	110
6	12	S II a	222
7	13	S III a	93
8	17	S I c	112

Co - Coconut oil cake
G - Groundnut oil cake
S - Sesamum oil cake

I - Southern region
II - Central region
III - Northern region

a - February - March
b - June - July
c - October - November

FIG. 5. PRODUCTION OF AFLATOXIN B_1 BY ISOLATES OF *Aspergillus niger* IN CULTURE MEDIUM.

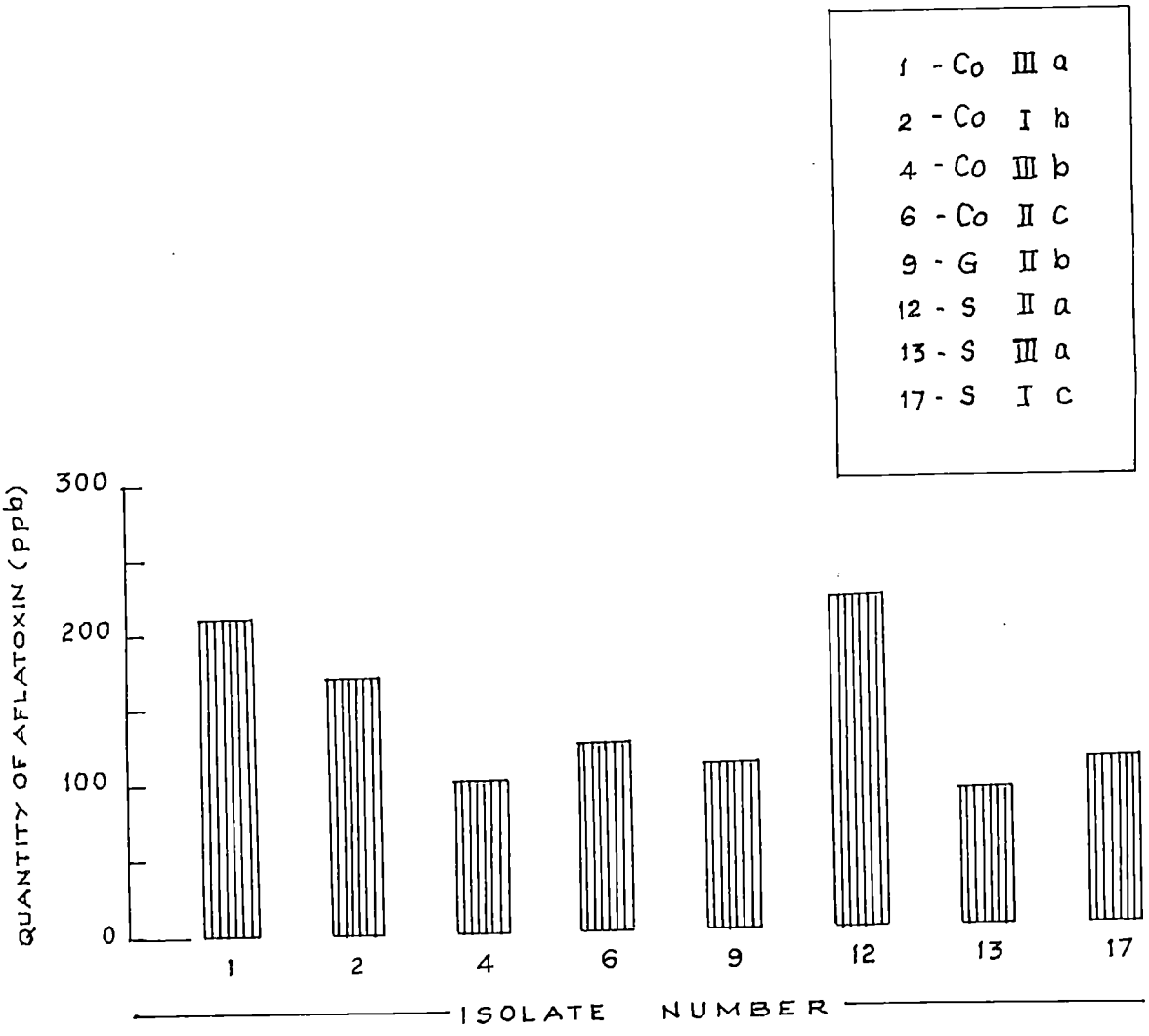


FIG. 6. PRODUCTION OF AFLATOXINS BY SPECIES OF *Aspergillus* IN COCONUT OIL CAKE.

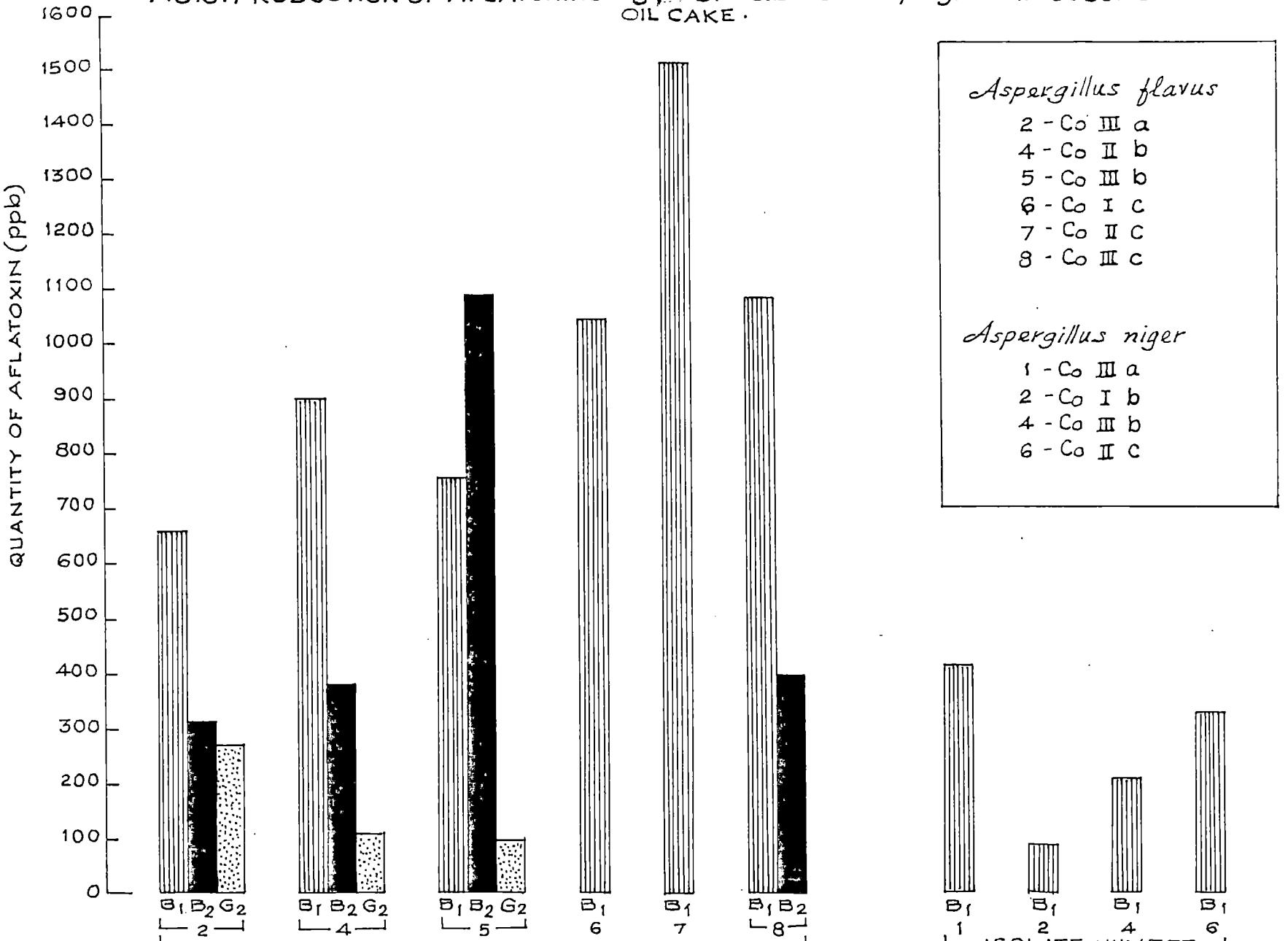
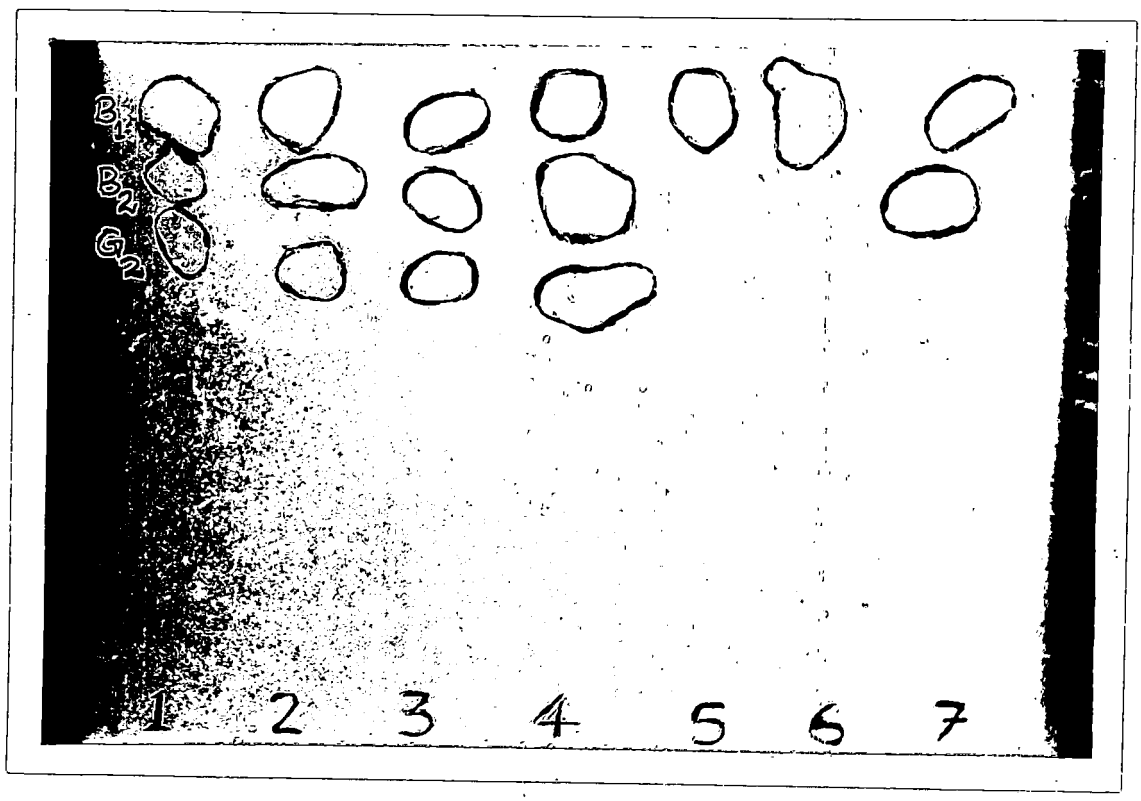


