INVESTIGATIONS ON THE MICROBIAL DETERIORATION OF COPRA

BY

SUSAMMA PHILIP

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Investigations on the microbial deterioration of copra" 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.

Susamma Philip

Vellayani,
30 August, 1978

SUSAMMA PHILLIP

CERTIFICATE

Certified that this thesis, entitled "Investigations on the microbial deterioration of copra" is a record of research work done independently by S.T. SUSANNA PHILIP, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

Vellayani,
30 August, 1978

30/8/78
(Dr. M. RAMANATHA MENON)
Chairman, Advisory Committee
Professor of Plant Pathology

Approved by:

Chairman

Dr.M. RATANATHA PILLAI

Members

Dr. M.C. NAIR

Dr. K.M. RAJAN

Dr. M.M.KOSHY

Dr. R.S. AIYAR

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INTRODUCTION

INTRODUCTION

Coconut has an eminent position among the oilseed crops in India. It is estimated that India produces 2.80 lakh tonnes of copra annually (Child, 1974).

Kerala State contributes more than 70.5 per cent of the total Indian production of coconuts. Out of this, about 70 per cent is directly consumed as food in the form of fresh kernel, ball copra or tender nuts, the remaining 30 per cent being used for copra making for oil extraction (Sreenulanathan and Nair, 1971). For the processing of copra sophisticated drying practices are not followed. Thus it can be seen that copra industry is one of the rural-based industries in the State giving gainful employment to several lakhs of people.

In Kerala the advantage of a tropical humid climate with the two monsoon periods renders the cultivation and production of coconuts fairly an easy operation. But this advantage turns into a disadvantage with respect to its post-harvest technology, viz., copra making for oil production both for industrial and domestic purposes.

Due to the intermittant rains, sun-drying of copra becomes very difficult except during the summer months of December to May. During the rainy season crude smoke

chambers are used for drying copra. The defective methods of processing and high moisture content of the copra are the major factors which favour the development of microorganisms resulting in its deterioration. The loss due to microbial infection varies depending upon the moisture content of copra (Aten et al., 1958 and Nathanael, 1965). Bacterial infection starts during the initial stages of processing followed by fungal infections (Subrahmanyan, 1965, Sreemulanathan and Nair, 1971).

The possibility of intermittent drying during the rainy season and the use of crude smokers to hasten the drying during such periods provides a conducive habitat for microbial infection and consequent spoilage resulting in both quantitative and qualitative loss in the oil expelled. The quantitative decrease in the yield of oil has been roughly estimated to be about 10 - 15 per cent (Sreemulanathan and Nair, 1971). However, no work has been done to evaluate the extent of this quantitative decrease in oil production due to spoilage by specific groups of microorganisms and combinations thereof. This is particularly relevant in finding out better methods of preparation and storage of copra. In terms of financial losses incurred due to such spoilage it can be safely guessed that the annual losses may be of the order of 10 - 15 crores. This loss, borne by the copra producers and oil mill owners will necessarily be

passed to the cultivator and the consumer. Any method which can prevent this quantitative loss, besides benefitting these sections of people will result in an overall increase in edible oil for which the country is desperately short of at the present juncture.

The use of unhygenic methods of storage and crude methods of processing stored copra results in oil with a rancid smell, incorporating toxic products due to microbial infection. Such inferior quality oil is finally passed on to the consumer. Further the oil cakes processed from such infected copra when used as an animal feed enters the food chain of human beings once again. This problem of production of toxins in copra and its products especially the aflatoxins is of national importance. Aflatoxin production in coconut and copra is already reported (Schindler and Koehler, 1965; Arseculeratne and Sosilva, 1971 and Samarajeewa, 1972). Aflatoxins are a group of highly toxic and carcinogenic metabolites produced by strains of Aspergillus flavus associated with the spoilage of agricultural commodities (Goosmore et al., 1964; Allcroft, 1964; Alsock et al., 1968 and Hogan, 1968).

The most important factors in the growth of fungi and production of aflatoxin is the moisture content of the substratum and the relative humidity of the atmosphere that

surrounds it (Mustafa and Ayerst, 1963). Hence improperly dried copra forms an ideal medium for the production of aflatoxins and causes toxic hazards to human beings and animals consuming copra and its products. In addition to Aspergillus flavus, other species of fungi are also known to produce potent toxins (Jono and Jono, 1972 and Duff and Hamilton, 1975) on several moistened substrates.

Though sporadic investigations have been conducted on the microbial spoilage of copra in India and other countries, no systematic work encompassing various aspects relating to microbial infection of copra have been conducted so far. The magnitude of the health hazards due to the use of such infected copra has also not been highlighted in literature.

Taking all these aspects into consideration and in view of national priorities on post-harvest technology of commercial agricultural produce, systematic work was planned with the following main objectives.

(i) Continuous monitoring of microflora associated with the deterioration of copra in relation to environmental factors for a period of one year to identify the major groups of organisms responsible for spoilage and to pinpoint the favourable environmental factors for microbial deterioration.

(ii) Keeping quality of copra under different storage conditions to locate the best methods of storage

(iii) Evaluation of the biochemical changes in copra and coconut oil with reference to the quality of oil by artificially infecting copra separately with specific fungi identified as being involved in deterioration

(iv) Fungal enzymes involved and mycotoxins elaborated in copra by infecting fungi and their bioassay to define the extent of toxicity

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

Fungal infection of copra

Fishlock (1928; 1929) observed fungi as more destructive than bacteria since different species attack copra at different stages of drying and during storage. They recorded Aspergillus niger and A. flavus as the most important moulds of copra on insufficiently dried material and Penicillium spp. on stored copra in Gold Coast. Passmore (1931) studied the depreciation of prepared copra due to moulds and insects in England and isolated A. niger, A. flavus, A. tamarii, A. chevalieri, Rhizopus nigricans, Scopulariopsis sp., Syncephalastrum cinereum, P. candidofulvum and Mucor racemosus. Thompson (1933) reported the common fungi on copra in Malaya as six species of Aspergillus and occasionally observed P. glaucum, Trichothecium roseum, R. nigricans, species of Cephalosporium, Colletotrichum, Diplodia and Ceratostomella adiposum.

Cooke (1932) in his investigations on coconuts and coconut products in Malaya reported the presence of A. niger, A. flavus oryzae and A. tamarii on wet material and A. cinnamonensis on dried copra. Dyre (1932) isolated nine species of Aspergillus, Mucor racemosus, Syncephalastrum sp. and Penicillium sp. from stored copra. Ward (1937) made

elaborate studies on the deterioration of copra caused by moulds in Malaya and recorded A. wentii, A. ochraceous, A. tamarii, A. flavus oryzae, A. glaucus, P. glaucum, a Saccharomycete, R. nigricans, Ceratostomella adiposa, Trichothecium roseum and Colletotrichum sp. Moulds like A. flavus and A. tamarii penetrated deep into copra and caused more damage than others. Inoculation experiments with A. flavus showed better penetration of coconut meat than A. niger or A. tamarii. Inoculation of A. flavus with previously inoculated bacteria inhibited germination of mould spores. But when inoculations were made simultaneously the spores germinated and caused penetration.

Wollenweber and Hochapfel (1943) reported the presence of eight species of Diplodia. They observed Diplodia palmarum Cke Wrn Comb. (Syn. Sphaeropsis palmarum Cke) on unripe coconuts in East Africa. Botryodiplodia sp. showed negative or very weak activity.

Griebel (1951) isolated from rancid coconuts a species of Mucor from Berlin. Reese et al. (1955) reported that in Massachussetts fungi like A. terreus, A. ustus, Chaetomium spirale and Cunninghamella bertholletiae developed profusely on coconut oil. Subramanian (1956) in his studies on marketed copra and coconut oil from South India reoorded that Aspergillus spp. caused considerable deterioration

while Penicillium sp. caused only superficial growth. The most frequently observed fungus was A. niger. Subrahmanyan (1965) reported the presence of A. flavus oryzae and R. nigricans at the early stages of drying and A. tamarii, A. niger, A. glaucum and Penicillium at later stages of drying of coconut kernels. Paul (1969) isolated A. niger, A. flavus, A. ustus, Trichoderma viride, Cunninghamella verticillata, Syncephalastrum racemosum, Rhizopus sp., Penicillium sp. and Diplodia sp. from infected copra collected from the different districts of Kerala State. All the fungi except Rhizopus sp. were able to grow well when oil was used as the carbon source. Infection of copra by A. niger was found to be faster at higher relative humidities. It was further noted that under conditions of high humidity copra containing very little moisture was also attacked by this fungus. Fungal population was predominant on copra samples collected from oil mills.

Nair and Sreemulanathan (1970) observed spoilage of well dried copra with moisture content below 4.0 per cent by P. frequentans. The studies with this fungus have shown it as xerophytic and preferred low moisture conditions. On stored copra its growth was superficial and does not cause significant quantitative loss; but caused off-flavour and rancidity to oil. Sreemulanathan and Nair (1971) isolated

species of Aspergillus, Penicillium, Diplodia, Monilia and Rhizopus from dried copra. Among the fungi R. nigricans, A. niger and A. flavus were found to be very destructive.

Rao et al. (1971) studied the post harvest infection of coconut kernel by Botryodiplodia theobromae. The fungus present on the eyes and adjoining tissues spreads into the internal region through cracks formed during storage and was responsible for the blackening and spoilage of kernels.

Bacterial infection of copra

Cooke (1932) observed that bacterial infection started first during the initial stages of processing itself. Moulds do not generally appear until a bacterial slime was established on the surface of copra. This process occupies about two days from the time the nuts are split and exposed to the air. The sequence of invasion by bacteria and moulds is determined by the appearance of certain degradation products of the complex glycerides, proteins and carbohydrates present in coconut meat. Ward and Cooke (1932) observed that at relative humidity of 80 per cent and temperature of 30°C or below the multiplication of bacteria occurred rapidly on coconut kernel and within about four hours developed a surface slime. The slime continued to develop and within 48 hours penetrating moulds made their

appearance. Ward (1937) found two species of rod shaped bacteria associated separately and together with the slime on the wet kernel during drying. Subrahmanyan (1965) observed Staphylococcus aureus, Bacilli and yeasts within 12 to 36 hours after opening the nut at 20 to 50 per cent moisture which caused discolouration and softening of the surface, development of off-flavours and pro-oxidants leading to rancidity of oil. Paul (1969) obtained a Bacillus sp. and one gram negative coccus from infected copra samples. Bacterial population was higher on copra samples taken immediately after drying than on stored copra. Sreemulanathan and Nair (1971) isolated Aerobacter sp., Bacillus sp. and Serratia marcescens from infected kernel. At higher moisture levels they caused discolouration and softening of the surface to a depth of 2 to 5 mm within 2 to 3 days, depending upon the rate of drying, with the production of rancidity and off-flavour.

Coconut oil cake

Rajasekharan et al. (1960), in their shelf life studies on coconut oil cake, observed that cake could be stored without spoilage upto about six months in alkathene lined bags. Under natural conditions the cake readily absorbs moisture during storage and is consequently prone to mould attack.

Moisture content and spoilage of copra

The pioneering contributions of Walker (1906) and Brill et al. (1917) have thrown light on the influence of moisture content on the nature and extent of spoilage of coconut material.

Fishlook (1928; 1929) studied the moisture content and its relation to fungi in stored copra. When separate lots of carefully dried copra having an average moisture content of 5.87 per cent were stored, the time taken for 100 per cent mould to develop ranged from 36 to 187 days. Most of the lots increased in weight during storage as a result of absorption of moisture from the air. Cruz (1930) correlated the moisture content of copra with the length of time required for mould appearance. At 53.19 per cent moisture, it took three days for the moulds to appear while at 26.85, 20.75 and 10.79 per cent moisture, the period required was 7, 8 and 13 days respectively under sun-dried conditions. Under the taphan method of drying the length of time required for the moulds to develop was 3, 2 and 5 days with 25.87, 25.11 and 17.73 per cent moisture respectively. In the case of desiccated coconuts with 7.53 per cent moisture, moulds appeared after 11 days, while at 6.60 per cent moisture 14 days were required.

Cooke (1932) studied the moisture loss during successive stages of sun-drying and reported an excess moisture of over eight per cent as the determining factor in the initiation of deterioration in copra. Ward (1937) studied the moisture relations and fungal growth on copra and found that A. glaucus group and P. glaucum preferred a moisture content of about seven per cent while A. tamarii, A. wentii and A. ochraceous groups preferred about 12 per cent and A. niger, R. nigricans and Ceratostomella adiposa preferred a moisture level between 15 and 20 per cent. He found that the sclerotial forms of A. flavus was the most important species on copra owing to its tolerance of a wide range of moisture from 7 to 15 per cent. Prepared copra deteriorated rapidly under the combined action of bacteria and penetrating fungi chiefly A. flavus, when the moisture content was over 12 per cent and stored at room temperature between 28 - 30°C and relative humidity over 80 per cent. Below 12 per cent moisture, A. flavus grew superficially but penetration of tissues was restricted to isolated areas where the bacteria were able to establish themselves prior to fungus invasion. Under the same conditions, no bacterial development occurred on copra dried to 6 per cent moisture content, while bacterial development was artificially produced on copra with a moisture content of 6 and 8 per cent and

stored at room temperature under 43°C and humidity over 80 per cent.

Child (1940) observed that at 1.8 per cent moisture content, coconut stored in sealed containers showed no deterioration for two months while at 3.65 per cent moisture it was in fairly good condition and at 6.73 per cent it was mouldy.

The copra processing in rural industries and the effect of moisture content on microbial infection during storage have been studied (Anon., 1958). On coconut meat which contained 20 to 50 per cent moisture, penetration of bacteria followed by yeasts and fungi like A. flavus, A. niger and R. nigricans were observed. At 12 to 20 per cent moisture also the above fungi were recorded. At 8 to 12 per cent moisture A. tamarii was found to occur superficially and at less than 8 per cent moisture A. glaucum and P. glaucum were occasionally observed forming a superficial growth.

Marar and Padmanabhan (1960) studied the moisture content and method of storage of copra. They considered moisture as the important single factor that governed the deterioration of copra in places where humidity ranged from 92 to 95 per cent throughout the year.

Rajasekharan and Pandalai (1961) observed eight per cent moisture as the critical moulding moisture and worked out the equilibrium moisture content of copra at higher relative humidities prevalent in Kerala. Subrahmanyan (1965) in his studies on the agencies, responsible for the spoilage and destruction of coconut material observed a relation between the occurrence of different fungi and moisture content of copra. At 20 to 50 per cent moisture some bacilli, yeasts and Staphylococcus aureus caused discolouration and softening of the kernel. At 15 per cent and above A. flavus oryzae and R. nigricans penetrated deep into the kernel and produced heat, gases and rancidity. At 8 to 12 per cent moisture A. tamarii and A. niger appeared which were followed by A. glaucum and Penicillium sp. at 8 per cent moisture.

Nathanael (1965) observed moisture as the single factor that governed the initiation and progress of deterioration. Bacterial fermentation and fungal action on copra required the presence of moisture. The intensity of attack varied with the precise moisture content. The insect pests which survived on copra also preferred fairly moist and mouldy conditions where they can feed on intermingled fungal mycelia and spores. Sreemulanathan and Nair (1971) studied the degree of dehydration required to prevent fungus infection in closed storage conditions and

found that copra cups and segments dried to a moisture content of 3 to 3.5 per cent before storage were free of infection.

BIOCHEMICAL CHANGES IN COPRA AND COCONUT OIL DUE TO INFECTION

Brill et al. (1917) narrated the effect of mould action on the quantity and free acidity of oil in copra. Rhizopus sp., the white mould, survived on the wet meat and under favourable conditions destroyed a high percentage of oil and resulted in higher production of free fatty acids.

Stoke (1923) reported that the rancidity of coconut oil caused by P. palitans was essentially due to the presence of methylamyl, methylheptyl and methylnonyl ketons.

Fishlock (1929) observed that Penicillium spp. growing on copra do not cause the production of free fatty acids in oils. Horowitz-vlassoma 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. The lipolysis induced by fungal activity is characterised by marked rise in acid number. A reduction in iodine number and rise in refractive index were also noticed.

Subramanian (1956) investigated on the seasonal and

tract variations in oopra and coconut oil marketed in South India. In all the samples studied, the July and October samples showed higher moisture content than January and April samples showing that the inoidenoe of moulds was more frequent in July samples than in others and related to the variation in aoid values. He also observed that less number of samples were mouldy in January. There were no marked changes in the saponification value and refractive index of coconut oil.

Rajasekharan and Pandalai (1961) in their studies on the seasonal and tract variations in the milling copra of South Canara, Malabar and Travancore-Cochin, obtained higher acid values in July, October and April samples than those of other periods. Unnikrishnan (1963) recorded changes in odour and oolour of the oil extracted from oopra infected by fungi. An increase in specific gravity, refractive index and iodine value was noted, whereas saponification value decreased due to infection. Subrahmanyam (1965) reported rancidity of oil due to bacterial and fungal infection.