Plate XIV. *Thin layer chromatogram showing aflatoxins produced by different isolates of* Aspergillus flavus *in coconut oil cake.*



1. Standard aflatoxins
2. Isolate 2 (CoIIIa)
3. " 4 (CoIIb)
4. " 5 (CoIIIb)
5. " 6 (CoIc)
6. " 7 (CoIIc)
7. " 8 (CoIIIc)

Table 15. Production of aflatoxins by species of *Aspergillus* in groundnut oil cake

Isolate		Quantity of aflatoxin (ppb)		
Number	Code	B_1	B_2	G_2
Aspergillus flavus				
9	G II a	255	1011	115
10	G II b	1210	310	96
11	G II c	1011	510	211
Aspergillus niger				
9	G II b	238	-	-

G - Groundnut oil cake

I - Southern region
II - Central region
III - Northern region

a - February - March
b - June - July
c - October - November

FIG.7. PRODUCTION OF AFLATOXINS BY SPECIES OF *Aspergillus* IN GROUND NUT OIL CAKE.

Aspergillus flavus	*Aspergillus niger*
9 - G II a	9 - G II b
10 - G II b	
11 - G II c	

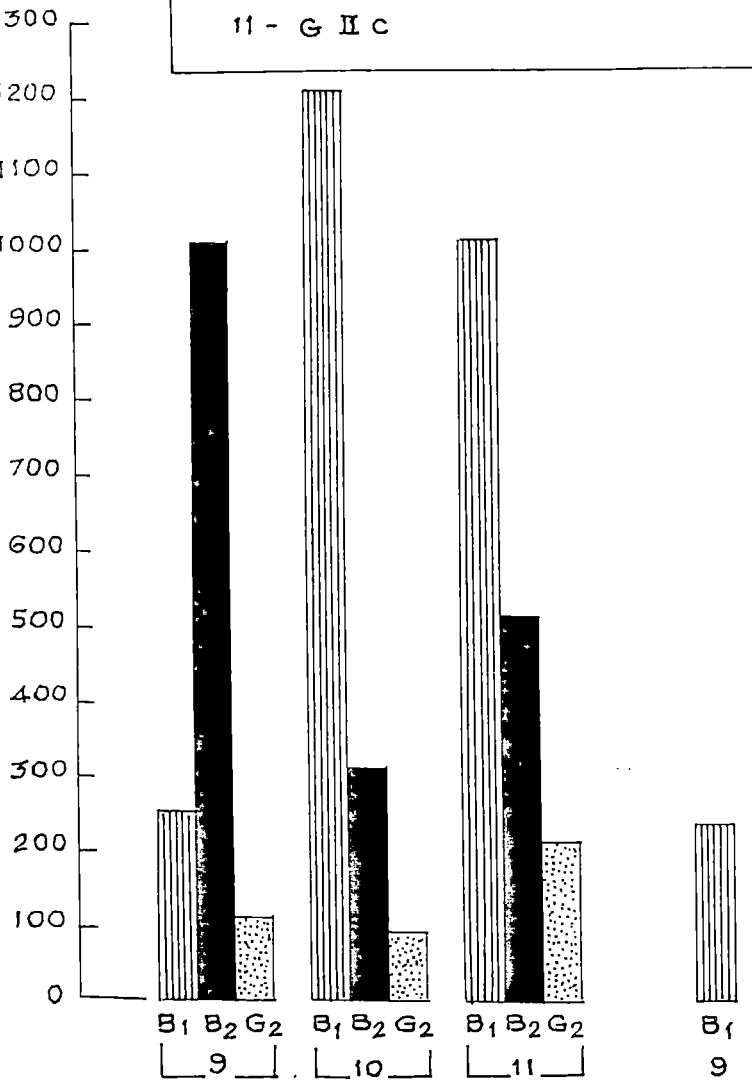


FIG.8. PRODUCTION OF AFLATOXINS BY SPECIES OF *Aspergillus* IN SESAMUM OIL CAKE.

Aspergillus flavus	*Aspergillus niger*
14 - S III a	12 - S II a
15 - S I b	13 - S III a
16 - S II b	17 - S I c
17 - S III b	
20 - S III c	

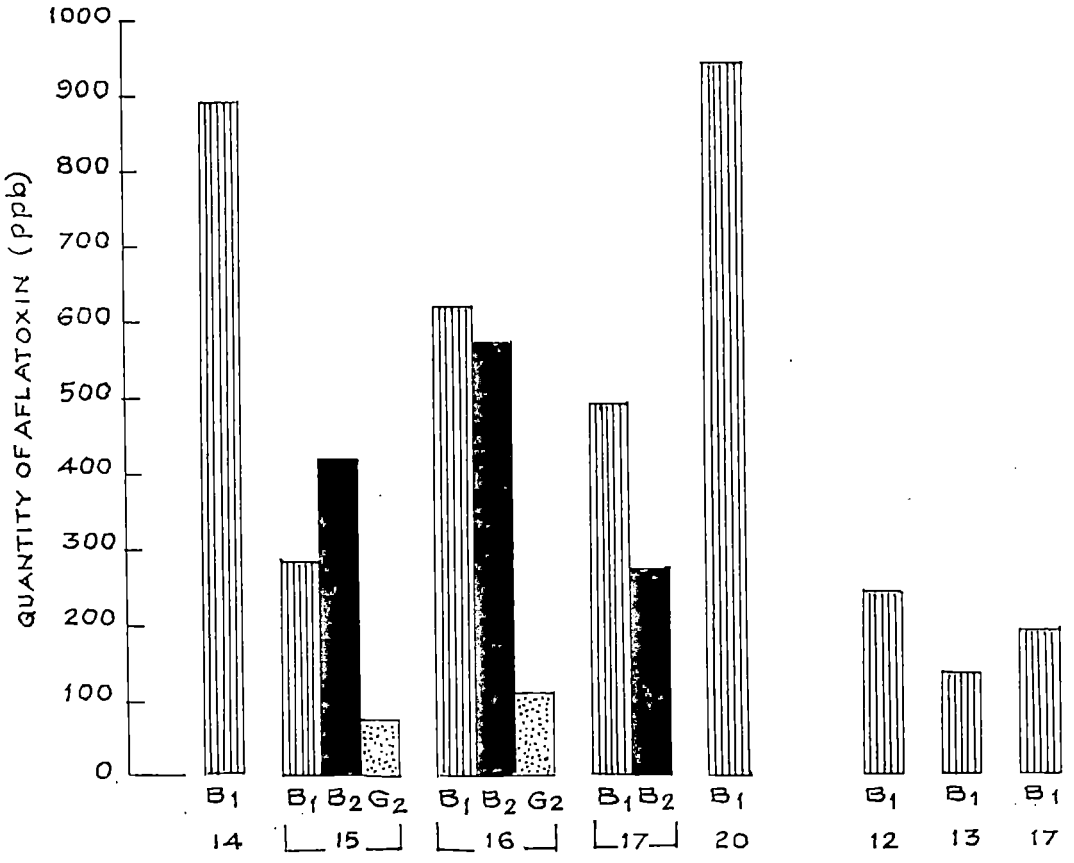


Table 16. Production of aflatoxins by species of Aspergillus in sesamum oil cake.

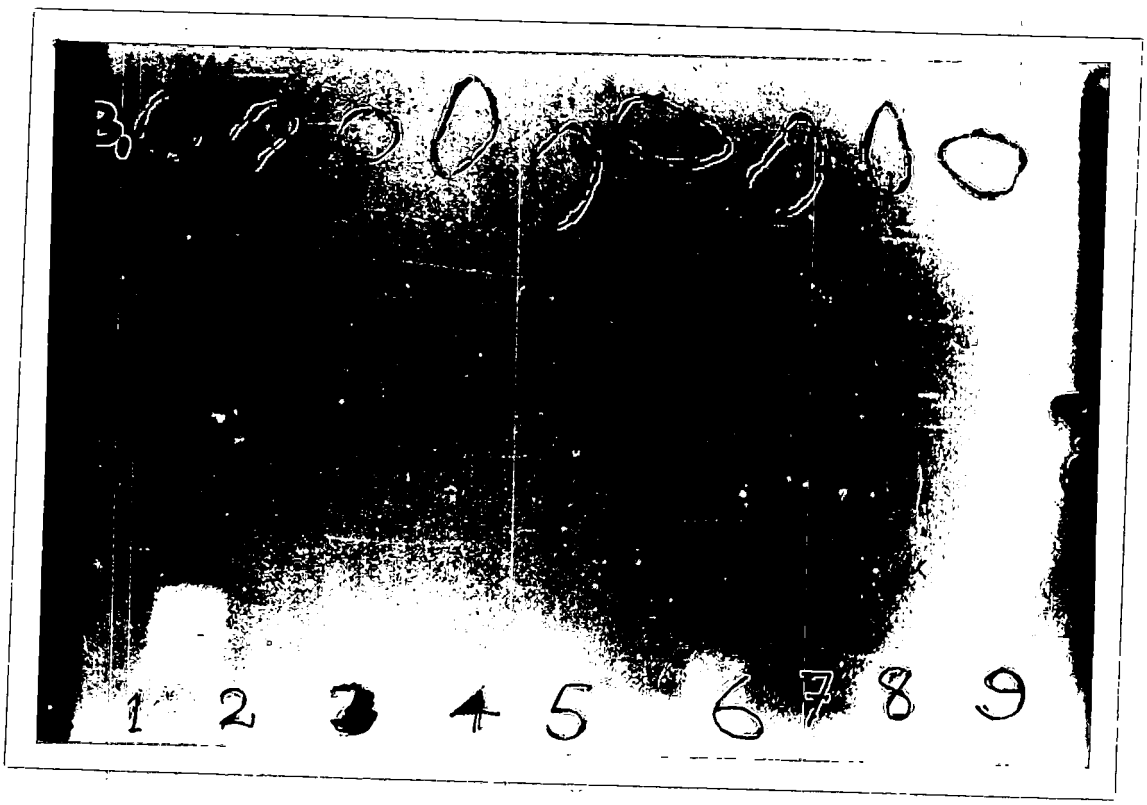
Isolate		Quantity of aflatoxin (ppb)		
Number	Code	B_1	B_2	G_2
Aspergillus flavus				
14	S III a	896	-	-
15	S I b	285	422	76
16	S II b	622	577	110
17	S III b	495	278	-
20	S III c	948	-	-
Aspergillus niger				
12	S II a	246	-	-
13	S III a	138	-	-
17	S I c	198	-	-

S - Sesamum oil cake

I - Southern region
II - Central region
III - Northern region

a - February - March
b - June - July
c - October - November

Plate XV. *Thin layer chromatogram showing aflatoxin B_1 produced by different isolates of* Aspergillus niger *in oil cakes.*



1. Standard aflatoxin B_1
2. Isolate 1 (CoIIIa)
3. " 2 (CoIb)
4. " 4 (CoIIIb)
5. " 6 (CoIIc)
6. " 9 (GIIb)
7. " 12 (SIIa)
8. " 13 (SIIIa)
9. " 17 (SIc)

The maximum quantity of the three aflatoxins were produced by isolates 20 (S III c), 16 (S II b) and 16 (S II b) respectively. Isolates of A. niger produced 138 to 246 ppb of B_1 alone (Table 16 and Fig.8).

5.1. Effect of chemicals on the deterioration of oil cakes by fungi

Oil cakes treated with various chemicals and stored in gunny bags at room temperature (28±2 °C) did not show any fungus growth upto 60 days, whereas in the untreated cakes mycelial growth was visible during the first month itself. Those treated with calcium propionate (0.6 per cent) were free from fungus growth throughout the period (180 days) of observation (Table 17, Plate, XVI).

Observations on the population of fungal propagules in the oil cakes treated with chemicals revealed that viable propagules were present in the samples throughout the period of observation (Table 18). Wide variation was noticed in the population of fungi present in samples treated with different chemicals. Oil cakes treated with calcium propionate had the minimum number of fungal propagules. Samples kept as control showed higher population of fungi than that in the treatments at all periods of observation.

Table 17. Effect of chemicals on the growth of fungi on oil cakes.

Sl. No.	Treatment	Concentration (%)	Oil cake	Fungal growth after (days) 30	60	90	120	150	180
1	Boric acid	1.5	C	-	-	+	+	+	+
			G	-	-	-	+	+	+
			S	-	-	-	+	+	+
		2.0	C	-	-	+	+	+	+
			G	-	-	-	-	+	+
			S	-	-	-	-	+	+
2	Calcium propionate	0.3	C	-	-	-	+	+	+
			G	-	-	-	-	+	+
			S	-	-	-	+	+	+
		0.6	C	-	-	-	-	-	-
			G	-	-	-	-	-	-
			S	-	-	-	-	-	-
3	Sodium chloride	3.0	C	-	-	+	+	+	+
			G	-	-	-	+	+	+
			S	-	-	+	+	+	+
		4.5	C	-	-	-	+	+	+
			G	-	-	-	-	+	+
			S	-	-	-	+	+	+
4	Sodium carbonate	2.0	C	-	-	+	+	+	+
			G	-	-	-	+	+	+
			S	-	-	+	+	+	+
		3.0	C	-	-	-	+	+	+
			G	-	-	-	-	-	+
			S	-	-	-	-	+	+
5	Sodium bicarbonate	2.0	C	-	-	+	+	+	+
			G	-	-	-	+	+	+
			S	-	-	-	+	+	+
		4.0	C	-	-	-	-	+	+
			G	-	-	-	-	+	+
			S	-	-	-	-	+	+
	Control		C	+	+	+	+	+	+
			G	+	+	+	+	+	+
			S	+	+	+	+	+	+

C - Coconut
G - Groundnut
S - Sesamum

- Fungus growth absent
+ Fungus growth present

Table 18. Effect of chemicals on the population of fungi in oil cakes.