Paul (1969) obtained a progressive fall in the oil content of copra due to fungal infection. A. niger and A. flavus penetrated deeper into the tissues and brought about maximum reduction in oil content. Jaganathan (1970)

reported the results of analysis of samples of coconut refuse for moisture, oil content and free fatty acids in Ceylon. The results showed that there was wide variation in the oil content and free fatty acids of samples depending upon the duration of storage and concomitant microbiological deterioration.

FUNGAL ENZYMES IN THE DETERIORATION OF COPRA

Literature on the production of enzymes by fungi in causing deterioration of copra are very meagre.

Rao et al. (1971) studied the elaboration of hydrolytic enzymes by B. theobromae isolated from coconut kernel. It was found to secrete pectinase and cellulase in stationary as well as in shaken cultures. Chakrabarti and Nandi (1976) made qualitative estimation of enzymes produced by B. theobromae isolated from coconut kernel. Maceration activity was detected among other enzymes studied.

Eyre (1932) conducted elaborate studies on the lipolytic activity of 13 species of Aspergillus, Mucor racemosus, Syncephalastrum sp. and Penicillium sp. isolated from stored copra. A. flavus, A. niger, A. fumigatus and A. awamori were found to be actively lipolytic while A. nidulans, A. sulphureus, A. tamarii and A. ustus were less lipolytic and others non-lipolytic in their activity.

Rao et al. (1971) and Rao and Sreekantiah (1976) studied the protease activity of B. theobromae isolated from cocoonut kernel and was found to elaborate an acid protease.

Aflatoxin

The presence of mould toxins is potentially the most serious quality problem encountered by producers, manufacturers and handlers of food and food products. For many years moulds have been known to produce toxic metabolites but their effects were largely ignored. Forgacs and Carll (1962) reviewed the work on mycotoxins and reported many pathologioal cases of animals due to ingestion of feed contaminated by fungi including Aspergillus spp. With the development of Turkey "X" disease (Blount, 1961) the situation has altered drastically. The demonstration of aflatoxin B_1 as an extremely potent carcinogen evoked world wide attention on mycotoxins.

Sargeant et al. (1961 a, b) first demonstrated that the toxic principle in mouldy peanut meal can be removed by exhaustive extraction with methanol, partially purified by transfer into chloroform and resolved by paper chromatography as a single spot exhibiting bluish fluorescence under long-wave ultra violet illumination. Hartley et al.(1963) isolated and characterised four olosely

related aflatoxins and designated them as B_1, B_2, G_1 and G_2 in order of decreasing Rf value. Robertson *et al.* (1965) and Pons *et al.* (1966), determined aflatoxins in agricultural products using acetone for extraction.

Baur and Armstrong (1971) and Samarjeewa and Arseculeratne (1975) evaluated techniques for the detection of aflatoxin in coconut products. Some of the copra, poonac and coconut oil samples showed medium or high aflatoxin levels. The chemical methods available for the analysis of food and feeding stuff like copra, copra cake and copra meal for aflatoxin estimation was detailed by Jones (1972) and Anon. (1976).

Labadan (1969) studied the effect of moulds in the feeding value of copra meat and observed that coconut meat formed a good substrate for the production of aflatoxin. Lafort and Lafort (1970), Elnur and Ibrahim (1970), Richard and Cajsewski (1971), Halloin (1975) and Ilag (1975) studied the production of aflatoxins by strains of *A. flavus* on animal feeds, stored corn and cotton seeds. Strzeleckl and Gasiorowska (1975) estimated the aflatoxin content of cattle, sheep, poultry and pig feeds as well as protein concentrates. Presence of aflotoxin was observed in 12.7 per oent of the samples tested and the concentration was high in pig feeds as compared to others.

Several fungi besides A. flavus are known to produce aflatoxins. Basappa et al. (1967) reported aflatoxin B_1 and B_2 production by a strain of A. oryzae. Walbeek et al. (1968) cultured several fungi, isolated from food samples obtained from households, retail stores and processing plants, on shredded wheat and artificial media. Besides A. flavus, they recorded aflatoxin production by Rhizopus sp. and A. ochraceous.

Biological assay

Chick embryo

Platt et al. (1962) used chick embryo for the bioassay of aflatoxins as a simple, reproducible and sensitive technique. Verrett et al. (1964) and Choudhury and Manjrekar (1967) also observed the feasibility of chicken embryo as test organisms.

Assay using ducklings

Blount (1961), Asplin and Carnaghan (1961), Allcroft et al. (1961) and Sargeant et al. (1961 b) reported heavy mortalities of ducklings and pheasants due to the ingestion of feed contaminated by aflatoxins. The sensitivity of ducklings to aflatoxin injury and the immediate induction of bile duct hyperplasia has contributed to the widespread use of ducklings for aflatoxin assay.

The pathological changes due to aflatoxicoses have been described by Asplin and Carnaghan (1961), Blount (1961), Butler (1964), Jogan (1966), Purchase (1967) and Joffe (1970). Symptons mainly manifested in the liver as diffuse degenerative changes in the parenchymal cells. Higher doses of aflatoxins produced extensive haemorrhagic necrosis, the normal structure of the liver no longer being distinguishable. Kidney showed congestion and degeneration of the glomerules and tubules.

Assay using guinea pigs and rats

The pathology of aflatoxicosis in farm animals has been reviewed by several workers. Paget (1954) and Patterson et al. (1962) studied the histopathology of the liver of guinea pigs and observed toxicity due to ingestion of infected groundnut kernel as the cause of exudative hepatitis (Oedema disease) of guinea pigs. Butler (1966) confirmed this with detailed description of hepatic lesions with biliary proliferation in guinea pigs and rats. Vogan and Newberne (1967) and Newberne and Butler (1969) studied the characteristics of aflatoxin poisoning in rats and guinea pigs. Aflatoxin B_1 administered in male and female rats induced hepatocellular carcinoma and other liver lesions and surviving male animals developed persistent liver lesions. Samarjeewa et al. (1975) studied

the sportaneous and experimental aflatoxicosis in goats fed with a concentrate mixture containing defatted residue from grated coconut with a toxigenic strain of A. flavus. Hepatic lesions included bile duct hyperplasia and periportal fibrosis.

Penicillium citrinum toxin

Citrinin was first isolated from P. citrinum during 1931 and its structure was determined by Brown et al.(1949) and Friis et al. (1969). In Denmark a toxic nephropathy in swine was associated with the feeding of mouldy cereals infected with P. citrinum. Wilson (1964) isolated P. citrinum from mouldy feeds. P. citrinum was shown to produce the toxic metabolite citrinin on several moistoned food materials in the laboratory. Miyake and Saito (1965) isolated several strains of Penicillium of which three in particular were toxic, i.e., P. citreoviride, P. citrinum and P. islandicum from the rice imported into Japan, following World war II.

Uraguchi (1969) cites evidence that vitamins B1, and C play only a subordinate role in beriberi and suggested it to be a mycotoxicosis. Beriberi correlated well with the ingestion of mouldy rice. Ueno and Ueno (1972) supported the view by demonstrating a symptomatic and

toxicological correlation of citreoviridin and cardiac beriberi in animals.

Saito et al. (1971) observed rice contaminated with P. citrinum to produce nephropathy in mice and citrinin was isolated from infected rice. Scott et al. (1972) reported P. citrinum to be naturally occurring in contaminated wheat, rye and barley. Mirocha and Christensen (1974) reported that P. citrinum usually grows on polished rice in storage and causes a characteristic yellowish colour which fluoresces under UV irradiation. As a diagnostic sign, citrinin caused an enlarged turbid kidney in rats with degeneration and dilation of the lower nephrons and renal lesions resembling glomerulonephrosis.

Prevention of deterioration of copra - Use of chemicals

Various investigators have suggested different methods for minimising the spoilage and improving the quality of copra. Fishlock (1928) obtained promising results with sulphur dioxide fumigation at the rate of 1/2 lb for C.ft for a period of 36 hours.

Subrahmanyan (1965) in his studies on the control of infection and production of quality products elaborated the uses of several chemicals like calcium hydroxide, sodium chloride, mineral acids, ammonia, ammonium compounds,

salts of different metals, organic acids, formaldehyde, different sulphur compounds, benzoate, hypochlorite, sorbic acid and different solvents like hydrocarbons and terpenes for coating and dipping. At the concentrations tried most of the chemicals were not effective for more than 24 hours while some were effective upto 48 hour. The physical coats failed because of shrinkage and often due to the cracking of kernel during drying. Subrahmanyan et al. (1966) devised a simple and inexpensive method of controlling infection during sun-drying of coconut kernel by daboing the kernel with five per cent acetic acid and allowing to stand over night. The kernel was then split into small segments, dipped for 3 to 5 minutes in a mixture of five per cent sulphuric acid and seven per cent acetic acid. The copra bits were dried in the open for four days and then stored in heaps by which time they became white copra with a moisture content of 5 to 6 per cent.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDIES ON THE MICROFLORA ASSOCIATED WITH COPRA AND COCONUT OIL CAKE

Collection of samples of copra and oil cake

Samples of copra, 1000 g each, were selected at random, pooled and collected in plastic bags at monthly intervals (on the fifth day of every month) over a continuous period of 12 months from four selected oil mills located at Thiruvallam (M1), Karamana (M2), Ulloor (M3) and Sreekaryam (M4) in Trivandrum District. The lots from which the samples were collected consisted of copra supplied by local farmers to the oil mills. The copra was made by sun-drying coconut kernels for seven days. Samples of oil cake were also collected in the same manner from the same locations.

Quantitative and qualitative determination of microbial population in copra and coconut oil cake

The microbial population in the different samples of copra and oil cake was determined at each sampling, by the modified soil dilution plate technique. Samples of copra with a surface area of one sq.cm and weighing one g were cut into small pieces under aseptic conditions and were transferred to 99 ml of sterile distilled water

in a 250 ml conical flask and was shaken in a mechanical shaker for 30 minutes. Serial dilutions were made by transferring one ml aliquot to 99 ml of sterile distilled water blanks. Between each dilution the flask containing the suspension was shaken for five minutes. The final dilution used for the estimation of microbial population was one in one million for copra and one in ten thousand for coconut oil cake. One ml of this diluted suspension was pipetted into sterile petri-dishes and 15 ml of appropriate melted and cooled medium was added and allowed to solidify. Peptone-dextrose agar with rose bengal and streptomycin, nutrient agar and Ken-knights agar (Appendix I, II, III) were used for the culturing of fungi, bacteria and actinomycetes, respectively. Dilutions were plated in triplicates for each experimental sample and three separate samples of one g each were used for the study. The plates were incubated at room temperature. Counts of bacteria, fungi and actinomycetes were taken after 3, 7 and 12 days respectively of plating. The number of microorganisms was expressed on moisture free basis of the sample. The moisture content of the samples was also determined (A.O.A.C., 1960).

The fungal colonies were transferred to potato dextrose agar slants (PDA Appendix IV), purified by single spore isolation and maintained on PDA. The bacteria and

actinomycete colonies were transferred to nutrient agar slants and purified by single colony isolates. The fungi and bacteria were identified based on their morphological characters (Gilman, 1967; Raper and Fennel, 1965; Buchanan and Gibbons, 1975). The identity of fungi and bacteria was confirmed by C.M.I., London. Actinomycetes were identified upto genera based on morphological characters (Rangaswami et al., 1967).

Inoculation studies of fungi isolated from samples of copra

Freshcoconut kernels were cut into rectangular pieces of 3 x 2 cm and sterilised using 0.1 per cent mercuric chloride and serially washed thrice in sterile water. They were then kept in a hot air oven at 70°C. When the moisture was reduced to about 9.5 per cent, they were taken out and inoculated with fungi, bacteria and actinomycete isolated from copra and incubated under saturated humidity conditions in desiccators at room temperature (28 - 30°C). For inoculating Penicillium citrinum, copra was prepared with about five per cent moisture level. Pieces of copra as detailed above were inoculated with P. citrinum and kept in desiccators without providing humidity.

Data on atmospheric factors.

Data on atmospheric factors such as relative humidity, rainfall and minimum temperature were collected from the Meteorological Centre, Observatory Hills, Trivandrum for ten days prior to the collection of copra samples.

Path analysis of the effect of weather elements on fungal and bacterial population.

Influence of weather elements on the fungal and bacterial population of copra were studied through correlation coefficient and path coefficient analysis (Li, 1968).

Moisture content and microbial succession during different stages of copra making

One hundred nuts were opened and sun-dried for a period of seven days. Loss of moisture from the kernels during drying and microorganisms occurring on the same were assessed after every 24 hours. At each sampling the organisms were isolated and identified.

KEEPING QUALITY OF COPRA UNDER DIFFERENT STORAGE CONDITIONS

Keeping quality of copra under different storage conditions were studied by using copra prepared as in inoculation studies. Three replications were maintained for each treatment.

Effect of temperature

Pieces of copra, each weighing approximately 50 g with a moisture content of about five per cent, were stored for a period of 60 days at different temperatures. The samples were kept at 10°C (±1) and 20°C (±1) in a refrigerator and at 25°C (±1) in a B.O.D incubator. The control consisted of copra kept at room temperature (28 - 30°C). The associated fungi were observed at 7, 15, 30 and 60 days interval. The moisture content was determined at the end of the experimental period.

Effect of relative humidity

Pieces of copra, each weighing approximately 50 g with a moisture content of about five per cent, were kept in desiccators maintained at relative humidities ranging from 50 - 100 per cent by using different concentrations of KOH in water (Buxton, 1931). The control consisted of copra kept at relative humidity obtainable at room temperature (80--82 per cent). The associated microbes were observed at 7, 15, 30 and 60 days interval and the moisture content was determined at the end of the experimental period.

Effect of moisture content

Pieces of copra each weighing 50 g with a moisture content of about 3-4, 5-6, 7-8 and 9-10 per cent were kept in desiccators for a period of 60 days. The associated

microbes and moisture content were determined at different intervals as in the previous experiment.

Effect of storage practices

Cups of copra with moisture content of about five per cent were prepared and stored in heaps, gunny bags and also in gunny bags lined with polythene paper (200 gauge) in godown for a period of six months. The associated microbes were observed at 30, 60, 90 and 180 days after storage and moisture content was determined at the end of the experimental period.

BIOCHEMICAL CHANGES IN COPRA AND COCONUT OIL DUE TO INFECTION

Pieces of copra prepared as in inoculation studies were inoculated similarly with Aspergillus niger, A. flavus, Rhizopus oryzae, Mucor hiemalis, Penicillium citrinum, Botryodiplodia theobromae and Curvularia senegalensis and incubated at room temperature for ten days. Control consisted of copra propared in the same manner without inoculation. Three replications were kept for each of the biochemical tests listed below.

Copra

Changes in total sugars due to fungal infection

The total sugars of copra infected by fungi after incubation for periods of 7, 14 and 21 days were estimated following the procedure of Yemm and Willis (1954) using anthrone reagent. The percentage of transmittance was recorded using Spectronic 20 (B & L) Colorimeter at 630 mμ against a reagent blank and the respective optical density values were determined

from the conversion Table. The concentration was then calculated with reference to a standard curve prepared with known levels of glucose and results expressed as mg of glucose per g of copra on moisture free basis.

Effect of infection by fungi on protein content.

The total nitrogen content of the copra samples infected by different fungi as detailed above was determined by Kjeldahl method (A.O.A.C., 1960) at 7, 15, 30 and 60 days after incubation and results expressed on moisture free basis. The protein content was obtained by multiplying the nitrogen content by 6.25.

Effect of infection by fungi on amino acid content.

Samples of copra were prepared as in inoculation studies and infected with different fungi. One hundred mg of defatted samples were hydrolysed with 6 N HCl at 110°C in sealed evacuated tubes. After hydrolysis it was evaporated in a rotary vacuum evaporator and taken in 0.05 N acetate buffer. The amino acids were determined in an automatic amino acid analyser (Perkin Elmer Model KLA-3B) and were expressed as g of amino acid per 100 g of protein (The analysis was done by the courtesy of Tamil Nadu Agricultural University, Coimbatore).

Coconut oil

Samples of copra, inoculated with cultures of

different fungi individually, ~~and~~ were collected at 7, 15, 30 and 60 days, finely powdered and extracted the oil by using a mechanical press. The oil obtained was centrifuged at 2000 rpm for 30 minutes and filtered through a fluted filter paper. This oil was used for the determination of the physical and chemical constants of the oil.

Physical constants

Colour of the oil.

Variation in the intensity of colour due to infection was assessed by using standard Lovibond tintometer. The colour of the oil was expressed as red and yellow units. The sample of oil was taken in the tintometer cell and the colour of the oil was matched with various combinations of red and yellow and expressed as Y + 5R units.

Odour of the oil

The odour of the oil was assessed by smelling and was expressed as pleasant or rancid.

Refractive index

The refractive index of the oil was read using Abbe' refractometer at 40^{o}C and expressed as a number (A.O.A.C., 1960).

Chemical constants

The changes in the fat constants such as acid value, saponification value and iodine value at 7, 15, 30 and 60 days

after inoculation of copra by the different fungi were determined (A.O.A.C., 1960) and were expressed as percentage on moisture free basis of copra.

Changes in the properties of oil on storage

Freshly extracted coconut oil (1000 g) was collected from an oil mill and preserved in a polythene bottle. This was kept under laboratory conditions for a period of 12 months. Samples of oil were taken after periods of 3, 6, 9 and 12 months and the physical and chemical changes in oil due to storage were studied.