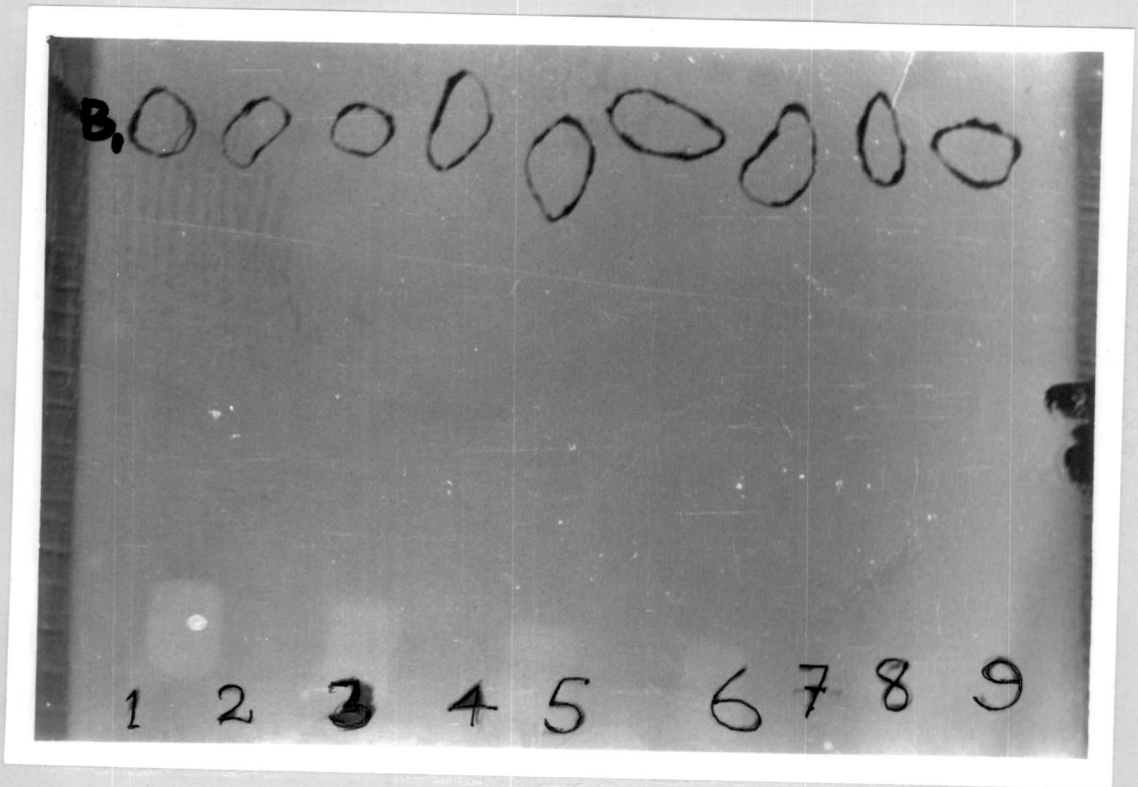
Treatment	Concentration (%)	Fungal population (10^4/g) after (days)												Mean
		0			60			120			180			
		C	G	S	C	G	S	C	G	S	C	G	S	
Boric acid	1.5	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	3.86 (2.20)	3.67 (2.16)	4.19 (2.28)	18.61 (4.43)	17.97 (4.36)	19.98 (4.58)	36.66 (6.14)	31.01 (5.66)	21.21 (4.71)	13.40 (3.79)
	2.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	4.61 (2.37)	3.24 (2.06)	2.79 (1.95)	18.99 (4.47)	9.77 (3.28)	12.10 (3.62)	34.36 (5.95)	29.07 (5.48)	24.81 (5.08)	11 95 (3.60)
Calcium propionate	0.3	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	2.84 (1.96)	2.90 (1.97)	1.23 (1.49)	10.22 (3.35)	3.49 (2.12)	13.21 (3.77)	22.95 (4.89)	17.11 (4.26)	20.35 (4.62)	8.17 (3.02)
	0.6	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	1.00 (1.41)	2.74 (1.93)	1.40 (1.55)	10.41 (3.38)	2 07 (1.75)	6.07 (2.66)	13.32 (3.78)	12.24 (3.64)	16.52 (4.19)	5.79 (2.61)
Sodium chloride	3.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	8.20 (3.03)	5.20 (2.49)	3.77 (2.18)	22.08 (4.70)	22.95 (4.89)	19.68 (4.55)	33.66 (5.89)	29.76 (5.55)	24.03 (5.00)	14.34 (3.92)
	4.5	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	9.72 (3.27)	6.89 (2.81)	4.12 (2.26)	20.93 (4.68)	10.10 (3.33)	11.68 (3.56)	32.38 (5.78)	23.63 (4.96)	24.04 (5.00)	12.27 (3.64)
Sodium carbonate	2.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	6.03 (2.65)	4.91 (2.42)	4.19 (2.28)	23.98 (4.99)	25.88 (5.18)	11.68 (3.56)	39.11 (6.33)	27.74 (5.36)	23.22 (4.92)	14.20 (3.90)
	3.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	7.97 (3.00)	3.23 (2.06)	4.33 (2.31)	16.65 (4.20)	13.86 (3.86)	12.17 (3.63)	25.17 (5.12)	20.22 (4.61)	22.92 (4.89)	10.85 (3.44)
Sodium bicarbonate	2.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	5.62 (2.57)	5.80 (2.61)	4.40 (2.32)	21.14 (4.70)	15 71 (4.09)	12.79 (3.71)	32.84 (5.82)	23.45 (4.94)	25.41 (5.14)	12.57 (3.68)
	4.0	0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	8.49 (3.08)	5.10 (2.47)	2.49 (1.87)	15.93 (4.11)	10.49 (3.39)	8.32 (3.05)	28.08 (5.39)	12.23 (3.64)	20.48 (4.64)	9.61 (3.25)
Control		0.75 (1.32)	1.75 (1.65)	1.25 (1.50)	16.75 (4.21)	16.20 (4.15)	16.69 (4.21)	31.91 (5.74)	26.64 (5.26)	33.97 (5.91)	42.96 (6 63)	38.94 (6.32)	36.68 (6.14)	22.04 (4.80)

Figures in parentheses are transformed values.

C - Coconut
G - Groundnut
S - Sesamum

CD at (0.05) level.
Between oil cakes - 0.195
Between chemicals - 0.218

Plate XV. *Thin layer chromatogram showing aflatoxin B_1 produced by different isolates of* Aspergillus niger *in oil cakes.*



1. Standard aflatoxin B_1
2. Isolate 1 (CoIIIa)
3. " 2 (CoIb)
4. " 4 (CoIIIb)
5. " 6 (CoIIc)
6. " 9 (GIIb)
7. " 12 (SIIa)
8. " 13 (SIIIa)
9. " 17 (SIc)

Plate XVI. Efficacy of calcium propionate for the control of deterioration of oil cake by fungi.

a) Coconut oil cake

1. Calcium propionate 0.6%
2. Control

b) Groundnut oil cake

1. Calcium propionate 0.6%

Statistical analysis of the data revealed that the effect of chemicals, periods of observation and the interaction between oil cakes, chemicals and periods of observation were significant (Appendix, III). Calcium propionate (0.6 per cent) was significantly superior to all the other chemicals tested.

5.2. Effect of storage containers on the deterioration of oil cakes by fungi

All the oil cakes stored in polythene lined gunny bags from May to November were free from visible growth of fungi upto 30 days (Table 19). Those stored from November to May did not show fungus growth upto 90 days (Table 20).

Population of fungal propagules in all the oil cakes was generally low when stored in polythene lined gunny bags. Comparatively higher population was noticed in samples stored in gunny bags (Table 21 and 22). Statistical analysis of the data revealed that polythene lined gunny bags were significantly superior to all the other containers tested for storage of oil cakes (Appendix, IV and V).

Table 19. Effect of storage containers on the growth of fungi on oil cakes. Stored from May to November 1987.

Treatment	Oil cake	Fungal growth after (days)						
		0	30	60	90	120	150	180
Gunny bag	C	-	+	+	+	+	+	+
	G	-	+	+	+	+	+	+
	S	-	+	+	+	+	+	+
Polythene bag	C	-	-	+	+	+	+	+
	G	-	+	+	+	+	+	+
	S	-	-	+	+	+	+	+
Polythene lined gunny bag	C	-	-	+	+	+	+	+
	G	-	-	+	+	+	+	+
	S	-	-	+	+	+	+	+
High Density Poly Ethylene (HDPE) woven bag	C	-	+	+	+	+	+	+
	G	-	+	+	+	+	+	+
	S	-	+	+	+	+	+	+

C - Coconut G - Groundnut S - Sesamum

\- Fungus growth absent

\+ Fungus growth present

Table 20. Effect of storage containers on the growth of fungi on oil cakes. Stored from November 1987 to May 1988.

Treatment	Oil cake	Fungal growth after (days)						
		0	30	60	90	120	150	180
Gunny bag	C	-	-	-	+	+	+	+
	G	-	-	-	+	+	+	+
	S	-	-	-	+	+	+	+
Polythene bag	C	-	-	-	-	+	+	+
	G	-	-	-	-	+	+	+
	S	-	-	-	-	-	+	+
Polythene lined gunny bag	C	-	-	-	-	+	+	+
	G	-	-	-	-	-	+	+
	S	-	-	-	-	-	+	+
High Density Poly Ethylene (HDPE) woven bag	C	-	-	-	-	+	+	+
	G	-	-	-	-	+	+	+
	S	-	-	-	+	+	+	+

C - Coconut G - Groundnut S - Sesamum

\- Fungus growth absent

\+ Fungus growth present

Table 22. Effect of containers on the population of fungi in oil cakes. Stored from November 1987 to May 1988.

Treatment	Oil cake	Fungal population (10^4/g) after (days)				
		0	60	120	180	Mean
Gunny bag	C	1.64 (1.63)	18.78 (4.45)	33.30 (5.86)	36.91 (6.16)	22.66 (4.86)
	G	1.40 (1.55)	9.60 (3.26)	23.92 (4.99)	47.28 (6.95)	20.55 (4.64)
	S	1.78 (1.67)	14.50 (3.94)	23.95 (5.00)	35.29 (6.02)	18.88 (4.46)
Polythene bag	C	1.64 (1.63)	17.58 (4.31)	19.21 (4.50)	24.32 (5.03)	15.69 (4.09)
	G	1.40 (1.55)	9.63 (3.26)	22.25 (4.82)	38.31 (6.27)	17.90 (4.35)
	S	1.78 (1.67)	5.81 (2.61)	12.97 (3.74)	25.96 (5.19)	11.19 (3.49)
Polythene lined gunny bag	C	1.64 (1.63)	9.98 (3.31)	19.31 (4.51)	21.65 (4.76)	13.15 (3.76)
	G	1.40 (1.55)	8.22 (3.04)	14.65 (3.96)	31.66 (5.71)	13.98 (3.87)
	S	1.78 (1.67)	5.92 (2.63)	9.43 (3.23)	26.99 (5.29)	11.03 (3.47)
High Density Poly Ethylene (HDPE) woven bag	C	1.64 (1.63)	12.60 (3.69)	25.63 (5.16)	29.32 (5.51)	17.30 (4.28)
	G	1.40 (1.55)	15.45 (4.06)	20.29 (4.61)	46.30 (6.88)	20.86 (4.68)
	S	1.78 (1.67)	15.40 (4.05)	20.65 (4.65)	30.65 (5.63)	17.12 (4.26)

(Figures in parentheses are transformed values)

C - Coconut G - Groundnut S - Sesamum

CD at (0.05) level

DISCUSSION

DISCUSSION

Oil cakes are subject to deterioration and spoilage by a large number of fungi during transit and storage . In the present investigation, fungi such as Aspergillus flavus, A. niger, A. terreus and Penicillium pinophilum were found commonly associated with coconut, groundnut and sesamum oil cakes.

In addition, Acremonium implicatum, Aspergillus aculeatus, A. caesiellus, A. fumigatus, A. versicolor, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, Pestalotiopsis palmarum, Rhizomucor pusillus and Rhizopus stolonifer were present in coconut oil cake, A. versicolor, Gliocladium sp., Rhizopus oryzae and Rhizopus stolonifer in groundnut oil cake and A. candidus, A. fumigatus, A. tamarii, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Pestalotiopsis palmarum and Rhizopus oryzae in sesamum oil cake.

Earlier workers reported A. flavus, A. niger, and A. versicolor from coconut oil cake (Philip, 1978 and Nusrath and Nahdi, 1983) and A. flavus, A. niger and A. terreus from

groundnut oil cake (Nusrath and Nahdi, 1983 and Reddy et al., (1986).

Among the fungi isolated from oil cakes during the present investigation, Acremonium implicatum, Aspergillus aculeatus, A. caesiellus, A. fumigatus, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, P. pinophilum, Pestalotiopsis palmarum and Rhizomucor pusillus isolated from coconut oil cake, A. versicolor, Gliocladium sp., Penicillium pinophilum, Rhizopus oryzae and Rhizopus stolonifer from groundnut oil cake and A. candidus, A. fumigatus, A. tamarii, A. terreus, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Penicillium pinophilum, Pestalotiopsis palmarum and Rhizopus oryzae from sesamum oil cake have not been reported earlier.

In regard to the occurrence of different fungi in the samples of oil cakes collected during different seasons from different regions, it was noticed that A. flavus and A. niger registered 100 per cent frequency in groundnut and sesamum oil cakes. In coconut oil cake, A. flavus and A. niger occurred at frequencies of 88.90 and 77.80 per cent respectively. Nusrath and Nahdi (1983) also reported high frequency of occurrence of the above fungi in coconut and

groundnut oil cakes. A. terreus and Penicillium pinophilum were also obtained at higher frequencies than other fungi from all the three oil cakes.

Highest population of fungi in oil cakes from all the three regions was obtained during June-July. This coincided with the receipt of high rainfall and consequent increase in the atmospheric humidity. Subramanian (1956) reported that the incidence of fungi in copra marketed from South India was more in samples collected during July, than those in other months. Philip (1978) observed that maximum microbial population in copra coincided with the monsoon seasons of the year. Niza (1981) noted high population of fungi in copra during June-July, followed by October-November.

The oil cakes collected from northern and central regions yielded higher population of fungi than those from the southern region during June-July and October-November. The high rainfall and humidity conditions prevailing in these regions during the monsoon periods can be attributed as plausible explanation for the above observation.

Positive and significant correlation could be obtained between weather parameters and population of fungi in different oil cakes. Maximum correlation was noticed in

relation to high rainfall conditions with consequent increase in relative humidity and decrease in daily average temperature. Bottomley et al. (1950) reported that the composition of microflora of corn varied with moisture, temperature and oxygen concentration in the atmosphere. Marar and Padmanabhan (1960) recorded maximum microbial deterioration of copra during June-July, when the relative humidity ranged between 92 and 95 per cent. Philip (1978) noticed positive correlation between atmospheric humidity and microbial population of copra.

Among the oil cakes, coconut cake registered higher population of fungi than groundnut and sesamum cakes. The high carbohydrate content of coconut oil cake is believed to be the major factor which supported good growth of fungi, as reported by Goldblatt (1969).

In the experiment conducted to study the effect of storage conditions on the growth of fungi on oil cakes, it was noticed that maximum mycelial growth occurred on oil cakes incubated at 27, 29 and 32°C for 45-60 days under saturated humidity conditions. When different percentages of relative humidity were tested at room temperature, it was noticed that good mycelial growth was produced at and above 92.9 per cent. The above observations clearly indicated that

relative humidity could be considered as an important factor governing the growth of fungi on oil cakes.

Ward (1937) reported rapid deterioration of copra by fungi over 90 per cent relative humidity at 28-30°C. Coconut oil cake stored at 76 to 79 per cent relative humidity did not show mould attack (Rajasekharan et al., 1960 and Nair, 1968). Maximum infestation with Aspergillus flavus was noticed in copra kept under 100 per cent relative humidity and at 30°C (Niza, 1981). Sesamum seeds stored at 90 per cent relative humidity and 20°C, showed very high percentage of infestation by fungi (Nandi et al., 1982). Aibara et al. (1985) reported that Aspergillus parasiticus in shelled peanuts could not grow during storage at 25°C if the RH was less than 79 per cent.