Quantitative loss of oil due to infection

Changes in oil content due to infection by various fungi individually as well as by mixed infection excluding P. citrinum on equal mycelium basis were studied at 15, 30, 45, 60, 75 and 90 days after incubation by the Soxhlet extraction procedure (A.O.A.C., 1960).

STUDY OF FUNGAL ENZYMES IN THE DETERIORATION OF COPRA

The enzyme extract for the in vitro production by different fungi viz., A. niger, A. flavus, R. oryzae, M. hiemalis, P. citrinum, B. theobromae and G. senegalensis were prepared by growing them in liquid medium separately. Details are given separately under each item.

Thirty ml medium was taken in 250 ml conical flasks and autoclaved. The flasks were inoculated with discs (5 mm diameter) cut with a sterile cork borer from the growing edge of a five day old culture on PDA medium. The flasks were incubated at room temperature (28 - 30°C). After a specific period of incubation the cultures were harvested, filtered and centrifuged at 10,000 g for 20 minutes at 6°C. The supernatant was decanted, dialysed against distilled water for 16 hours at laboratory temperature and used for enzyme studies. Control consisted of autoclaved culture filtrates.

For in vivo production of enzymes the different fungi were inoculated on copra and incubated under laboratory conditions. The enzyme extract was prepared by grinding five g of infected copra in ten ml of distilled water. The tissue homogenates were squeezed through several layers of cheese cloth. The extract was centrifuged and dialysed as in in vitro studies. The extract from uninfected copra treated as above served as control.

The cell free extract so obtained was used for the determination of the activity of various enzymes detailed below. For in vitro studies the method adopted was the same except that the nature of the medium was different depending on the enzyme studied. Three replications were maintained

for each treatment.

Macerating enzyme activity

Macerating enzyme activity was studied by growing the fungi in a liquid medium (Kannaiyan et al., 1975 Appendix V). Two separate sources of nitrogen viz., sodium nitrate (S_1) and ammonium sulphate (S_2) were used. The enzyme activity in the cell free extracts (in vitro and in vivo (S_3) were studied after 4, 8 and 16 days of incubation (P_1, P_2, P_3 respectively) by the method described by Brown (1915). Potato discs 10 mm diameter and 50μ thickness were placed inside sterile petridishes containing 15 ml of the cell free extract. The end point was taken as the time when the discs gave no resistance on gentle pressing. Results are expressed as reciprocal of the time taken in minutes multiplied by 1000.

Estimation of pectin methyl esterase (PME)

Pectin methyl esterase activity in vitro (S_1, S_2) and in vivo (S_3) was determined following the method of Hancock et al. (1964) by using the cell free extracts prepared in the previous experiment.

The culture filtrate 4.5 ml was incubated with 30 ml of 1 per cent apple pectin solution (pH 5.5). The exact pH immediately after the addition of the culture filtrate

was determined. The solution was kept at room temperature for a period of three hour and the change in pH was noted. The reaction mixture was adjusted to original pH with 0.02 N NaOH. Data are expressed as micro-equivalents of NaOH absorbed per hour per ml of filtrate or extract.

Estimation of polygalacturonase and polygalacturonase transeliminase

The enzymes polygalaoturonase and polygalacturonase transeliminase were assessed by the method of Ayres et al. (1966).

For the in vitro studies the different fungi were grown in modified Czapk's solution (Appendix VI) containing one per cent apple pectin and 0.1 per cent glucose. Two separate sources of nitrogen viz., sodium nitrate (S_1) and ammonium sulphate (S_2) were used. The enzyme activity was studied at 4, 8 and 16 days after incubation (P_1, P_2, P_3 respectively).

Polygalacturonase

The enzyme reaction mixture contained 4 ml of one per cent polygalacturonic acid (pH 8), 1 ml of 0.1 M acetate buffer (pH 5), 1 ml of 0.01 M Ca Cl_2 and 4 ml of enzyme preparation. Following incubation at $30^0$0 for 4 hours the enzyme reaction was stopped and enzyme protein and excess substrate were precipitated by adding 0.6 ml of 9 per cent

zinc sulphate ($Zn\ SO_4 \cdot 9\ H_2O$) and 0.6 ml of 0.5 N NaOH. The mixture was vigorously shaken and centrifuged at 10,000 g for 20 minutes at 6°C.

Five ml each of the clarified reaction mixture was then added to tubes containing 3 ml of 0.04 M thiobarbituric acid (TBA), 1.5 ml of 1 N HCl and 0.5 ml distilled water. The tubes were then placed in boiling water bath for 30 minutes and then cooled. The per cent transmittance was determined at 515 mμ using a Spectronic 20 colorimeter. The readings were made against blanks prepared from identical mixtures with autoclaved culture filtrate.

Transeliminase

The procedure described above was followed for the assay of transeliminase. The absorbance was determined at 550 mμ.

Study of cellulolytic enzymes elaborated by fungi *in vitro* and *in vivo*

For the *in vivo* studies cell free extracts were prepared by the same method as detailed under pectic enzymes. For *in vitro* studies the cell free extracts were prepared by growing the fungi in a liquid medium (Kannaiyan *et al.*, 1975, Appendix VII) using calcium nitrate (S_1) or sodium nitrate (S_2) as sources of nitrogen and enzyme extract was prepared as described earlier. The enzyme activity was studied at

4, 8 and 16 days after incubation (P_1, P_2, P_3 respectively.

Cellulase (C1)

The assay of the enzyme oellulase (C1) was carried out in the cell free extracts for both in vitro and in vivo studies by adopting the procedure of Norkrans (1950) (as quoted by Vidhyasekaran et al., 1971) at 4, 8 and 16 days of incubation (P_1, P_2, P_3 respectively).

The assay mixture contained 1 ml of cellulose solution, 4 ml of 0.5 M citrate buffer (pH 7.0) and 5 ml of culture filtrate. The concentration of cellulose solution was adjusted to give approximately an absorbance of 0.85. The absorbance of the assay mixture at 610 mμ was determined immediately upon the addition of the enzyme preparation and again after an incubation period of 24 hours at 30°C.
The enzyme C1 activity is expressed as the difference in absorbance (0.01 = 1 unit) before and after incubation period of 24 hours. Control consisted of autoclaved culture filtrate which is used as blank.

Cellulase (CX)

The enzyme CX was assayed viscometrically. One ml each of enzyme sample prepared as in the previous study were

added to 5 ml of 1.2 per cent carboxy methyl cellulose (CMC) in 0.1 M citrate buffer and incubated at 30°C for 2 hours (Rai and Dhawan, 1976). The flow rate of the reaction mixture was measured using an Ostwald viscometer and the percentage reduction in viscosity of the reaction mixture was calculated (Morrall et al. 1972).

$$\text{Per cent loss in viscosity} = \frac{Fi-Fx \times 100}{Fi-Fw}$$

Where Fi = flow time for 1 ml of control + 5 ml of CMC solution

Fx = flow time for the reaction mixture at the designated time interval

Fw = flow time for water

Lipolytic enzymes elaborated by fungi in vitro

Lipase

For in vitro studies the cell free extracts were prepared by growing the fungi on Czapek's solution (S_1) and Richard's solution (S_2) (Appendix VI, VIII) where the carbon source was replaced by an equivalent quantity of coconut oil. The method of enzyme preparation was the same as that adopted for in vitro studies of other enzymes described earlier. The enzyme activity was studied for different periods, viz., 8, 12 and 16 days after incubation (P_1, P_2, P_3 respectively) following the titrimetric method of Bier (1955) with slight modifications.

The reaction mixture contained one ml of the culture filtrate, 5 ml of citrate phosphate buffer (pH 7.0) and 5 ml of substrate (10 ml coconut oil emulsified with 90 ml of 1 per cent egg albumen). The reaction mixture was incubated for 5 hours at 37°C with occasional shaking. Then 25 ml of 1:1 alcohol:acetone mixture (V/V) was added and the liberated fatty acids were titrated with 0.05 N NaOH using phenolphthalein as indicator. In the controls the culture filtrates were added just before titration and results expressed as lipolytic units (1 ml of 0.05 N NaOH = 1 lipase unit).

Proteolytic enzymes elaborated by pathogens in vitro

In vitro production of proteolytic enzymes elaborated by different fungi were estimated by growing them in a liquid medium (Kirchoff, 1929 - Appendix IX) where asparagine was substituted with peptone (S_1) and casein (S_2) as nitrogen sources.

The presence of proteolytic enzymes was assessed on 7, 14 and 21 days after incubation (P_1, P_2, P_3 respectively) following the method of Kunitz (1947) as described by Hancock and Millar (1965).

Reaction mixture contained 1 ml of cell free extract and 1 ml of 1 per cent casein in 0.1 M phosphate buffer (pH 7.5). The enzyme substrate mixture was incubated at 35°C and the residual protein was precipitated at 0 and 40 minutes

with 3 ml of 5 per cent trichloroacetic acid (TCA). After incubating the preparation at room temperature for 1 hour, it was centrifuged at 10,000 g for 20 minutes at 6°C. The optical density of the supernatant was measured at 280 mμ using UV spectrophotometer against a blank that consisted of the supernatant fluid from the reaction mixture precipitated with TCA at 0 time. The enzyme activity was expressed as units and one unit equal to an increase in optical density of 0.01/40 minutes.

STUDIES ON MYCOTOXINS IN INFECTED COPRA

Aflatoxin

Microbiological assay of a aflatoxin in copra infected with fungi

The ability to produce aflatoxins by fungi causing deterioration, in copra were studied by employing Bacillus megaterium as the test organism (Clements, 1968). Ten gram of copra with visible fungal infection were finely ground with 20 ml of sterile water. The contents were filtered through several layers of cheese cloth and centrifuged at 3000 rpm for half an hour. The supernatant was decanted and used for toxin assay.

Nutrient agar medium (Appendix II) seeded with 1×10^{10} spores per ml of B. megaterium was poured into

sterile petridishes to a thickness of 5 mm. Wells of 5 mm diameter were scooped out at the centre using a sterile cork borer. Toxin mixture of 0.5 ml was added to this central well, and the dishes were incubated at 28 - 30°C for 48 hour; Control consisted of extracts of copra without infection. Three replications were maintained.

Estimation of aflatoxin produced by fungi in copra

Samples of 1000 g of copra freshly collected from an oil mill in Trivandrum during the month of May 1977 (average moisture content eight per cent) was used for the assay of aflatoxin following the procedure of Waltking et al. (1968).

Fifty g of copra were blended in a high speed grinder for 5 minutes with 500 ml of methanol and water (55:45 v/v), 200 ml of hexane and 4 g of sodium chloride. After blending, the slurry was centrifuged at 2000 rpm for five minutes. Then 25 ml of aqueous methanol layer was transferred to a separating funnel and extracted thrice with 25 ml each of chloroform. Then the bottom chloroform layer was drawn off into a conical flask through a bed of anhydrous sodium sulphate in a funnel. The chloroform layer was then evaporated to dryness and the residue dissolved in 2 ml of distilled chloroform. With a micropipette the solution was spotted in a line 2 cm from either side on thin layer chromatography plates in incandescent light at a concentration of 2-10 µl

along with the standard aflatoxin B_1 (supplied by the courtesy of Makor Chemicals, Israel). The plates were then kept in chromatography tanks containing 10:90 acetone-chloroform mixture (v/v) until the solvent front has run 10-14 cm from the baseline. It was then taken out and examined in a dark room at a distance of 30 cm from an ultraviolet lamp and observed for the presence or absence of a fluorescent spot corresponding to the standard aflatoxin B_1. Noted the volume of sample extract which was just visible under the Uv light. Equated this volume to the concentration of standard aflatoxin required to give minimum fluorescence and the aflatoxin B_1 content was calculated as parts per million.

$$\text{ppm of aflatoxin } (\mu g/g) = \frac{\text{Total volume of extract (ml)} \times \text{Standard toxin for minimum fluorescence } (\mu g)}{\mu l \text{ of sample extract which gives minimum fluorescence} \times \text{Weight of sample (g)} \times 25} \times 1000 \times 250$$

<u>Aflatoxin levels in infected copra at different periods of the year</u>

Samples of copra were collected at bimonthly intervals for a period of one year from an oil mill in Trivandrum. They were assayed for aflatoxin as in the previous experiment.

In this case the assay was confined to detect whether the samples contained aflatoxin above the critical level of 1 ppm only.

Screening of fungi for aflatoxin production

Pieces of copra were artificially infected by different fungi viz., A. niger, A. flavus, R. oryzae, M. hiemalis, P. citrinum, B. theobromae and C. senegalensis as in inoculation studies and assayed for aflatoxin production as in the previous study.

Biological assay of aflatoxin

Chick embryo

Five hundred mg of an A. flavus infected sample of copra (aflatoxin content 2 ppm) was extracted with 100 ml of methanol and evaporated to dryness. The dry residue was dissolved in one ml of absolute alcohol and diluted to 1:10, 1:20, 1:30 and 1:40 with alcohol. The diluted solutions were used for inoculating the eggs following the procedure of Verrett et al. (1964).

Fertile white leghorn eggs were cleaned with alcohol and incubated for four days. On the fifth day the eggs were taken out and inoculated with the toxin preparation by drilling the shell at the broad end by using a hypodermic syringe. The needle was passed through the air sac into the chorio allantoic membrane. An aliquot of 0.2 ml each of the

different concentrations of the toxin preparation was injected and the eggs were kept in a vertical position for 15 minutes. The drilled hole on the egg was smeared with alcohol and sealed with hot paraffin and incubated. Three eggs were used for each treatment. Eggs injected with 0.2 ml alcohol served as control. Eggs were candled daily for a period of 21 days so as to note the movement of the embryo. The number of days taken for the death of the embryo was noted and the observations were continued for 21 days.

Ducklings

Pieces of copra were artificially inoculated with *A. flavus* in bulk and stored under saturated humidity conditions at 28 - 30°C for 10 days. They were then finely powdered using a blender, made into pellets of one g each, autoclaved and kept ready for feeding trials.

A group of 37 numbers of one day old local variety of ducklings (approximate weight 50 g) were housed in well ventillated brooders along with control birds. The birds were force fed daily with the above sample of copra (one g each). Control groups were fed with one g of autoclaved uninoculated copra. This was in addition to normal feed. For the first four days the birds were fed on whole milk powder solution and mashed boiled eggs, after that they were maintained on starter ration for ducklings (Appendix X).

Daily observations on the casualities were recorded. The weight of the surviving birds was recorded at the end of the experimental period, viz., on the 30th day, and were killed by stunning. Liver and kidney of all the dead and killed birds were preserved in formaldehyde-saline mixture (Appendix XI) for histopathological studies.

Guinea pigs

Two groups of three guinea pigs each, weighing 400-500 g, were housed in cages kept in a well ventillated room. They were fed with germinated bengal gram and Hind lever ready made concentrate (Appendix XII). 1 g of copra infected by A. flavus as detailed earlier was force fed in addition to the normal feed for a continuous period of thirty days. Control group of animals were fed with the same quantity of uninfected copra and normal feed. Daily observations on casualities were recorded. At the end of the experimental period the surviving animals were killed by severing the jugular veins on either side. Autopsy was done for both dead and killed animals and liver and kidney were preserved in formaldehyde-saline mixture for histopathological studies.

Rats

Young male albino rats (Sprague-Dawley strain) with an average weight of 130 g were used. They were fed

with 2.5 g of copra infected with A. flavus for a period of thirty days. The control animals were fed with the same quantity of uninfected copra. They were maintained on a diet mixture (Appendix XIII). At the end of the experimental period the animals were killed and the liver and kidney removed and preserved in formaldehyde-saline mixture for histopathological studies.

Histopathological studies

Histopathological studies were conducted by the method of Humason (1972). After dehydration, clearing and embedding, microtome sections were taken and stained using Harris's alum haematoxylin and eosin (H & E) stain and examined under the microscope for lesions.

Penicillium citrinum toxins

Biological assay

Chick embryo

Five hundred mg of a P. citrinum infected sample (Citrinin content not known) was extracted with methanol and dilutions were prepared and used for inoculation purposes as in aflatoxins detailed earlier.

Ducklings

Pieces of copra were artificially inoculated in bulk

with P. citrinum as in inoculation studies for a period of ten days. They were then finely powdered in a waring blendor, made into pellets of one g each, autoclaved and used for feeding trials as in bioassay studies of aflatoxin in ducklings. Casualities were recorded each day. Surviving birds were destroyed on the 30th day. Liver and kidney of dead as well as killed were preserved in formaldehyde-saline mixture for histopathological studies.

Mixed infection of A. flavus and P. citrinum

Biological assay

Ducklings

Samples of copra in bulk were artificially inoculated with A. flavus and P. citrinum separately as in the previous studies. They were then macerated, mixed in equal quantities made into pellets of one g each autoclaved and fed to ducklings as detailed earlier. Casualities were recorded each day. Surviving birds were destroyed on the 30th day. Liver and kidney of dead as well as killed were preserved in formaldehyde-saline mixture for histopathological studies.

different concentrations of the toxin preparation was injected and the eggs were kept in a vertical position for 15 minutes. The drilled hole on the egg was smeared with alcohol and sealed with hot paraffin and incubated. Three eggs were used for each treatment. Eggs injected with 0.2 ml alcohol served as control. Eggs were candled daily for a period of 21 days so as to note the movement of the embryo. The number of days taken for the death of the embryo was noted and the observations were continued for 21 days.

Ducklings

Pieces of copra were artificially inoculated with *A. flavus* in bulk and stored under saturated humidity conditions at 28 - 30°C for 10 days. They were then finely powdered using a blender, made into pellets of one g each, autoclaved and kept ready for feeding trials.

A group of 37 numbers of one day old local variety of ducklings (approximate weight 50 g) were housed in well ventillated brooders along with control birds. The birds were force fed daily with the above sample of copra (one g each). Control groups were fed with one g of autoclaved uninoculated copra. This was in addition to normal feed. For the first four days the birds were fed on whole milk powder solution and mashed boiled eggs, after that they were maintained on starter ration for ducklings (Appendix X).

Daily observations on the casualities were recorded. The weight of the surviving birds was recorded at the end of the experimental period, viz., on the 30th day, and were killed by stunning. Liver and kidney of all the dead and killed birds were preserved in formaldehyde-saline mixture (Appendix XI) for histopathological studies.

Guinea pigs

Two groups of three guinea pigs each, weighing 400-500 g, were housed in cages kept in a well ventillated room. They were fed with germinated bengal gram and Hind lever ready made concentrate (Appendix XII). Four g of copra infected by A. flavus as detailed earlier was force fed in addition to the normal feed for a continuous period of thirty days. Control group of animals were fed with the same quantity of uninfected copra and normal feed. Daily observations on casualities were recorded. At the end of the experimental period the surviving animals were killed by severing the jugular veins on either side. Autopsy was done for both dead and killed animals and liver and kidney were preserved in formaldehyde-saline mixture for histo-pathological studies.

Rats

Young male albino rats (Sprague-Dawley strain) with an average weight of 130 g were used. They were force fed

with 2.5 g of copra infected with A. flavus for a period of thirty days. The control animals were fed with the same quantity of uninfected copra. They were maintained on a diet mixture (Appendix XIII). At the end of the experimental period the animals were killed and the liver and kidney removed and preserved in formaldehyde-saline mixture for histopathological studies.

Histopathological studies

Histopathological studies were conducted by the method of Humason (1972). After dehydration, clearing and embedding, microtome sections were taken and stained using Harris's alum haematoxylin and eosin (H & E) stain and examined under the microscope for lesions.

Penicillium citrinum toxins

Biological assay

Chick embryo

Five hundred mg of a P. citrinum infected sample (Citrinin content not known) was extracted with methanol and dilutions were prepared and used for inoculation purposes as in aflatoxins detailed earlier.

Ducklings

Pieces of copra were artificially inoculated in bulk

with P. citrinum as in inoculation studies for a period of ten days. They were then finely powdered in a waring blendor, made into pellets of one g each, autoclaved and used for feeding trials as in bioassay studies of aflatoxin in ducklings. Casualities were recorded each day. Surviving birds were destroyed on the 30th day. Liver and kidney of dead as well as killed were preserved in formaldehyde-saline mixture for histopathological studies.

Mixed infection of A. flavus and P. citrinum

Biological assay

Ducklings

Samples of copra in bulk were artificially inoculated with A. flavus and P. citrinum separately as in the previous studies. They were then macerated, mixed in equal quantities made into pellets of one g each autoclaved and fed to ducklings as detailed earlier. Casualities were recorded each day. Surviving birds were destroyed on the 30th day. Liver and kidney of dead as well as killed were preserved in formaldehyde-saline mixture for histopathological studies.

PREVENTION OF DETERIORATION

Use of food additives, antibiotics and chemicals

The following food additives, antibiotics and chemicals were tried to prevent the deterioration of copra by different microorganisms.

(a)	Common salt	1.0, 1.5, 2.0 per cent
(b)	Citric acid	,,
(c)	Tartaric acid	,,
(d)	Sodium metabisulphate	0.5, 1.0, 1.5 per cent
(e)	Sodium benzoate	,,
(f)	Streptocycline	250, 500, 1000 ppm
(g)	Nebasulf	,,
(h)	Aureofungin	,,
(i)	Penicillin	,,
(j)	Calcium carbonate	0.5, 1.0, 1.5 per cent
(k)	Calcium chloride	,,
(l)	Sodium carbonate	4.0, 5.0, 5.5 per cent
(m)	Acetic acid	4.0, 5.0, 6.0 per cent

Copra was dipped in the above solutions for half an hour on the third day when the kernel separated from the shell and again sun-dried till the moisture reduced to about five per cent. After that they were stored under laboratory conditions in gunny bags lined with polythene

paper (200 gauge). Observations were made at periodical intervals of 15, 30, 60 and 90 days. The observations were confined to visual examination of the samples for fungal infection.

ASSAY OF MAMMALIAN TOXICITY OF ADDED PRESERVATIVES

Short term studies on feeding of treated copra to rabbits, guinea pigs and rats were conducted for a continuous period of 60 days (Rao et al., 1964 and Kamble and Schonie, 1968). They were grouped and each group contained three animals. The animals were fed on normal diet (Appendix XII, XIII) and force fed with different quantities of copra treated with the highest concentration of chemicals used for preservation studies.

Treatment No.	Details of treatments
Group I	Normal diet + copra treated with 1000 ppm streptocycline
Group II	Normal diet + copra treated with 5.5 per cent sodium carbonate
Group III	Normal diet + copra treated with six per cent acetic acid

Rats, guinea pigs and rabbits were given treated copra at the rate of 1.5, 2.5 and 3.5 g respectively. Control animals were fed the same quantity of untreated copra. The initial weights of the animals were recorded. Casualities were observed. The weights of the animals were recorded after the end of the experimental period. The animals that survived the

experimental period were killed and post mortem examination was conducted for gross lesions. Liver and kidney were preserved in formaldehyde-saline mixture for histopathological studies.

RESULTS

RESULTS

Quantitative and qualitative determination of the microbial population in copra and coconut oil cake

Copra.

Fungal population

From the ANOVA given in Appendix XIV and Fig. 1a it is observed that the effects of mill (source), period and interaction between period and mill on the fungal population are significant. The mean values of the fungal population are given in Table 1. The maximum fungal population is observed during the month of August. The population recorded during the months of July, September and November are not significantly different from that of August. The population recorded for the months of March, April and May are significantly lower than those for the period from June to December. The lowest population is recorded in February and it is on par with that of January. Among the mills, significant difference in the population of fungi is observed. The fungi (Plate I, II) observed on copra during different periods are

1. *Aspergillus niger* Van Tiegh
2. *A. flavus* Link ex Fries
3. *A. flavus* Link ex Fr. Sclerotial isolate

Table 1. Mean fungal population in copra collected from different sources (oil mill) during different periods of the year (log count 10^{-6}/g copra)

(January - December 1976)

Period	Source*				
	M 1	M 2	M 3	M 4	Mean
January	0.8303	0.8431	0.8319	0.9625	0.8796
February	0.6927	0.8833	1.0166	0.7483	0.8353
March	0.8958	0.9929	1.0144	0.8725	0.9189
April	1.0080	0.9981	1.0570	1.2577	1.0803
May	0.9015	0.9776	1.0955	0.9272	0.9922
June	1.2953	1.2938	1.3692	1.3958	1.3385
July	1.5224	1.4541	1.4262	1.4891	1.4730
August	1.3952	1.4819	1.4747	1.5704	1.4806
September	1.3897	1.4689	1.4661	1.4586	1.4464
October	1.3143	1.4230	1.3336	1.3551	1.3569
November	1.3326	1.4283	1.4036	1.5428	1.4279
December	1.2396	1.3276	1.3906	1.4076	1.3539
Mean	1.1611	1.2061	2.4470	1.2491	

*Copra collected from different oil mills

C.D. periods = 0.0658
C.D. mills = 0.0380
C.D. combinations of period and mill = 0.1317

4. Rhizopus stolonifer (Ehren. ex Fr.)Lind
5. R. oryzae Went & Prinsen Geerligs
6. Muoor hiemalis Wehmer
7. Botryodiplodia theobromae Pat
8. Penicillium citrinum Thom
9. Curvularia senegalensis (Speg.) Subram
10. Cochliobolus lunatus Nelson & Haasis
11. Paecilomyces lilacinus (Thom) Samson
12. A. ochraceous Wilhelm
13. A. tamarii Kita
14. A. chevalieri (Mangin) Thom & Church
15. A. oryzae Ahlburg Cohn
16. A. fumigatus Fresenius

*

Bacterial population

From the ANOVA in Appendix XV and Fig. 1b it is observed that the effects of period and interactions between mill (source) and period are significant. The mean values on bacterial population are given in Table 2. The maximum bacterial population is observed during November and the minimum during January. The population recorded during January, February, March and April is not significantly different from each other. The population for the rest of

---

*The most commonly encountered are the first nine fungi listed above.

Table 2. Mean bacterial population in copra collected from different sources (oil mill) during different periods of the year (log count x 10^{-6}/g copra)

Period	Source*				
	M 1	M 2	M 3	M 4	Mean
January	1.6193	1.6540	1.7313	1.7166	1.6804
February	1.8369	1.9095	1.6994	1.6895	1.7838
March	1.7075	1.6978	1.7656	1.6010	1.6930
April	1.7220	1.7042	1.6551	1.7616	1.7155
May	1.8766	1.8162	1.8944	1.8973	2.0378
June	2.2402	2.1906	2.1851	2.2124	2.2071
July	2.3022	2.1441	2.1210	1.9978	2.1404
August	2.2987	2.2641	2.3593	2.3117	2.3084
September	2.2214	2.1678	2.3595	2.2110	2.2399
October	2.1890	2.1747	1.9757	2.0024	2.0854
November	2.4138	2.3767	2.2777	2.2176	2.3215
December	2.2479	2.1124	1.9871	2.0685	2.1040
Mean	2.0563	2.0174	1.9993	1.9756	

*Copra collected from different oil mills

C.D. periods = 0.1117

FIG.1 a. FUNGAL POPULATION ON COPRA COLLECTED FROM DIFFERENT SOURCES (OIL MILL) DURING DIFFERENT PERIODS.

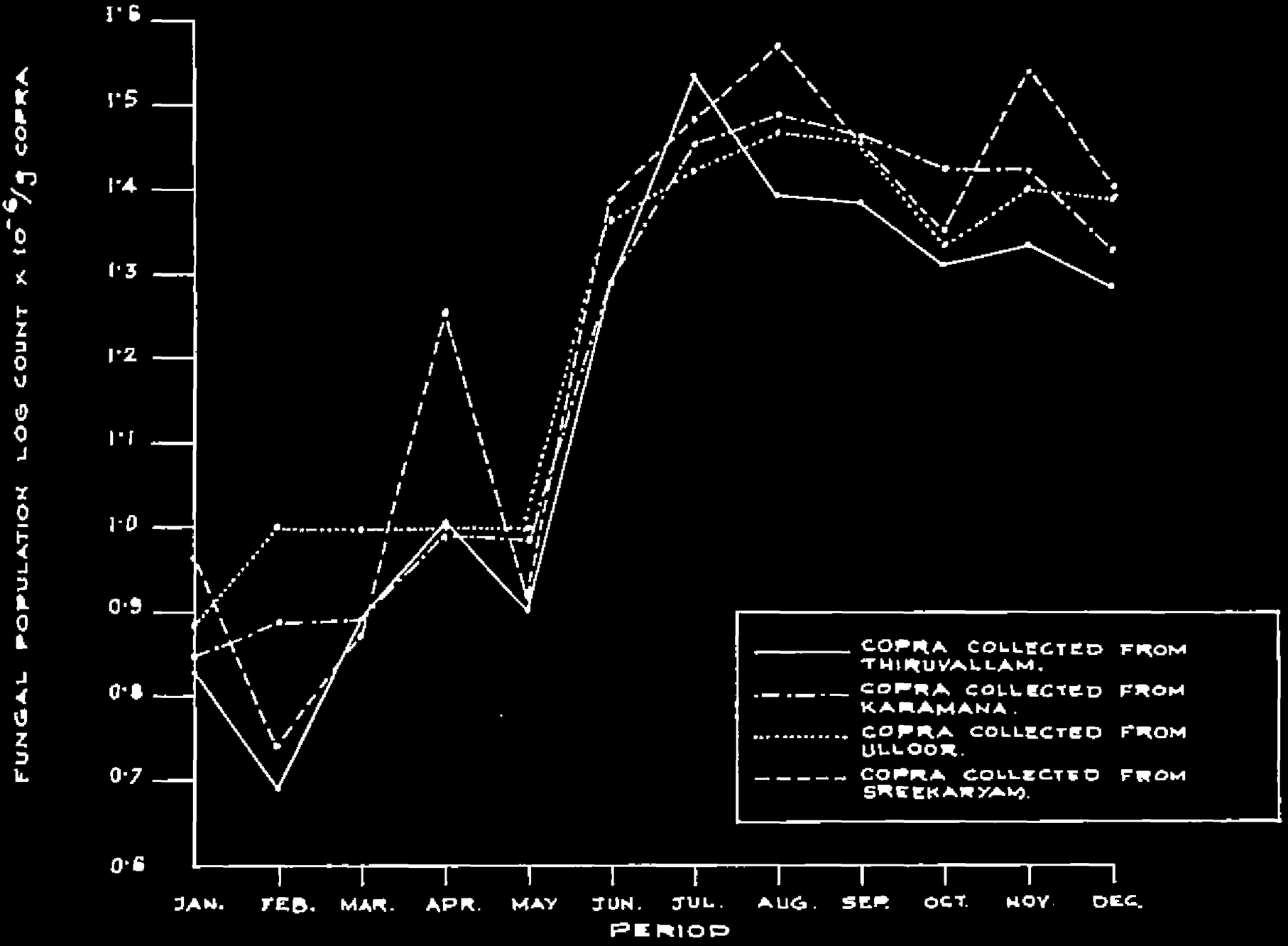


FIG.1 b. BACTERIAL POPULATION ON COPRA COLLECTED FROM DIFFERENT SOURCES DURING DIFFERENT PERIODS.

the period is significantly higher. The population fluctuated and did not reveal any definite pattern. However, the bacterial population during August, September and November is significantly higher than other periods of observation.

The effect of mill (source) on the bacterial population is not significant. The predominant bacteria are:-

1. Bacillus subtilis (Ehrenberg) Cohn
2. Enterobacter aerogenes (Kruse) Hormaeche & Edwards
3. E. aerogenes non-aerogenic strain
4. Pseudomonas fluorescens (Trevisan) Migula Biotype of G of Stainer et al.
5. Staphylococcus aureus Rosenbach
6. Serratia marcescens Bizio
7. Saroina lutea Schroeter

Actinomycete population

The incidence of the actinomycete population is presented in Table 3. The data could not be statistically analysed as the actinomycetes are not present during certain periods. There is no definite pattern in the occurrence of actinomycetes and its population fluctuated widely. The population is minimum during November and is high during June, the mean count being 0.9999 and 3.4166 respectively.

Table 3. Mean actinomycete population in copra collected from different sources (oil mill) during different periods of the year (log count x 10^{-6}/g copra)

Period	Source*				
	M 1	M 2	M 3	M 4	Mean
January	1.0000	2.6666	1.3333	1.6666	1.6666
February	2.6666	4.6666	2.0000	3.0000	3.0833
March	2.0000	0.0000	2.6666	0.0000	1.8333
April	0.6666	5.3333	1.0000	4.3333	2.8333
May	1.6666	2.6666	1.3333	2.3333	1.9999
June	3.6666	3.0000	4.3333	2.6666	3.4166
July	4.0000	1.3333	3.3333	1.0000	2.4166
August	0.0000	2.0000	2.0000	1.6666	1.4166
September	3.0000	1.6666	1.0000	1.3333	1.7499
October	0.6666	3.0000	1.3333	2.0000	1.7499
November	1.6666	0.0000	1.0000	1.3333	0.9999
December	2.6666	1.0000	1.0000	1.0000	1.4166
Mean	1.9721	2.4999	1.8610	1.8610	

*Copra collected from different oil mills

Coconut oil cake

Fungal population.

Results are presented in Appendix XVI and Table 4. There is no significant difference in the fungal population of oil cake during the different periods. The effect of mill (source) on the fungal population is also not significant.

The predominant fungi are:-

1. Aspergillus niger Van Tiegh
2. A. flavus Link ex Fries
3. Rhizopus stolonifer (Ehren. ex. Fr.) Lind
4. Penicillium citrinum Thom

Bacterial population.

Results are presented in Appendix XVII and Table 5. Significant differences in the bacterial population of oil cake are observed during different periods. Maximum bacterial population is observed during the month of July. The population recorded during the months of June, September, October, November and December are not significantly different. The population for the rest of the period is low. The bacteria observed are:-

1. Bacillus subtillis (Ehrenberg) Cohn
2. Staphylococcus aureus Rosenbach

Actinomycete population.