Fungi inoculated individually and in combination caused considerable reduction in the oil content of the oil cakes. Maximum reduction was noticed due to the growth of Pestalotiopsis palmarum in coconut oil cake, Rhizopus stolonifer in groundnut oil cake and Fasarium pallidoroseum in sesamum oil cake. In the case of combinations, A. flavus, A. niger and Penicillum pinophilum together caused maximum reduction of oil in coconut oil cake. In groundnut oil cake, combined growth of A. flavus, A. niger and A. terreus caused

maximum reduction in oil whereas, A. niger and P. pinophilum effected maximum reduction of oil in sesamum oil cake. However, the differences in the per cent reduction in oil content caused by the growth of fungi individually and in combination were not pronounced.

Eventhough P. palmarum and F. pallidoroseum caused maximum reduction in oil in coconut and sesamum oil cake respectively, these were not included for testing their combined effect on oil content, since fungi which were constantly noted in all the three oil cakes were only included in the experiment.

Fungi like Aspergillus niger, Rhizopus sp., Aspergillus sp. and Penicillium sp. (Nair, 1968), A. flavus and A. niger (Paul et al., 1980 and Niza, 1981) have been reported to cause considerable reduction in the oil content of copra. While studying the utilization of oil as a source of carbon for growth of fungi invading copra, Paul (1969) noted maximum mycelial growth of Penicillium sp. followed by A. niger in Czapek's solution incorporated with coconut oil as carbon source.

Invasion by Aspergillus tamarii (Ward and Diener, 1961) A. flavus (Lalithakumari et al., 1971,a; Basha and Pancholy, 1986)

caused considerable reduction in the oil content of groundnut seeds. A. flavus, A. ustus and Penicillium sp. have been found to utilize groundnut oil as a source of carbon in Czapek's solution (Paul, 1969).

Considerable reduction in the oil content of sesamum seeds have been reported due to the growth of A. flavus and A. tamarii (Sharma, 1981) and A. flavus, A. niger and Fusarium moniliforme (Saxena,1988). Maximum mycelial growth of A. flavus, followed by A. ustus, Penicillium sp. and A. niger was reported in sesamum oil incorporated Czapek's solution (Paul, 1969).

Oil cakes inoculated with different fungi showed considerable reduction in most of the nutrients. Total carbohydrates, crude protein, free amino nitrogen, crude fibre and ash were reduced to the extent of 6.11 to 76.95, 4.28 to 68.03, 14.91 to 92.52, 1.25 to 92.55 and 0.17 to 65.16 per cent ~~reduction~~ respectively. In the case of mineral nutrients like phosphorus, potassium, magnesium, calcium, copper and iron, reduction ranging from 15.07 to 75.54, 23.13 to 94.41, 10.89 to 63.37, 28.78 to 90.20, 52.52 to 97.12 and 0.32. to 60.77 per cent, respectively was noticed. Similar observations have been reported by Mallick and Nandi (1979) in rice grains, Sinha and Chauhan (1981) in

gram seeds, Bilgrami (1985) in copra, Prasad and Pathak (1986) in cereals and Airede and Esuruoso (1987) in oil palm kernels for total carbohydrates; Nagel and Semenuik (1947) in shelled corn, Ward and Diener (1961), Cherry et al. (1975), Cherry and Beuchat (1976), Basha and Pancholy (1986) in peanuts, Bilgrami et al. (1980) in maize seeds, Sinha and Chauhan (1981) in gram seeds, Vijayakumari and Karan (1981) in cowpea seeds, Prasad and Pathak (1986) in cereals and Ogundero (1987) in feed samples in regard to crude protein, free amino nitrogen and crude fibre and Hegde and Munjal (1971) in bean pods and Sinha and Chauhan (1981) in gram seeds for various mineral constituents.

Eventhough the effect of fungal growth on the oil cakes was a general reduction in the various chemical constituents, increase in crude fibre, ash and iron was noticed in certain treatments. No defenite reason could be attributed to this. Hegde and Munjal (1971) observed increase in the iron content of bean pods infected by Colletotrichum gloeosporioides whereas a general reduction was noticed in the other mineral components studied.

Of the 20 isolates of Aspergillus flavus tested, 14 produced one or more of the aflatoxins B_1, B_2 and G_2 in SMKY liquid medium. Production of aflatoxin in culture medium

by isolates of A. flavus has been reported by Diener and Davis (1969), Thomson and Mehdy (1978), Sinha (1980) and Bilgrami (1985). Austwick and Ayerst (1963), Codner et al. (1963), Diener and Davis (1969), Detroy et al. (1971) and Sinha et al. (1988) noted that all strains of A. flavus did not possess the capacity to elaborate aflatoxins.

Eight out of the 19 isolates of Aspergillus niger produced aflatoxin B_1 in the medium. Production of this aflatoxin by A. niger has been reported by Kulik and Holaday (1967) and Bilgrami (1985).

Among the three aflatoxins produced by isolates of A.flavus, isolate 7 (Co II c), isolate 5 (Co III b) and isolate 2 (Co III a) from coconut oil cake produced maximum quantity of B_1, B_2 and G_2 respectively. In the case of A. niger, isolate 12 (S II a) from sesamum oil cake produced maximum quantity of B_1.

When the isolates of A. flavus and A. niger were tested for their ability to produce aflatoxins in their respective host material, it was noticed that maximum quantity of B_1 was produced by isolate 7 (Co II c) from coconut, isolate 10 (G II b) from groundnut and isolate 20 (S III c) from sesamum oil cake, maximum quantity of B_2

was produced by isolate 5 (Co III b) from coconut, isolate 9 (G II a) from groundnut and isolate 16 (S II b) from sesamum oil cake and maximum quantity of G_2 was produced by isolate 2 (Co III a) from coconut, isolate 11 (G II c) from groundnut and isolate 16 (S II b) from sesamum oil cake respectively.

Production of aflatoxin B_1 by A. flavus has been reported by Gopal et al. (1968), Reddy et al. (1984) and Balasubramanian (1985) in groundnut oil cake and Niza (1981) in coconut, groundnut and sesamum oil cakes. Neelakantan et al. (1981) noted B_1 and B_2 in groundnut oil cake. Bilgrami (1985) detected aflatoxins B_1, B_2 and G_2 in copra samples. Production of aflatoxin G_2 in oil cakes has not been reported so far.

The present investigation further revealed that the isolates which produced maximum quantity of aflatoxin in culture medium could also produce similarly in their host material, thereby indicating their inherent ability for aflatoxin production. It was also noticed that the highest amounts of all the three aflatoxins were produced in coconut oil cake. Labadan (1969) noticed that coconut oil cake formed a good substrate for the production of aflatoxin. Goldblatt (1969) stated that high carbohydrate substrate supported luxuriant growth of A. flavus with consequent produ-

ction of large quantities of aflatoxin. In the present investigation coconut oil cake showed higher carbohydrate content than groundnut and sesamum oil cakes (Table 8). The fact that coconut oil cake favoured the production of aflatoxin by A. flavus, it is essential to avoid feeding cattle and poultry with oil cakes contaminated by the fungus.

Out of the five chemicals tested for their efficacy to check deterioration and spoilage of oil cakes during storage, calcium propionate (0.6% w/w) was found to be the most effective. Treatment with this chemical could keep the oil cakes without fungal deterioration throughout the period (180 days) of observation. Other chemicals tested were able to keep the cakes free from fungal growth only upto 60 days of storage. Viable propagules of fungi could, however, be detected in the oil cakes treated with the chemicals. Those treated with calcium propionate had minimum number of fungal propagules while those kept as control had maximum. The fact that there was no fungal growth upto 180 days in calcium propionate treated oil cakes and upto 60 days in those treated with the other chemicals indicated that these chemicals were able to suppress the growth and spread of contaminating fungi to varying periods of time; calcium propionate being the most effective in this regard. Paster (1979) and Vanselow et al. (1985) reported suppression

of fungal growth due to the addition of calcium propionate in poultry feed materials.

In the experiment to study the effect of storage containers in checking/reducing the deterioration of oil cakes by fungi, it was noticed that polythene lined gunny bags were superior to the other containers tested. The oil cakes stored in polythene lined gunny bag had the least population of fungi. Those stored in ordinary gunny bag which is most commonly used for the storage and transport had very high population of fungi.