The actinomycetes are not observed during any period

Table 4. Mean fungal population in coconut oil cake collected from different sources (oil mill) during different periods of the year (log count x 10^{-4}/g cocoonut oil cake)

Period	Moisture per cent	Source* M 1	M 2	M 3	M 4	Mean
January	2.92	0.3766	0.3096	0.4857	0.3075	0.3698
February	2.92	0.2304	0.4346	0.5717	0.3096	0.3865
March	2.92	0.3096	0.2304	0.3118	0.2304	0.2705
April	2.96	0.3096	0.3139	0.3747	0.3096	0.3269
May	2.98	0.3766	0.4857	0.3784	0.5315	0.4430
June	3.41	0.4857	0.3766	0.3784	0.4857	0.4316
July	3.47	0.7356	0.5315	0.3747	0.4346	0.5191
August	3.05	0.4330	0.4857	0.3747	0.4346	0.4320
September	3.42	0.7348	0.6454	0.5705	0.6776	0.6570
October	3.50	0.7067	0.7356	0.6767	0.7396	0.7146
November	3.58	0.5302	0.4654	0.4362	0.5729	0.5010
December	3.42	0.5302	0.5302	0.5514	0.5315	0.5358
Mean	3.21	0.4799	0.5462	0.4570	0.4637	

*Coconut oil cake collected from different oil mills

Table 5. Mean bacterial population in cocoonut oil cake collected from different sources (oil mill) during different periods of the year (log count x 10^{-4}/g coconut oil cake)

Period	Moisture per cent	Source* M 1	M 2	M 3	M 4	Mean
January	2.92	1.1458	1.0927	1.0820	0.9763	1.0742
February	2.92	1.1548	1.3096	1.0824	1.0042	1.1377
March	2.92	1.0070	1.0973	1,0973	1.1221	1.0809
April	2.96	1.1466	1.1489	1.1331	1.1015	1.1325
May	2.98	1.2742	1.3122	1.3105	1.2920	1.2972
June	3.41	1.3150	1.3128	1.3765	1.3163	1.3301
July	3.47	1.3211	1.3564	1.3966	1.3026	1.3441
August	3.05	1.1436	1.0051	1.1238	1.0955	1.0920
September	3.42	1.2717	1.3118	1.2155	1.1553	1.2385
October	3.50	1.3151	1.3422	1.3417	1.3002	1.3248
November	3.58	1.2733	1.3370	1.3575	1.3096	1.3187
December	3.42	1.3060	1.2934	1.2446	1.2581	1.2755
Mean	3.21	1.2228	1.2432	1.2301	1.1861	

*Cocoonut oil cake collected from different oil mills

C.D. periods = 0.1125

of the year in the samples collected from different mills (source).

Inoculation studies of microorganisms isolated from samples of copra

All the microorganisms isolated from copra were inoculated separately on fresh copra and the results recorded. The following microorganisms are found to be capable infecting copra resulting in its deterioration.

Fungi

1. Aspergillus niger
2. A. flavus
3. A. tamarii
4. A. ochraceous
5. A. chevalieri
6. A. oryzae
7. Mucor hiemalis
8. Rhizopus oryzae
9. R. stolonifer
10. Penicillium citrinum
11. Botryodiplodia theobromae
12. Curvularia senegalensis

Bacteria

1. Staphylococcus aureus
2. Bacillus subtilis
3. Enterobacter aerogenes

4. Pseudomonas fluorescens

It is observed that Streptomyces sp. isolated could not infect copra on artificial inoculation.

INFLUENCE OF WEATHER ELEMENTS AND MOISTURE CONTENT OF COPRA ON THE MICROBIAL POPULATION

Path coefficient studies

The data on rainfall, relative humidity, minimum temperature (average of ten days prior to the collection of samples) and moisture content of copra along with microbial population over a continuous period of 12 months are presented in Table 6, Fig. 2. It is observed that relative humidity of the atmpsphere and moisture content of copra are the important factors which influenced the occurrence of the microflora on copra. Relative humidity and moisture content of copra are in turn influenced by rainfall (Table 39, 40, 41, 42).

Moisture content and succession of microflora during copra making

The results are presented in Table 7. At the time of splitting the coconuts the kernel is sterile and recorded a moisture content of about 51.16 per cent. On sun-drying for a day (about six hours) the moisture content is reduced to about 28.39 per cent and bacteria alone are observed on the kernels. On further drying, the moisture level decreases

Table 6. Relation of fungal and bacterial population in copra to the weather elements (average of ten days prior to the collection of samples) and the moisture content of copra during different periods

Period	Microbial population (10^{-6}/g copra)		Rainfall (mm)	Per cent relative humidity	Minimum temperature ^{o}C	Per cent moisture content
	Fungal	Bacterial				
January	0.8796	1.6804	0.00	76.40	21.25	6.10
February	0.8353	1.7838	0.00	73.50	22.09	5.91
March	0.9189	1.6930	0.00	72.20	21.84	5.70
April	1.0803	1.7155	3.40	78.50	24.28	6.41
May	0.9922	2.0378	0.00	77.10	25.20	6.41
June	1.3385	2.2071	1.85	78.30	24.20	7.24
July	1.4730	2.1404	4.12	83.80	23.08	7.33
August	1.4806	2.3034	0.51	87.80	23.61	7.11
September	1.4464	2.2399	10.80	91.40	22.73	7.58
October	1.3569	2.0854	3.02	80.90	23.62	7.95
November	1.4279	2.3215	9.06	82.80	23.20	6.90
December	1.3539	2.1040	9.04	90.10	23.28	6.42

FIG.2. RELATION OF WEATHER ELEMENTS (AVERAGE OF TEN DAYS PRIOR TO THE COLLECTION OF SAMPLES) AND MOISTURE - CONTENT OF COPRA DURING DIFFERENT PERIODS.

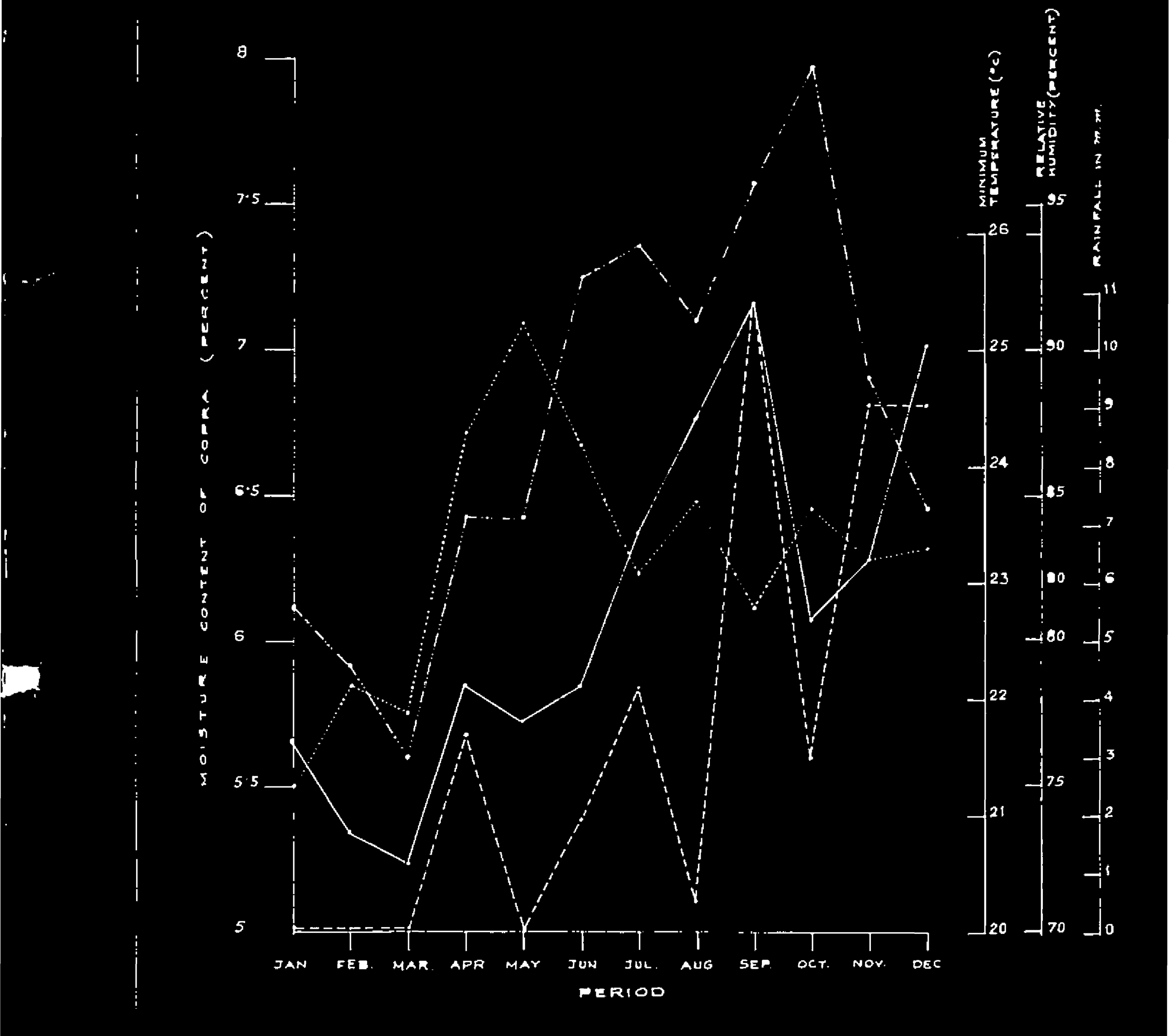


Table 7. Moisture content of coconut kernel (whole cups) and succession of microflora during copra making (100 nuts sun-dried for one week)

Time of observation (Hour)	Temperature °0		Moisture content (per cent)	Succession of microflora
	maximum	Minimum		
0	-	-	51.16	-
24	30.5	21.3	28.39	Serratia marcescens Staphylococcus aureus
48	31.5	21.3	20.31	Rhizopus stolonifer R. oryzae Mucor hiemalis
72	30.6	22.1	17.50	Aspergillus niger A. flavus
96	31.1	23.0	13.58	A. oryzae A. tamarii A. ochraceous A. chevalieri
120	31.1	21.2	10.75	Botryodiplodia theobromae Curvularia senegalensis
144	32.2	19.9	7.50	,,
168	30.0	20.8	5.00	Penicillium citrinum

followed by a decrease in the bacterial population. A concomitant incidence of a fungal population consisting of Mucor hiemalis and Rhizopus spp. is observed (Plate III). As the moisture content decreases further incidence of Aspergillus spp. predominated (Plate IV) followed by B. theobromae and C. senegalensis (Plate V). Their incidence continued to be present till copra attains a moisture content of 7.5 per cent. On fully dried kernels P. citrinum alone is observed. The results also show that a moisture content of 20.31 per cent is favourable for the incidence of species of Mucorales while a moisture per cent of 17.50 to 7.50 is highly suited for the luxuriant growth of Aspergillus spp. On dried kernels (copra) having five per cent moisture, only P. citrinum could survive (Plate VI).

KEEPING QUALITY OF COPRA UNDER DIFFERENT STORAGE CONDITIONS

Effect of temperature

The data on the effect of temperature on the natural infection of copra by fungi are presented in Table 8. The fungal infection is minimum at low temperature. The intensity of infection by fungi increases with increase in temperature.

Table 8. Effect of temperature on the keeping quality of copra. About five per cent moisture level (Mean of three replications)

June - July 1977

Temperature $^{o}C \pm 1$	Period of observation (day)				Per cent moisture content of copra after 60 days
	7	15	30	60	
10	-	-	-	-	5.4
20	-	-	+	+	5.6
25	-	+	++	++	5.8
(Control 28-30°C Laboratory conditions)	+	++	+++	+++	8.3

\- No fungal infection on the surface of copra

\+ Surface fungal infection on copra upto 5 per cent

++ " " 20 "

+++ " " 50 per cent and above

In samples kept at 10°C (± 1) invasion by fungi is not noticed even after 60 days of storage, while in samples kept at 20°C (± 1) P. citrinum appeared by 30 days of storage and continued to be present. Samples kept at 25°C (± 1) also showed invasion of P. citrinum after 15 days of storage. The intensity of infection increased with storage. Storage of samples (control) at room temperature (28 - 30°C) also showed invasion by P. citrinum at 7 and 15 days followed by A. flavus, A. tamarii and A. niger during 30 to 60 days of storage.

Effect of relative humidity

Results are presented in Table 9. No fungal infection is noticed when copra is stored at an atmospheric humidity of 50 per cent. At 60 per cent humidity slight infection by P. citrinum is noticed by 60 days of storage. At 70 per cent relative humidity infection by P. citrinum is noticed from 30 days onwards while at 80 and 90 per cent relative humidity P. citrinum is noticed by the 7th day itself. By 15th day Aspergillus spp. viz., A. flavus, A. niger, A. tamarii as well as B. theobromae and C. senegalensis showed their appearance. In control as well as samples kept under saturated humidity conditions (100 per cent) P. citrinum is noticed at first followed by Aspergillus spp., B. theobromae and C. senegalensis.

Table 9. Effect of relative humidity on the keeping quality of copra. About five per cent moisture level (Mean of three replications)

June - July 1977

Per cent relative humidity	Period of observation (day)				Per cent moisture content of copra after 60 days storage
	7	15	30	69	
50	-	-	-	-	5.00
60	-	-	-	+	5.05
70	-	-	+	++	6.82
80	+	++	+++	+++	8.20
90	+	++	+++	+++	8.35
Saturated humidity (100)	+	+++	+++	+++	8.30
Control (80 - 82)	+	++	+++	+++	8.25

\- No fungal infection on the surface of copra

\+ Surface fungal infection on copra upto 10 per cent

++ ,, ,, 30 per cent

+++ ,, ,, 50 per cent and above

Effect of moisture content of copra

The observations are presented in Table 10. No infection is noticed during storage of copra having 3-4 per cent moisture content. At 5-6 per cent moisture content, slight infection by P. citrinum is noticed after 30 days of storage and thereafter.

At 7-8 per cent moisture level infection is slight after 7 days and increased considerably with storage period. Fungi observed included A. flavus, A. niger, A. tamarii, B. theobromae and C. senegalensis. At the moisture level of 9-10 per cent severe infection is noticed from the seventh day onwards. A. flavus, A. niger, B. theobromae and C. senegalensis are found associated.

Effect of storage practices

Results are presented in Table 11. Maximum infection is noticed in copra heaped in godowns. Infection by P. citrinum is observed by the first month itself. Infection increased with the period of storage and reached maximum by 60 days. At 60, 90 and 180 days of observation the fungi present included A. niger, A. flavus, A. tamarii and B. theobromae. Copra kept in gunny bags showed lesser infection, the microflora obtained being P. citrinum at 30 days of storage and A. flavus and A. niger are noted after 60, 90 and 180 days of storage. Copra kept in gunny bags lined with polythene

Table 10. Effect of moisture content of copra on the keeping quality. (Mean of three replications)

June - July 1977

Moisture content (per cent)	Period of observation (day)				Moisture per cent after 60 days storage
	7	15	30	60	
3 - 4	-	-	-	-	4.20
5 - 6	-	-	+	+	5.50
7 - 8	+	++	+++	+++	8.43
9 - 10	+++	+++	+++	+++	8.59

\- No fungal infection on the surface of copra

\+ Surface fungal infection on copra up to 5 per cent

++ " " 25 "

+++ " " 50 per cent and above

Table 11. Effect of storage practices on the keeping quality of copra. Above five per cent moisture level (Mean of three replications)

June - November

Storage practice	Period of observation (day)				Moisture per-cent after 180 days storage
	30	60	90	180	
Heaped in godown	++	+++	+++	+++	8.55
Kept in gunny bags	+	++	++	++	8.00
Kept in gunny bags lined with polythene paper	-	+	+	++	5.8

\- No fungal infection on the surface of copra

\+ Surface ,, copra upto 10 per cent

++ ,, ,, ,, 25 ,,

+++ ,, ,, ,, 50 per cent and above

paper was free of infection upto 30 days and thereafter showed slight to moderate infection by P. citrinum alone.

Increase in moisture content is noticed in all samples kept under different storage practices (Table 8, 9, 10, 11) as evidenced from the final moisture content.

BIOCHEMICAL CHANGES IN COPRA AND COCONUT OIL DUE TO FUNGAL INFECTION

Copra

Changes in total sugars due to fungal infection on copra.

From the ANOVA presented in Appendix XVIII and Fig. 3a it is evident that the effects of period of incubation and fungi on total sugars of copra are significant. The mean values of the total sugar content of copra are given in Table 12. Significant difference in total sugars due to infection by different fungi as compared to control are observed. The sugar content is reduced as the period of inoubation is increased. The effects of infection of copra by A. flavus, R. oryzae and B. theobromae are on par. Similarly A. niger, M. hiemalis and C. senegalensis are also similar in their effects and are on par.

Effect of fungal infection on protein content

The results are presented in Appendix XIX and Table 13. There is no significant difference in the protein content of

Table 12. Mean value of total sugars due to fungal infection of copra (mg/g) of copra on moisture free basis)

Fungus tested	Days after incubation			
	7	14	21	Mean
A. niger	0.2132	0.1152	0.0592	0.1292
A. flavus	0.1140	0.0534	0.0377	0.0670
R. oryzae	0.0913	0.0539	0.0337	0.0596
M. hiemalis	0.1718	0.1397	0.0257	0.1124
P. citrinum	0.1937	0.1937	0.1718	0.1864
B. theobromae	0.0456	0.0350	0.0305	0.0370
C. senegalensis	0.2370	0.0926	0.0558	0.1284
Control	0.2470	0.2470	0.2470	0.2470
Mean	0.1642	0.1163	0.0321	

C.D. periods = 0.0677
C.D. fungi = 0.0429

FIG. 3a. EFFECT OF INFECTION OF COPRA BY FUNGI ON THE TOTAL SUGAR CONTENT

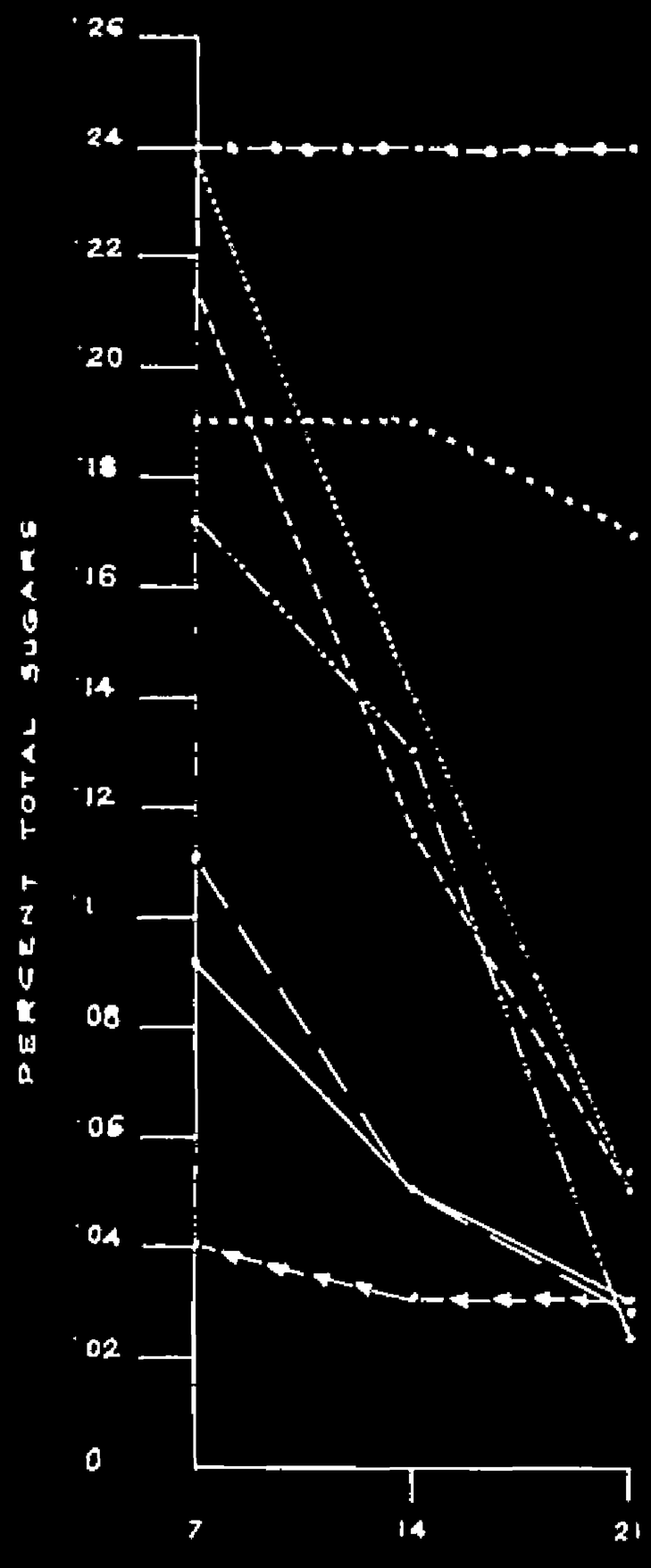
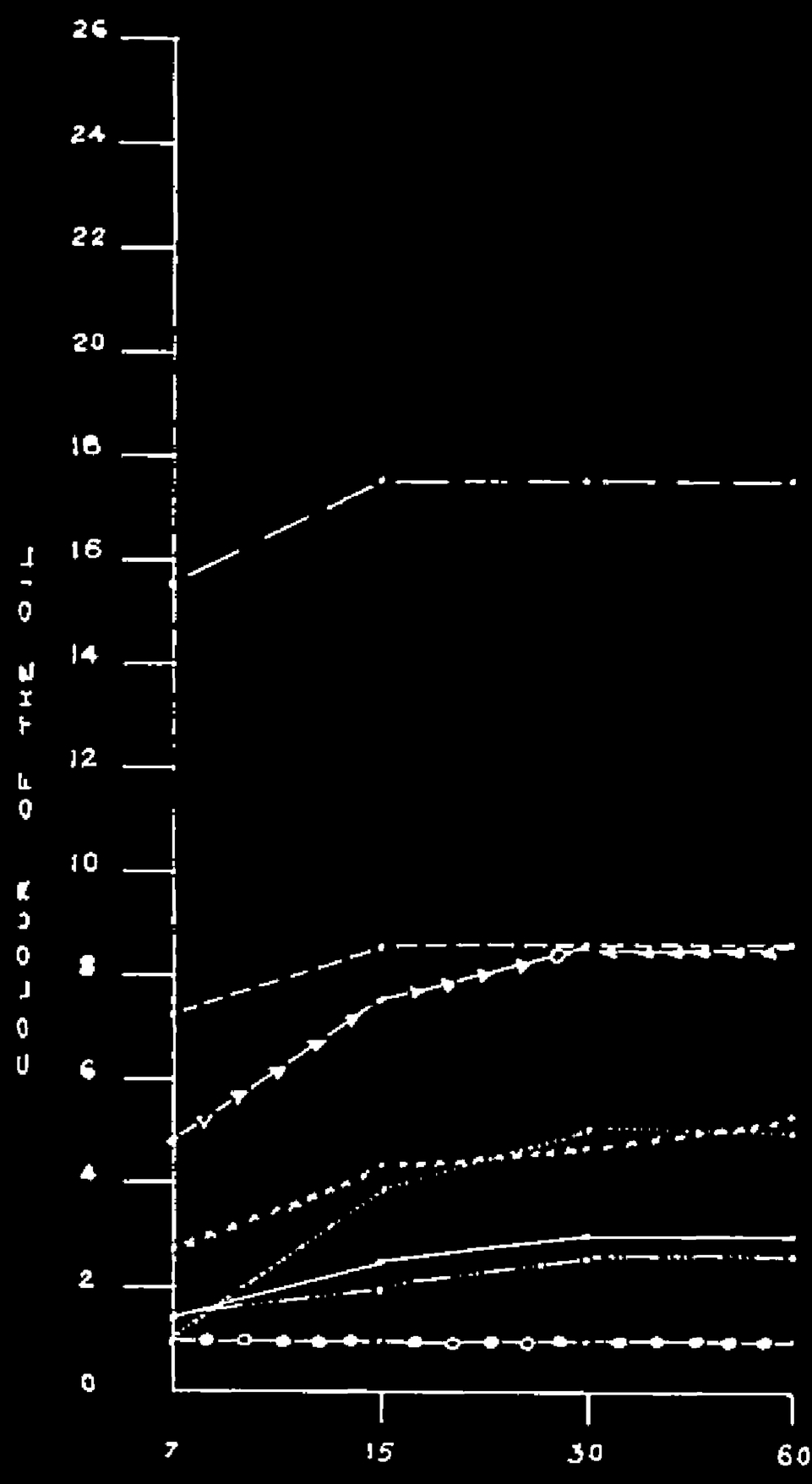


FIG. 3b. EFFECT OF INFECTION OF COPRA BY FUNGI ON THE COLOUR OF OIL



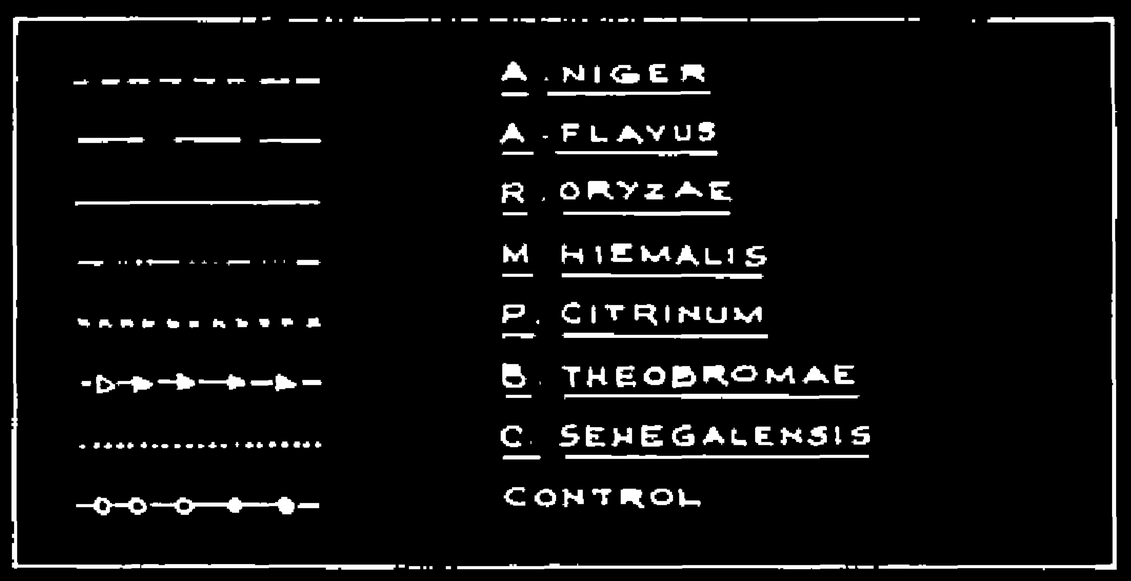


Table 13. Mean value of protein content (in per cent) due to fungal infection of copra N X 6.25

Fungus tested	Period of incubation (day)				
	7	15	30	60	Mean
A. niger	8.1000	8.2450	8.5000	8.6323	8.3693
A. flavus	8.1250	8.4375	8.5735	8.6854	8.4553
R. oryzae	7.8125	8.1250	8.2575	8.3523	8.1368
M. hiemalis	7.5000	8.1250	8.5350	8.7521	8.2280
P. citrinum	8.2500	8.3128	8.4875	8.5030	8.3882
B. theobromae	8.0000	8.1235	8.2535	8.3015	8.1696
C. senegalensi	8.1250	8.2500	8.5000	8.6000	8.3687
Control	8.2500	8.2500	8.2500	8.2500	8.2500
Mean	8.0203	8.2335	8.4196	8.5095	

C.D. periods = 0.3870

copra due to infection by different fungi. A slight reduction in protein content is observed after 7 days of incubation. After 15, 30 and 60 days of incubation of copra inoculated with different fungi, an increase in the protein content is observed and it is on par.

Effect of fungal infection on amino acid content

The amino acid content of copra samples due to fungus infection is given in Table 14. A statistical comparison is not possible in this case. Hence a comparison with control is made based on a variation of 20 per cent on either side of the control value. Values which have a positive variation of 20 per cent or above are marked with + sign and values having a negative variation of 20 per oent or above are marked with - sign.

It can be seen that B. theobromae infected samples does not have variation with control to the tune of 20 per-cent in respect of any of the amino acids. Copra samples infected by M. hiemalis and C. senegalensis showed a similar amino acid pattern and these samples recorded lower amino acid contents than the control. Samples infected by R. oryzae, in general, is found to have greater quantities of amino acids when compared to the control (Table 15). Samples infected by A. niger is found to have a similar pattern compared to the control and show greater quantities of

Table 14. Effect of infection by fungi on the amino acid content of copra (g amino acid per 100 g protein)

	I	II	III	IV	V	VI	VII	VIII
Aspartic acid	8.1357	5.5747	9.2163	3.2403		7.1818	4.0129	7.1804
Glutamic acid	21.2583	18.0829	17.7173	8.2366	29.1826	18.1331	7.0951	17.7684
Hydroxy proline	—	—	—	—				
Threonine	3.5754	2.3879	3.4770	1.1200	3.2536	2.5319	1.5309	2.1551
Serine	3.3801	2.7090	3.6302	1.4058	4.0105	3.2742	1.7993	2.8372
Proline								
Alanine	4.1999	3.5425	5.2004	1.7653	4.4667	3.6420	2.2707	3.9540
Glycine	10.2003	8.9407	6.1453	4.1607	12.4760	4.6543	2.4266	4.4183
Valine			5.9632			4.4455	1.2276	3.9157
Isoleucine	3.4433	2.0923	4.8877	1.7243	4.1692	3.2330	1.7082	2.7127
Leucine	6.3705	6.9555	8.0265	2.6176	7.4421	6.0748	3.2465	5.4872
Tyrosine	2.2724	1.7688	2.8901	1.5238	1.8597	2.3442	1.7378	2.8403
Phenylalanine	5.0481	3.8978	5.6749	2.2903	4.9597	4.8000	2.3152	5.8833
Lysine	4.0990	3.0410	3.9990	1.6275	3.6020	3.8399	1.4603	4.6085
Histidine	0.00000	0.8257	1.0779	0.0000	0.4433	1.6644	0.3950	1.9178
Ammonia (*)	9.1212	5.3289	8.8913	8.4224	6.9046	5.5907	3.9172	6.7545
Arginine	14.2833	13.5786	18.2393	6.5741	14.5456	17.5039	3.8094	20.7667

(*) Ammonia is expressed as ammonium chloride Instrument: PERKIN ELMER Model KLA 3B

I. A. niger
II. A. flavus
III. R. oryzae
IV. M. hiemalis
V. P. citrinum
VI. B. theobromae
VII. C. senegalensis
VIII. Control

Table 15. Effect of infection by fungi on the amino acid content of copra (after grading)

Fungus	Amino acid												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. niger	0	+	0	0	+	-	0	0	0	0	0	-	-
A. flavus	0	0	0	0	0	-	+	-	-	-	-	-	-
R. oryzae	0	+	+	+	+	+	+	0	0	0	-	+	0
M. hiemalis	-	-	-	-	-	-	-	-	-	-	-	+	-
P. citrinum	0	+	+	0	+	+	+	-	0	-	0	-	-
B. theobromae	0	0	0	0	0	0	0	0	0	0	0	0	0
C. senegalensis	-	-	-	-	-	-	-	-	-	-	-	-	-

Grades

0 = Within ± 20% of control

\- = Below 80% of control

\+ = Above 120% of control

1. Aspartic acid
   Glutamic acid
2. Hydroxy proline
   Threonine
3. Serine
   Proline
4. Alanine
5. Glycine
   Valine
6. Isoleucine
7. Leucine
8. Tyrosine
9. Phenyl alanine
10. Lysine
11. Histidine
12. Ammonia
13. Arginine

hydroxy proline and threonine, glycine and valanine and isoleucine and decreased quantities of arginine. Arginine content of copra samples is reduced due to fungus infection in all cases. It is also significant to note that histidine is not detected in samples of copra infected by A. niger and M. hiemalis (Table 14).

In the 6 N HCl hydrolysate apart from the amino acids estimated ammonia present as ammonium chloride has also been determined and expressed as g of ammonium chloride per 100 g protein.

Coconut oil

Colour of the oil

From the ANOVA in Appendix XX and Fig. 5b it is observed that the effects due to periods and fungi are significant on colour production. The mean values are presented in Table 16. There is significant change in the colour of the oil as the period of incubation is increased.

Among the fungi tested A. flavus produced the maximum colour to the oil and it is significant over the colour produced by other fungi. R. oryzae and M hiemalis produced the minimum colour change of oil. All the fungi produced significantly higher variation in colour as compared to the control.

Table 16. Effect of infection of copra by fungi on the colour of oil (Y 5R) units.(Mean of three replications)

Fungus tested	Colour of the oil Period of incubation (day)				Mean
	7	15	30	60	
A. niger	7.4	8.4	8.4	8.4	8.15
A. flavus	15.5	17.5	17.5	17.5	17.00
R. oryzae	1.5	2.5	3.0	3.0	2.50
M. hiemalis	1.5	2.0	2.5	2.5	2.13
P. citrinum	2.8	4.2	4.7	5.2	4.23
B. theobromae	4.9	7.4	8.4	8.4	7.28
C. senegalensis	1.0	3.9	5.0	5.0	3.72
Control	1.0	1.0	1.0	1.0	1.0
Mean	4.48	5.86	6.31	6.38	

C.D. period = 1.12

C.D. fungi = 0.79

(b) Odour of oil

The results are presented in Table 17. A rancid smell is detectable in all most all the samples of oils extracted from copra infected by various fungi. This is evident seven days after incubation in the case of A. flavus. The oil obtained from copra infected with M. hiemalis produced a smell reminiscent of fermented material. The rancid smell is prominent only after 15 days of incubation in the case of A. niger, R. oryzae and B. theobromae. Copra infected with P. citrinum and C. senegalensis have normal smell after incubation for seven days. However the smell is slightly rancid by 15 days of incubation onwards. The data clearly revealed that oil extracted from copra infected with fungi rapidly assumes a ranoid smell.