Marar and Padmanabhan (1960) noted that copra stored in alkathene lined gunny bags remained in good condition for six months. Philip (1978) and Niza (1981) reported that polythene lined gunny bag was superior to ordinary gunny bag for the storage of copra as there was less contamination by fungi in those stored in polythene lined gunny bags. Absorption of moisture and development of high humidity during rainy and humid weather conditions could be attributed to the presence of higher population of fungi in oil cakes stored in gunny bags. It is therefore imperative that polythene lined gunny bags should be used for storage and transport of oil cakes in order to minimise deterioration by fungi and to avoid health hazards to cattle, poultry etc

which are likely to be caused by the production of aflatoxin by contaminating species of Aspergillus.

The foregoing considerations clearly indicate that deterioration and spoilage of oil cakes by fungi can be prevented or reduced to the minimum by treatment with 0.6 per cent calcium propionate (w/w) and by using polythene lined gunny bag for storage and transport.

SUMMARY

SUMMARY

Fungi causing deterioration of coconut, groundnut and sesamum oil cakes were studied. Aspergillus flavus, A. niger, A. terreus and Penicillium pinophilum were isolated from all the three oil cakes. In addition to the above, Acremonium implicatum, Aspergillus aculeatus, A. caesiellus, A. fumigatus, A. versicolor, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, Pestalotiopsis palmarum, Rhizomucor pusillus and Rhizopus stolonifer from coconut oil cake, A. versicolor, Gliocladium sp., Rhizopus oryzae and Rhizopus stolonifer from groundnut oil cake and A. candidus, A. fumigatus, A. tamarii, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Pestalotiopsis palmarum and Rhizopus oryzae from sesamum oil cake were also isolated.

Acremonium implicatum, Aspergillus aculeatus, A. caesiellus, A. fumigatus, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, P. pinophilum, Pestalotiopsis palmarum and Rhizomucor pusillus isolated from coconut oil cake, A. versicolor, Gliocladium sp., Penicillium pinophilum,

Rhizopus oryzae and Rhizopus stolonifer from groundnut oil cake and A. candidus A. fumigatus, A. tamarii, A. terreus, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Penicillium pinophilum, Pestalotiopsis palmarum and Rhizopus oryzae from sesamum oil cake have not been reported earlier.

Aspergillus flavus and A. niger were obtained from all the samples of groundnut and sesamum oil cakes. In coconut oil cake, these two fungi were present in 88.89 and 77.78 per cent of the samples. A. terreus was isolated from 66.67 per cent of groundnut and 55.56 per cent of coconut and sesamum oil cake samples. A. candidus was obtained in 50 per cent samples of sesamum oil cake only. Penicillium pinophilum was present in 66.67 per cent of groundnut, 44.44 per cent of sesamum and 27.78 per cent of coconut oil cakes. Rhizopus stolonifer was isolated from 50 per cent of coconut and groundnut oil cakes, while R. oryzae was present in 50 per cent of sesamum and 11.11 per cent of groundnut oil cake samples.

Wide variation was noted in the population of fungal propagules present in the oil cakes collected from different regions during different periods of the year. Oil cakes collected during June-July, had the highest population of

fungi. Samples collected from central and northern regions showed higher population of fungi than those from the southern region.

Positive and significant correlation could be obtained between weather parameters and population of fungi in different oil cakes. Maximum correlation was noticed in relation to total rainfall.

Good mycelial growth of fungi was obtained in all the oil cakes incubated at 27, 29 and 32°C. Relative humidity had a profound effect on fungal growth on oil cakes. Maximum fungal growth was noticed at 100 per cent RH, in all the oil cakes. This was followed by 96.1 and 92.9 per cent in the descending order.

The oil content of oil cakes was considerably reduced due to the growth of all the fungi tested individually and in combination. Maximum reduction was noticed due to the growth of Pestalotiopsis palmarum in coconut oil cake, Rhizopus stolonifer in groundnut and Fusarium pallidoroseum in sesamum oil cake. In the case of combinations, Aspergilus flavus, A. niger and Penicillium pinophilum together caused maximum reduction of oil in coconut oil cake. In groundnut oil cake, combined growth of A.

flavus, A. niger and A. terreus caused maximum reduction in oil whereas, A. niger + P. pinophilum effected maximum reduction of oil in sesamum oil cake.

Oil cakes inoculated with different fungi showed considerable reduction in most of the nutrients. Total carbohydrates, crude protein, free amino nitrogen, crude fibre and ash were reduced to the extent of 6.11 to 76.95, 4.28 to 68.03, 14.91 to 92.52, 1.25 to 92.55 and o.17 to 65.16 per cent respectively. In the case of mineral nutrients like phosphorus, potassium, magnesium, calcium, copper and iron, reduction ranging from 15.07 to 75.54, 23.13 to 94.41, 10.89 to 63.37, 28.78 to 90.20, 52.52 to 97.12 and 0.32 to 60.77 per cent respectively was noticed.

Fourteen out of the twenty isolates Aspergillus flavus produced aflatoxins, B_1, B_2 and G_2 in culture medium. Quantities upto 1210,1040 and 151 ppb of B_1, B_2 and G_2 were produced by A. flavus isolates 7 (Co II c), 5 (Co III b) and 2 (Co III a) respectively from coconut oil cake. Eight out of nineteen isolates of A. niger elaborated aflatoxin B_1, with the maximum quantity of 222 ppb produced by isolate 12 (S II a) from sesamum oil cake.

When tested for the ability to produce aflatoxin in host material, the maximum quantity of B_1 produced by A.

flavus in coconut oil cake was 1517 ppb by isolate 7 (Co II c); in groundnut oil cake, 1210 ppb by isolate 10 (G II b) and in sesamum oil cake, 948 ppb by isolate 20 (S III c). Quantities of B_2 upto 1092, 1011 and 577 ppb were produced by the isolates of A. flavus, 5 (Co III b), 9 (G II a) and 16 (S II b) in coconut, groundnut and sesamum oil cake respectively. A. flavus isolates, 2 (Co III a), 11 (G II c) and 16 (S I b) in coconut, groundnut and sesamum oil cakes produced G_2 upto 272, 211 and 110 ppb respectively.

Isolates of A.niger, 2 (Co III a), 9 (G II b) and 12 (S II a) produced B_1 to the extent of 419, 246 and 238 ppb respectively in coconut, groundnut and sesamum oil cake.

The isolates which produced maximum quantities of the aflatoxins in culture medium could produce highest quantities of the same in their respective host material.

Oil cakes treated with calcium propionate (0.6 per cent, w/w) were free from fungus growth throughout the period (180 days) of observation and showed minimum number of fungal propagules whereas, those kept as control had higher population of fungi than the treated ones, at all periods of observation.

Oil cakes stored in polythene lined gunny bag had the least population of fungi, whereas those stored in ordinary gunny bag had very high population of fungi.

These results revealed that fungal deterioration and spoilage of oil cakes could be prevented or reduced to the minimum by treatment with 0.6 per cent calcium propionate and by using polythene lined gunny bags for storage and transport.

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APPENDICES

APPENDIX I

Interaction between oil cakes, regions, periods and isolations on the population of fungi.

Oil cake		Region			Period			Isolation		Mean
		Southern	Central	Northern	Feb-Mar	Jun-Jul	Oct-Nov	I	II	
Coconut		71.93	100.13	92.83	43.39	131.64	89.86	30.55	146.05	88.30
Sesamum		70.02	87.05	87.29	43.12	123.83	77.38	26.30	136.60	81.45
Isolations	I	17.30	31.36	36.63	13.59	44.20	27.50	--	--	28.43
	II	124.66	155.82	143.49	72.93	211.28	139.76	--	--	141.32
Mean		70.98	93.59	90.06	43.26	127.74	83.62	28.43	141.32	

CD at (0.05) level

Oil cakes, Isolations	- 6.612
Regions, Periods	- 8.098
Oil cake X Region, Oil cake X Period Region X Isolation Period X Isolation	-11.453
Region X Period	-14.027
Oil cake X Isolation	- 9.351

APPENDIX II

Interaction between oil cakes regions and isolations on the population of fungi.

Oil cake		Period			Isolation		Mean
		Feb-Mar	Jun-Jul	Oct-Nov	I	II	
Coconut		45.00	134.53	120.87	40.09	160.18	100.14
Groundnut		38.34	131.80	91.00	22.62	151.47	87.05
Sesamum		47.70	169.13	79.65	27.45	170.20	98.83
Isolation	I	10.83	48.20	30.98	--	--	30.05
	II	76.52	242.10	163.22	--	--	160.62
Mean		43.68	145.15	96.88	30.05	160.62	

CD at (0.05) level

Oil cakes, Periods - 18.550
Isolation - 15.150
Oil cake X Period - 32.130
Oil cake X Isolation,
Period X Isolation - 26.230

APPENDIX III

Interaction between oil cakes, chemicals and period of observation on the population of fungi.

Oil cake	Boric acid (1.5%)	Boric acid (2.0%)	Calcium propionate (0.3%)	Calcium propionate (0.6%)	Sodium chloride (3.0%)	Sodium chloride (4.5%)	Sodium carbonate (2.0%)	Sodium carbonate (3.0%)	Sodium bicarbonate (2.0%)	Sodium bicarbonate (4.0%)	Control	Mean
Coconut	4.08	3.87	3.21	2.79	4.24	4.07	4.19	3.74	3.98	3.86	5.17	3.93
Groundnut	3.70	3.54	2.82	2.46	3.74	3.23	3.62	3.37	3.52	3.06	5.12	3.47
Sesamum	3.87	3.45	2.71	2.37	4.04	3.61	3.98	3.12	3.64	2.95	5.13	3.53
Mean	3.88	3.62	2.91	2.54	4.01	3.64	3.93	3.41	3.71	3.29	5.14	

Oil cake	Period of observation (days)				Mean
	0	60	120	180	
Coconut	1.93	3.77	4.43	5.61	3.93
Groundnut	2.03	3.22	3.88	4.94	3.47
Sesamum	1.96	3.40	3.77	4.95	3.53
Mean	1.97	3.46	4.02	5.17	

CD at (0.05) level

Oil cakes - 0.195
Chemicals - 0.218
Oil cake X Chemical - 0.327
Oil cake X Period of observation - 0.238

APPENDIX IV

Interaction between oil cakes, containers and period of observation on the population of fungi.

May to November 1987.

Oil cake	Containers				Period of observation (days)				Mean
	Gunny bag	Polythene bag	Polythene lined gunny bag	HDPE woven bag	0	60	120	180	
Coconut	7.14	6.23	4.60	6.14	1.41	7.20	7.46	6.86	6.03
Groundnut	8.01	7.53	6.56	7.05	2.57	8.71	8.55	7.84	7.29
Sesamum	8.98	6.38	6.31	5.84	2.80	8.34	7.83	6.89	6.88
Mean	8.04	6.71	5.82	6.34	2.26	8.08	7.95	7.20	

CD at (0.05) level

Oil cakes - 0.221
Containers - 0.259
Oil cake X container - 0.436
Oil cake X period of observation-0.515

APPENDIX V

Interaction between oil cakes, containers and period of observation on the population of fungi.

November 1987 to May 1988.

Oil cake	Containers				Period of observation (days)				Mean
	Gunny bag	Polythene bag	Polythene lined gunny bag	HDPE woven bag	0	60	120	180	
Coconut	4.64	3.91	3.61	4.05	1.63	3.94	5.01	5.36	4.05
Groundnut	4.30	3.49	3.15	4.10	1.67	3.31	4.15	5.53	3.76
Sesamum	4.24	4.00	3.50	4.02	1.55	3.40	4.60	6.45	3.94
Mean	4.39	3.80	3.42	4.06	1.61	3.55	4.59	5.78	

CD at (0.05) level

Oil cakes	- 0.082
Containers	- 0.150
Oil cake X containers	- 0.247
Oil cake X period of observation	- 0.365

ABSTRACT

Fungi causing deterioration of coconut, groundnut and sesamum oil cakes were studied. Acremonium implicatum, Aspergillus aculeatus, A. flavus, A. fumigatus, A. niger, A. terreus, A. versicolor, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, P. pinophilum, Pestalotiopsis palmarum, Rhizomucor pusillus and Rhizopus stolonifer were obtained from coconut oil cake. Aspergillus flavus, A. niger, A. terreus, A. versicolor, Gliocladium sp. Penicillium pinophilum, Rhizopus oryzae and Rhizopus stolonifer were noticed in groundnut and Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. tamarii, A. terreus, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Penicillium pinophilum, Pestalotiopsis palmarum and Rhizopus oryzae in sesamum oil cake.

Of these, Acremonium implicatum, Aspergillus aculeatus, A. caesiellus, A. fumigatus, Bipolaris hawaiiensis, Curvularia clavata, Monascus ruber, Penicillium aurantiogriseum, P. pinophilum, Pestalotiopsis palmarum and Rhizomucor pusillus from coconut oil cake, Aspergillus versicolor, Gliocladium sp., Penicillium pinophilum, Rhizopus oryzae and R. stolonifer from groundnut and Aspergillus

candidus, A. fumigatus, A. tamarii, A. terreus, Curvularia clavata, Eurotium chevalieri, Fusarium pallidoroseum, Monascus ruber, Penicillium pinophilum, Pestalotiopsis palmarum and Rhizopus oryzae from sesamum oil cake have not been reported earlier.

Aspergillus flavus and A. niger were isolated from all the samples of groundnut and sesamum oil cakes. In coconut oil cake, these two fungi were present in 88.89 and 77.78 per cent of the samples. A. terreus was isolated from 66.67 per cent of groundnut and 55.56 per cent of coconut and sesamum oil cake samples. Penicillium pinophilum was obtained from 66.67 per cent of groundnut, 44.44 per cent of sesamum and 27.78 per cent of coconut oil cake samples.

Wide variation was noticed in the population of fungi present in the oil cakes collected from different regions during different periods of the year. Oil cakes collected during June-July had the highest population of fungi. The central and the northern regions recorded higher population of fungi than the southern region.

Positive and significant correlation could be obtained between weather parameters and population of fungi in different oil cakes. Maximum correlation was noticed in relation to total rainfall.

Good mycelial growth of fungi was obtained in all the oil cakes incubated at 27, 29 and 32°C. Maximum mycelial growth was noticed at 100 per cent relative humidity. This was followed by 96.1 per cent and 92.9 per cent in the descending order.

The oil content of the oil cakes was considerably reduced due to the growth of all the fungi tested individually and in combination. Maximum reduction was noticed due to the growth of Pestalotiopsis palmarum in coconut oil cake, Rhizopus stolonifer in groundnut and Fusarium pallidorosem in sesamum oil cake. In the case of combinations, Aspergillus flavus, A. niger and Penicillium pinophilum together caused maximum reduction in oil content of coconut oil cake. In groundnut, combined growth of A. flavus, A. niger and A. terreus caused maximum reduction in oil whereas, A. niger and P. pinophilum together effected maximum reduction of oil in sesamum oil cake.

Oil cakes inoculated with different fungi showed considerable reduction in total carbohydrates, crude protein, free amino nitrogen, crude fibre and ash to the extent of 6.11 to 76.95, 4.28 to 68.03, 14.91 to 92.52, 1.25 to 92.55 and 0.17 to 65.16 per cent respectively. In the case of mineral nutrients like phosphorus, potassium, magnesium,

calcium, copper and iron reduction ranging from 15.07 to 75.54, 23.13 to 94.41, 10.89 to 63.37, 28.78 to 90.20, 52.52 to 97.12 and 0.32 to 60.77 per cent respectively was noticed.

Fourteen out of 20 isolates of Aspergillus flavus produced aflatoxins B_1, B_2 and G_2 in culture medium with maximum quantities being 1210, 1040 and 151 ppb respectively by the isolates from coconut oil cake. Eight out of 19 isolates of A. niger elaborated B_1 upto 222 ppb by the isolate from sesamum oil cake. When grown on the respective host material, A. flavus isolates from coconut oil cake produced maximum quantity of B_1, B_2 and G_2, being 1517, 1092 and 272 ppb respectively. A. niger isolate from coconut oil cake produced B_1 upto 419 ppb.

Oil cakes treated with calcium propionate (0.6 per cent, w/w) were free from fungus growth throughout the period (180 days) of observation and showed minimum number of fungal propagules whereas, those kept as control had higher population of fungi than the treated ones, at all period of observation.

Oil cakes stored in polythene lined gunny bags had the least population of fungi, whereas those stored in ordinary gunny bag had very high population of fungi.

These results revealed that fungal deterioration and spoilage of oil cakes could be prevented or reduced to the minimum by treatment with 0.6 per cent calcium propionate and by using polythene lined gunny bags for storage and transport.