Refractive index

The results are presented in Table 18. The refractive indices of different samples of oil did not show any appreciable difference from the standard specifications of 1.4480 to 1.4490.

Acid value

From the ANOVA given in Appendix XXI, Table 19 and Fig. 4a it is observed that effects of period of incubation and fungal infection of copra on the acid value of oil are significant in all the infected samples; the acid value of

Table 17. Effect of infection of copra by fungi on the odour of oil (by smell)

Fungus tested	Odour of the oil			
	Period of incubation (day)			
	7	15	30	60
A. niger	Slightly rancid	Rancid	Rancid	Rancid
A. flavus	Rancid	Highly rancid	Highly rancid	Highly rancid
R. oryzae	Slightly rancid	Rancid	Rancid	Rancid
M. hiemalis	Fermented smell	Fermented smell	Fermented smell	Fermented smell
P. citrinum	-	Slightly rancid	Slightly rancid	Slightly rancid
B. theobromae	Slightly rancid	Rancid	Rancid	Rancid
C. senegalensis	-	Slightly rancid	Slightly rancid	Slightly rancid
Control	-	-	-	-

- = Normal smell

Table 18. Refractive index of oil at 40°C as affected by various fungi causing deterioration of copra (Mean of three replications)

Fungus tested	Refractive index			
	Period of incubation (day)			
	7	15	30	60
A. niger	1.4482	1.4484	1.4484	1.4485
A. flavus	1.4480	1.4482	1.4482	1.4484
R. oryzae	1.4487	1.4487	1.4488	1.4488
M. hiemalis	1.4487	1.4489	1.4489	1.4489
P. citrinum	1.4485	1.4485	1.4485	1.4485
B. theobromae	1.4480	1.4481	1.4481	1.4481
C. senegalensis	1.4488	1.4488	1.4489	1.4489
Control	1.4483	1.4483	1.4485	1.4485

Table 19. Effect of infection of copra by fungi on the acid value of the oil (Mean of three replications)

Fungus tested	Acid value (per cent)				
	Period of incubation (day)				
	7	15	30	60	Mean
A. niger	6.30	8.01	14.00	16.00	11.08
A. flavus	6.25	7.84	13.70	16.00	10.95
R. oryzae	6.16	8.01	11.76	18.20	11.03
M. hiemalis	8.00	11.80	18.25	34.90	18.24
P. citrinum	1.10	1.20	1.50	1.50	1.325
B. theobromae	4.00	6.20	8.50	10.25	7.24
C. senegalensis	2.18	3.75	4.50	5.62	4.01
Control	0.5	0.5	0.5	0.5	0.5
Mean	4.3111	5.9137	9.088	12.8712	

C.D. periods = 4.085

C.D. fungi = 5.780

Table 18. Refractive index of oil at 40°C as affected by various fungi causing deterioration of copra (Mean of three replications)

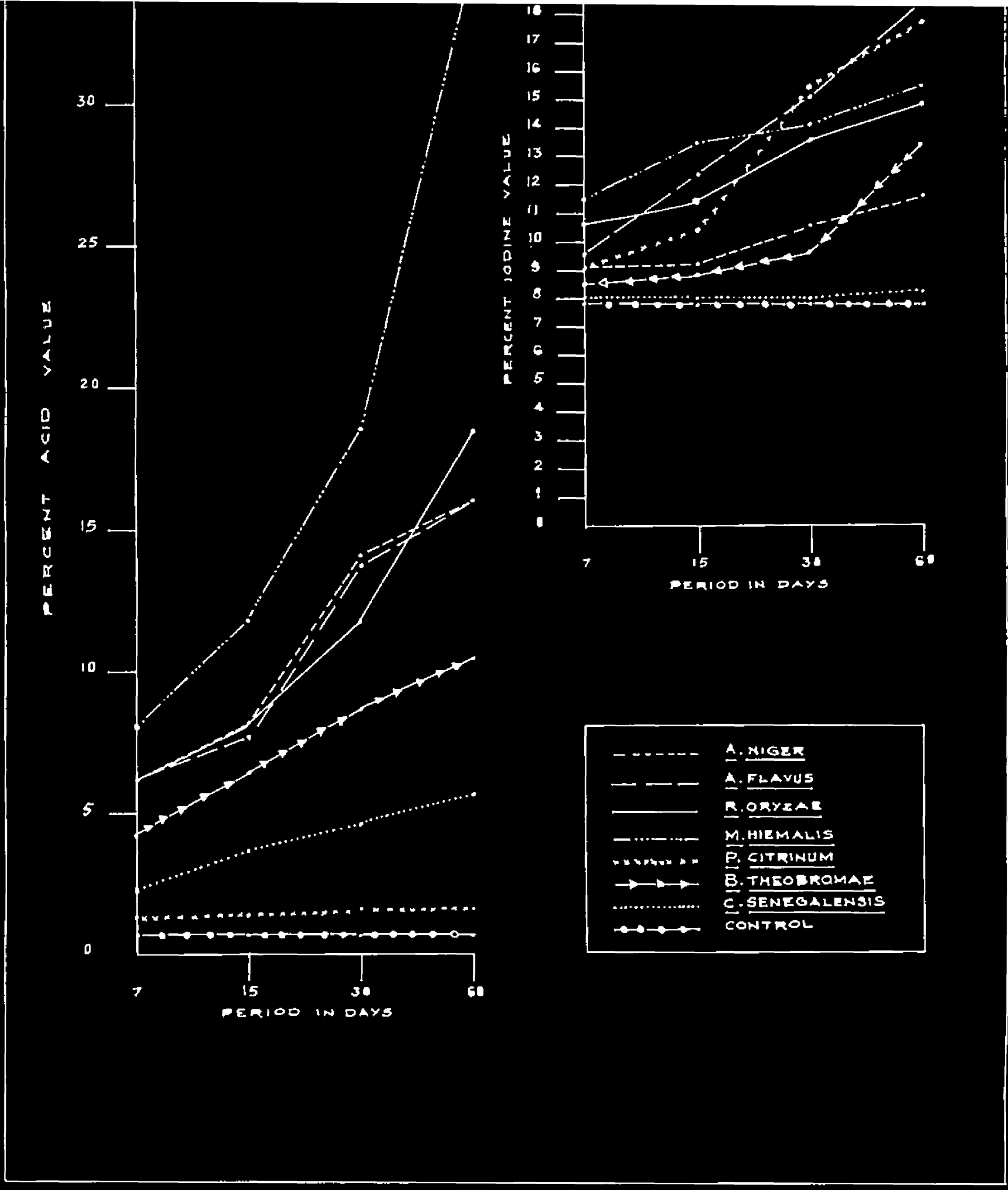
Fungus tested	Refractive index			
	Period of incubation (day)			
	7	15	30	60
A. niger	1.4482	1.4484	1.4484	1.4485
A. flavus	1.4480	1.4482	1.4482	1.4484
R. oryzae	1.4487	1.4487	1.4488	1.4488
M. hiemalis	1.4487	1.4489	1.4489	1.4489
P. citrinum	1.4485	1.4485	1.4485	1.4485
B. theobromae	1.4480	1.4481	1.4481	1.4481
C. senegalensis	1.4488	1.4488	1.4489	1.4489
Control	1.4483	1.4483	1.4485	1.4485

Table 19. Effect of infection of copra by fungi on the acid value of the oil (Mean of three replications)

Fungus tested	Acid value (per cent)				
	Period of incubation (day)				
	7	15	30	60	Mean
A. niger	6.30	8.01	14.00	16.00	11.08
A. flavus	6.25	7.84	13.70	16.00	10.95
R. oryzae	6.16	8.01	11.76	18.20	11.03
M. hiemalis	8.00	11.80	18.25	34.90	18.24
P. citrinum	1.10	1.20	1.50	1.50	1.325
B. theobromae	4.00	6.20	8.50	10.25	7.24
C. senegalensis	2.18	3.75	4.50	5.62	4.01
Control	0.5	0.5	0.5	0.5	0.5
Mean	4.3111	5.9137	9.088	12.8712	

C.D. periods = 4.085

C.D. fungi = 5.780


PERCENT ACID VALUE
30
25
20
15
10
5
0
7
15
30
60
PERIOD IN DAYS
PERCENT IODINE VALUE
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7
15
30
60
PERIOD IN DAYS
A. NIGER
A. FLAVUS
R. ORYZAE
M. HIEMALIS
P. CITRINUM
B. THEOBROMAE
C. SENEGALENSIS
CONTROL

oil steadily increased with the period of incubation. Maximum acid value is recorded by samples infected with M. hiemalis which is significant over other samples. A. niger, A. flavus and R. oryzae are similar in their effects and are on par. P. citrinum produced the minimum acid value of 1.325 which is on par with C. senegalensis and the effects of these two fungi are on par with the control.

Saponification value

The results on saponification value of oil are presented in Table 20. The saponification value of oil is not found to be affected due to infection of copra by fungi.

Iodine value

From the ANOVA presented in Appendix XXII, Table 21 and Fig. 4b it is observed that the effects of periods and fungi are significant. Maximum iodine value is recorded in oils extracted from copra which is incubated for 60 days. A gradual increase in iodine value is noted with increasing periods of incubation of inoculated copra. Incubation for 60 days showed significant difference in the iodine values as compared to those inoculated for 7 and 15 days. Among the fungi A. flavus induced the maximum and C. senegalensis the minimum iodine value. The effects of A. flavus, R. oryzae, M. hiemalis and P. citrinum are on par. Similarly the

Table 20. Effect of infection of copra by fungi on the saponification value of oil (Mean of three replications)

Fungus tested	Saponification value (per cent)			
	Period of incubation (day)			
	7	15	30	60
A. niger	258.75	260.20	260.00	260.00
A. flavus	258.00	258.36	258.55	259.00
R. oryzae	260.00	260.00	260.00	260.00
M. hiemalis	258.10	258.10	258.50	258.00
P. citrinum	257.05	257.50	257.85	257.90
B. theobromae	257.50	257.50	258.00	258.00
C. senegalensis	258.00	258.00	258.00	258.00
Control	258.00	258.00	258.00	258.00

Table 21. Effect of infection of copra by fungi on the iodine value of oil (Mean of three replications)

Fungi tested	Iodine value (per cent) Period of incubation (day) 7	15	30	60	Mean
A. niger	9.00	9.35	10.50	11.65	10.12
A. flavus	9.50	12.25	15.00	18.15	13.72
R. oryzae	10.50	11.20	13.50	14.75	12.49
M. hiemalis	11.25	13.25	14.00	15.25	13.44
P. citrinum	9.00	10.25	15.21	17.78	13.06
B. theobromae	8.55	8.89	9.50	13.12	10.01
C. senegalensis	8.00	8.00	8.00	8.13	8.03
Control	8.00	8.00	8.00	8.00	8.00
Mean	9.2250	10.1487	11.7137	13.3537	

C.D. period = 2.3088

C.D. fungi = 1.6286

iodine values of oil infected with A. niger and B. theobromae are also on par. Iodine value of oil infected by C. senegalensis is similar to that of the control. The results revealed that all fungi except C. senegalensis is capable of increasing the iodine value of oil due to infection.

Changes in the properties of oil on storage

Results are presented in Table 22. No appreciable changes in the properties of oil are noticed for the first three months. After six months of storage the oil becomes slightly rancid. An increase in acid value, and iodine value are noticed as the period of storage of oil is increased beyond six months. Refractive index and saponification value remain unchanged.

Effect of infection of copra by fungi on the oil content

Results are presented in Appendix XXIII, Table 23 and Fig.5.

It is observed that the effects of periods and fungi are significant. A steady decrease in oil content is noticed over different periods after inoculation by fungi. Among the fungi, infection by R. oryzae caused the maximum reduction in oil content which is on par with that of M. hiemalis. The effects of A. flavus, A. niger and B. theobromae in reducing the oil content are also on par with each other. P. citrinum

Table 22. Changes in the properties of oil on storage (Mean of three replications)

January - December 1976

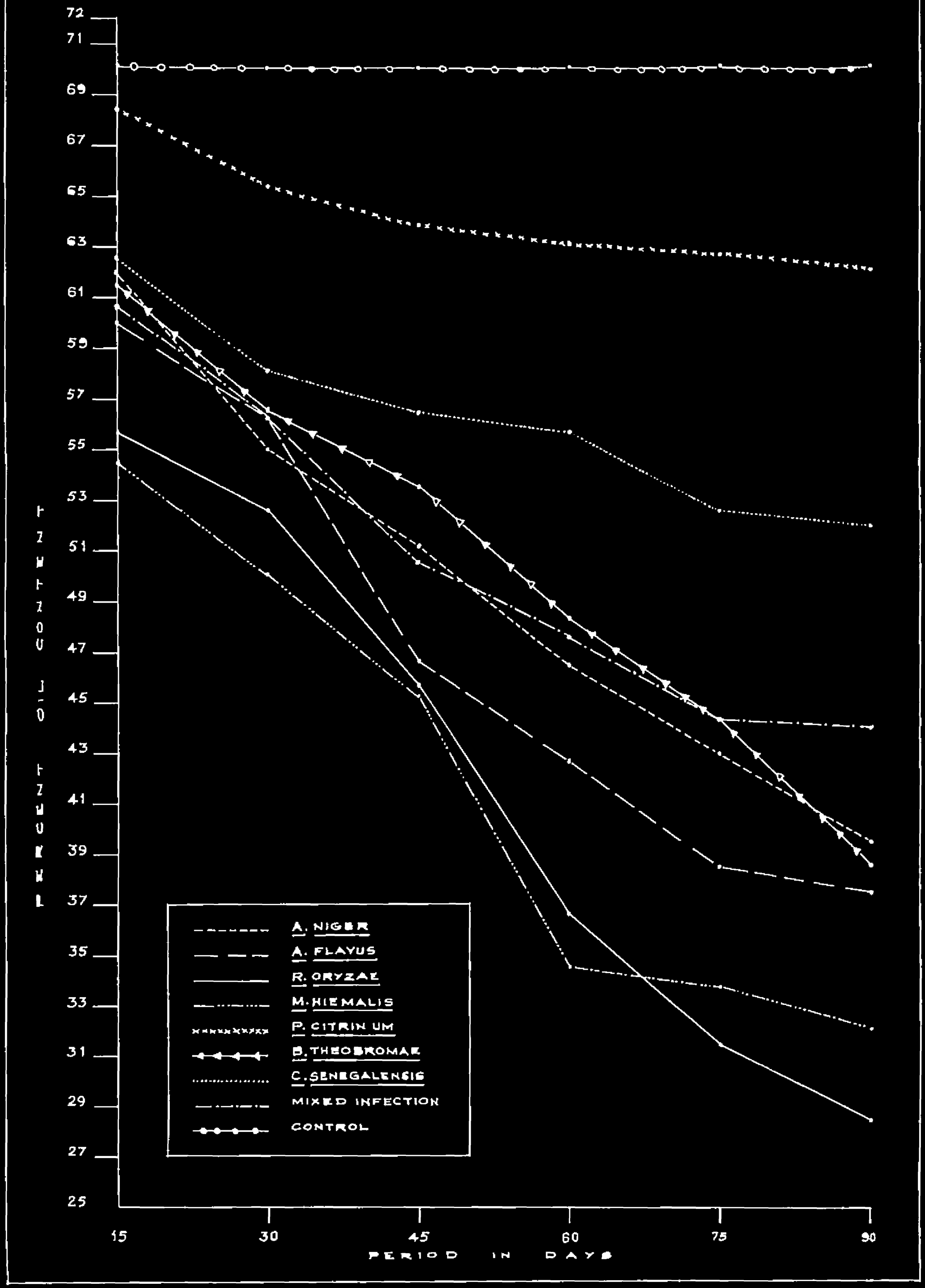
Properties of oil	Duration of storage (months)				
	Control	3	6	9	12
Colour	1.0000	1.0000	2.0000	2.5000	3.0000
Odour	Normal	Normal	Slightly rancid	Slightly rancid	Rancid
Refractive index	1.4484	1.4484	1.4487	1.4487	1.4489
Acid value	0.5000	0.5000	1.0000	1.2000	2.000
Saponification value	258.0000	258.0000	258.0000	258.0000	258.0000
Iddine value	8.0000	9.0000	10.2500	11.5000	12.1800

Table 23. Effect of infection by fungi on oil content of copra (Mean of three replications)

	Oil content (per cent)						
	Period of incubation (day)						
Fungi tested	15	30	45	60	75	90	Mean
A. niger	62.00	55.07	51.75	46.50	43.00	39.50	49.64
A. flavus	60.00	56.05	46.50	42.75	38.45	37.50	46.88
R. oryzae	55.50	52.75	45.75	36.70	31.40	28.50	41.77
M. hiemalis	54.25	50.00	45.15	34.50	33.75	32.20	41.64
P. citrinum	68.20	65.20	63.75	63.00	62.75	62.25	64.19
B. theobromae	61.50	56.75	53.50	48.27	44.10	38.50	50.43
C. senegalensis	62.50	58.07	56.50	55.75	52.50	52.00	56.22
Mixed infection	60.50	56.45	50.50	47.50	44.25	44.00	50.53
Control	70.00	70.00	70.00	70.00	70.00	70.00	70.00
Mean	61.0555	57.8155	53.7111	49.4411	46.6888	44.93	

C.D. periods = 4.60119
C.D. fungi = 3.75685

FIG. 5. EFFECT OF INFECTION BY FUNGI ON OIL CONTENT OF COPRA.



caused only minimum reduction in oil content. Infecting samples of copra by a mixed inoculum of six fungi reduced the oil content to a level that caused by the infection of the sample by B. theobromae.

ROLE OF FUNGAL ENZYMES IN DETERIORATION OF COPRA

Pectic enzymes elaborated by pathogens in vitro and in vivo

Macerating enzymes

Among the seven fungi tested only A. flavus, A. niger and R. oryzae show maceration of potato discs thereby revealing the production of macerating enzymes. On a comparison of the above three fungi it is observed that the effects of periods (P), treatments (S) and fungi (F) are significant. The interaction between period X treatment, period X fungi and treatment X fungi are also significant. The mean values are given in Table 24 and Appendix XXIV. After 16 days of inoubation there is practically no enzyme activity. The difference in enzyme activity after an inoubation of four days is significant over that of eight days. Among the fungi A. flavus showed the maximum activity which is significantly different from A. niger. The activities of A. flavus and R. oryzae as well as A. niger and R. oryzae are on par. A significantly higher enzyme activity is seen when the organism is grown in the medium having ammonium sulphate as the nitrogen source (S_2) as compared to that where sodium nitrate is used

Table 24. Production of macerating enzymes by fungi infecting copra (Reciprocal of the time taken in minutes x 1000)

Fungus tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A.niger	4.6031	5.7403	4.1930	4.8456	7.5210	7.0160	0
A.flavus	6.3880	6.9616	4.7752	6.0420	11.0650	7.0610	0
R.oryzae	4.8618	6.3603	4.9038	5.3753	9.1330	6.9930	0

	P_1	P_2	P_3	Mean
S_1	9.5779	6.2750	0	5.2846
S_2	10.7402	8.3221	0	6.3541
S_3	7.4010	6.4710	0	4.6240
Mean	9.2400	7.0230		

C.D. for P, S and F = 0.7686

C.D. for combinations of P and S, P and F and S and F = 1.3314

S_1 = *in vitro* studies where sodium nitrate is the nitrogen source

S_2 = " " ammonium sulphate "

S_3 = *in vivo* preparation

P_1 = Period of incubation 4 days

P_2 = " 8 days

P_3 = " 16 days

as the nitrogen source (S_1). *In vivo* preparations (S_3) showed least activity. Control did not show any activity.

Pectin methyl esterase (PME)

From the ANOVA in Appendix XXV it is observed that the effects of periods (P), treatments (S), fungi (F) and interaction between period X fungi, treatment X fungi and period X treatment are significant. The mean values are given in Table 25. Maximum PME activity is observed during the fourth day of incubation followed by eighth and sixteenth day respectively. Significant difference in enzyme production is noted between different periods. Between the different treatments maximum activity is noticed in medium (S_1), where sodium nitrate is used as the nitrogen source. The *in vivo* preparations (S_3) showed the least activity. Among the fungi tried, *A. flavus* showed the maximum enzyme activity and is on par with that of *A. niger*. Similarly the enzyme activity of *C. senegalensis* and *P. citrinum* is on par. However they show significantly lower activity than *A. flavus* and *A. niger*. Minimum activity is shown by *M. hiemalis*. The enzyme activity during the period of observation under any treatment is not revealed in the case of *B. theobromae*.

Polygalacturonase

From the ANOVA given in Appendix XXVI it can be seen that the effects of periods (P), treatments (S) and fungi (F)

Table 25. Production of Pectin methyl esterase by fungi infecting copra (Mean of three replications) Microequivelent of NaOH required/ hour/ml

Fungus tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A. niger	10.8500	7.1040	3.1560	7.0377	11.2480	6.7093	3.1560
A. flavus	10.4586	6.5120	7.1040	8.0248	11.0506	8.4853	4.5336
R. oryzae	4.9333	2.9586	2.7296	3.5405	4.9016	4.1440	1.5760
M. hiemalis	3.7493	2.7666	0	2.1729	4.2460	1.5786	0.6906
P. citrinum	6.8080	4.9333	1.9713	4.5708	7.1040	4.5366	2.0720
B. theobromae	0	0	0	0	0	0	0
C. senegalensis	6.4133	6.1173	1.7746	4.7684	8.9066	4.7360	2.6626
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0;0000
P_1	7.3260	6.5505	3.1701	5.6822			
P_2	5.7720	3.6260	1.9232	3.7737			
P_3	3.1080	1.2205	1.8250	1.8370			
Mean	5.4020	3.7990	2.0919				

C.D. for P and S = 0.6570
" F = 1.0729
C.D. for combinations of P and F } = 1.8583
" " S and F }
" " P and S = 1.1380

S_1 = in vitro studies where sodium nitrate is the nitrogen source
S_2 = " ammonium sulphate "
S_3 = in vivo preparation
P_1 = Period of incubation 4 days
P_2 = " 8 days
P_3 = " 16 days

are significant. The mean values are given in Table 26. The enzyme activity increased by eighth day of incubation and then decreased by the sixteenth day. There is significant difference in the enzyme activity between the first two periods. Between the different treatments, the in vivo preparation (S_3), show the maximum activity which is significantly higher than the other two treatments which are on par. The activity of A. flavus, R. oryzae, M. hiemalis and P. citrinum are on par. Similarly M. hiemalis, B. theobromae, C. senegalensis and A. niger are on par.

Polygalacturonase transeliminase (PGTE)

From the ANOVA in Appendix XXVII, it is observed that the effects of periods (P), treatments (S) and fungi (F) and interactions of period X fungi and treatment X fungi are significant. The mean values are presented in Table 27. The enzyme activity increased with the period of incubation and reached a maximum on the sixteenth day. Significant difference is noticed in the PGTE activity during different periods of incubation. Among the treatments, in vivo preparations (S_3) showed the maximum activity. Among in vitro studies the activity is significantly higher in medium (S_1), where sodium nitrate is used as the nitrogen source, as compared to the medium (S_2) where ammonium sulphate is used as the nitrogen source. Among the fungi P. citrinum exhibits the maximum

Table 26. Production of polygalacturonase by fungi infecting copra (Mean of three replications). Absorbance of TBA products at 515 mμ

Fungi tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A. niger	0.2804	0.4520	1.0106	0.5810	0.5176	0.5896	0.6357
A. flavus	0.7513	0.9233	1.1950	0.9565	0.7223	1.2543	0.8930
R. oryzae	0.6333	0.8320	1.2266	0.8973	0.8393	1.0683	0.7843
M. hiemalis	0.7680	0.3670	1.1290	0.7546	0.4850	0.8026	0.9763
P. citrinum	0.9213	0.7626	0.7150	0.7996	0.4370	1.0090	0:9530
B. theobromae	0.7243	0.6150	0.7493	0.6962	0.4663	0.8433	0.7790
C. senegalensis	0.3236	0.3262	0.8846	0.5115	0.2996	0.7916	0.4431
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	0.3597	0.3666	0.6101	0.4709			
P_2	0.7481	0.7908	0.8456	0.7948			
P_3	0.5429	0.4468	1.0590	0.6830			
Mean	0.5502	0.5347	0.8637				

C.D. for P or S = 0.1730

,, F = 0.2825

C.D. for combinations of P and S = 0.2996

,, ,, S and F or P and F = 0.4892

S_1 = in vitro studies where sodium nitrate is the nitrogen sourse

S_2 = ,, ,, ammonium sulphate ,,

S_3 = in vivo preparation

P_1 = Period of incubation 4 days

P_2 = ,, 8 days

P_3 = ,, 16 days

Table 27. Production of polygalacturonase transeliminase by fungi infecting copra (Mean of three replications)•Absorbance of TBA products at 550 mμ

Fungi tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A. niger	0.5563	0.4625	0.3800	0.6329	0.4150	0.4931	0.9906
A. flavus	0.5049	0.4150	1.0610	0.6603	0.4673	0.6273	0.8863
R. oryzae	0.9740	0.8446	0.8630	0.8938	0.7390	0.8836	1.0590
M. hiemalis	0.4983	0.3553	1.0043	0.6193	0.4765	0.5694	0.8120
P. citrinum	0.9613	0.8587	0.9060	0.9086	0.8046	0.9123	1.0090
B. theobromae	0.8256	0.7870	0.9443	0.8523	0.7453	0.8446	0.9670
C. senegalensis	0.4957	0.3933	1.2200	0.7040	0.5757	0.6380	0.8985
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	0.4415	0.3761	0.7672	0.5279			
P_2	0.5553	0.4735	0.9352	0.6218			
P_3	0.8103	0.6950	0.9780	0.8277			
Mean	0.6024	0.5145	0.8793				

C.D. for P or S = 0.0551
,, F = 0.0900
C.D. for combinations of P and F } = 0.1559
,, ,, S and F }
,, ,, P and S = 0.0955

S_1 = In vitro studies where sodium nitrate is the nitrogen source
S_2 = ,, ammonium sulphate ,,
S_3 = in vivo preparations
P_1 = Period of incubation 4 days
P_2 = ,, 8 days
P_3 = ,, 16 days

enzyme activity which is not significantly different from that of R. oryzae. M. hiemalis show the minimum enzyme activity which is on par with that of A. niger and A. flavus.

Cellulolytic enzymes

Cellulase (C1)

From the ANOVA in Appendix XXVIII it is observed that the effects of periods (P), treatments (S) and fungi (F) and interaction between treatment X fungi are significant. The mean values are given in Table 28. The enzyme activity increased with the period of incubation. The maximum activity is noticed on the sixteenth day which is significantly different from other periods. Among the treatments, in vivo (S_3) preparations show the minimum activity and the medium, in which the nitrogen source is sodium nitrate (S_2), show the maximum C1 enzyme activity but it is not significantly different from the other treatment. Among the fungi, the maximum activity is shown by B. theobromae, A. flavus and A. niger, all the three being on par. The activity of R. oryzae is significantly lower than that of B. theobromae but R. oryzae and P. citrinum are on par. M. hiemalis showed the minimum activity and the activity is on par with C. senegalensis.

Cellulase (Cx)

From the ANOVA in Appendix XXIX it is noticed that the

Table 28. Production of cellulase (Cl) by fungi infecting copra (Mean of three replications). Absorbance at 610 mμ

Fungi tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A. niger	0.8129	0.6790	0.1631	0.5517	0.2445	0.5711	0.8394
A. flavus	0.7135	0.8313	0.1547	0.5665	0.3167	0.5714	0.8114
R. oryzae	0.4656	0.7573	0.0829	0.4353	0.2883	0.4828	0.5347
M. hiemalis	0.0322	0.0951	0.0256	0.0676	0.0368	0.6745	0.0917
P. citrinum	0.4596	0.4610	0.0322	0.3177	0.1706	0.3511	0.4314
B. theobromae	0.8486	0.8563	0.0700	0.5916	0.4448	0.6136	0.7165
C. senegalensis	0.2165	0.1945	0.0597	0.1569	0.0918	0.1760	0.2030
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	0.2116	0.3443	0.0417	0.14921			
P_2	0.4990	0.4433	0.0774	0.3550			
P_3	0.6391	0.6199	0.1015	0.4535			
Mean	0.4499	0.4843	0.0735				

C.D. for P or S = 0.0778
,, F = 0.1318
C.D. for combinations of P and F = 0.2292
,, ,, P and S = 0.1404
,, ,, S and F = 0.2314

S_1 = *in vitro* studies where calcium nitrate is the nitrogen source
S_2 = ,, sodium nitrate ,,
S_3 = *in vivo* preparations
P_1 = Period of incubation 4 days
P_2 = ,, 8 days
P_3 = ,, 16 days

effects of periods (P), treatments(S) and fungi (F) and interaction between period X treatment, period X fungi and treatment X fungi are significant. The mean values are presented in Table 29. Significant difference in the (Cx) activity is noted between different periods. The activity increased with the period of incubation and reached a maximum on the 16th day. Between the different treatments, in vivo preparation show the minimum activity. Significant difference is not observed between the other treatments (in vitro). Among the fungi, C. senegalensis show the maximum activity. The activity of P. citrinum, R. oryzae and A. niger are on par. The enzyme activity of B. theobromae is significantly lower than that of other fungi.

III. Lipase

From the ANOVA given in Appendix XXX it is evident that the effects of period, fungi and interaction between period X fungi and treatment X fungi are significant. The mean values are given in Table 30. The activity of the enzyme increases with the incubation period, being maximum on the twelfth day and decreased by sixteenth day. The effect of media (S) on lipase production is not significant. Among the fungi tested, maximum lipase activity is shown by R. oryzae which is on par with that of A. niger. However the activity of A. niger is not significantly different from that of other

Table 29. Production of cellulase (CX) by fungi infecting copra (Mean of three replications).(per cent reduction in viscosity)

Fungi tested	S_1	S_2	S_3	Mean	P_1	P_2	P_3
A. niger	27.9366	24.8600	9.8700	20.8888	7.1166	17.2633	38.2866
A. flavus	25.4066	23.5233	10.1666	19.6988	7.0533	16.0633	35.9800
R. oryzae	25.8233	29.3900	11.9866	22.4000	8.9133	23.1466	35.1400
M. hiemalis	25.3700	18.2800	10.2100	18.1200	6.1066	16.8433	31.4100
P. citrinum	29.0966	28.1766	11.0733	22.7822	11.0633	5.3666	34.2500
B. theobromae	14.8833	16.4633	4.0233	11.7900	3.5433	12.5533	19.2733
C. senegalensis	35.1033	33.6066	5.5833	24.7644	7.1466	25.1900	42.6233
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	9.1675	7.8337	2.0975	6.3679			
P_2	22.7037	21.1287	6.4525	16.7616			
P_3	37.1737	36.3950	15.0425	29.5370			
Mean	23.0150	23.0375	7.8641				

C.D. for P or S = 2.5864

,, F = 4.2235

C.D. for combinations of P and S = 4.4810

,, ,, F and S or P and F = 7.3153

S_1 = in vitro studies where calcium nitrate is the nitrogen source

S_2 = ,, sodium nitrate ,,

S_3 = in vivo preparation

P_1 = Period of incubation 4 days

P_2 = ,, 8 days

P_3 = ,, 16 days

Table 30. Production of lipase by fungi infecting copra (Mean of three replications) 1 ml: 1 unit

Fungi tested	S_1	S_2	Mean	P_1	P_2	P_3
A. niger	1.6000	1.9333	1.7666	2.2500	2.5500	0.5000
A. flavus	1.4000	1.9333	1.6666	1.7500	2.5000	0.7500
R. oryzae	1.7600	2.0330	1.9000	1.8500	2.8500	1.0000
M. hiemalis	1.2000	2.0330	1.6166	1.7400	2.7500	0.4000
P. citrinum	1.2666	2.0000	1.6333	1.7000	2.6000	0.6000
B. theobromae	2.1666	1.1660	1.6666	1.7000	2.5000	0.8000
C. senegalensis	1.8666	1.3000	1.5833	1.7500	2.3000	0.7000
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	1.5375	1.6375	1.5875			
P_2	2.1000	2.4125	2.2562			
P_3	0.5875	0.6000	0.5937			
Mean	1.4083	1.5500				

C.D. for P = 0.1832
,, S = 0.1496
,, F = 0.2992
C.D. for combinations of P and F = 0.51836
,, ,, S and F = 0.4232
,, ,, P and S = 0.2591

S_1 = Fungi grown on Czapek's solution
S_2 = ,, Richard's solution
P_1 = Period of incubation 4 days
P_2 = ,, 12 days
P_3 = ,, 16 days

fungi. The activities of A. flavus, I. hiemalis, P. citrinum, B. theobromae and C. senegalensis are on par.

Proteolytic enzymes

From the ANOVA given in Appendix XXXI it is clear that the effects of periods, treatments, fungi and interaction between period X treatment and period X fungi are significant. The mean values are presented in Table 31. The activity of the enzyme is maximum on the fourteenth day of incubation and minimum on the twenty-first day. Significant difference is noticed between different periods. Significant difference is observed between the protein sources tried (S) for enzyme production. Medium, having casein (S_2) as the protein source, showed significantly higher enzymatic activity than the medium where peptone is used. Among the fungi maximum activity is shown by P. citrinum. Significant difference is noticed between the activity of P. citrinum and other fungi. There is no difference in the enzyme activity of A. flavus and M. hiemalis as well as M. hiemalis and B. theobromae. Minimum enzyme activity is observed in the case of C. senegalensis, R. oryzae and A. niger and is on par.

Metroglyph analysis of the enzyme activity of fungi

The metroglyph analysis of the various enzyme activities of different fungi are given in Appendix XXXII and Fig. 6.

Table 31. Production of proteolytic enzymes by fungi infecting copra (Mean of three replications)• Absorbance at 280 mμ

Fungi tested	S_1	S_2	Mean	P_1	P_2	P_3
A. niger	0.0433	0.0550	0.0491	0.0480	0.0725	0.0270
A. flavus	0.2613	0.2666	0.2640	0.3010	0.3125	0.1780
R. oryzae	0.0616	0.0796	0.0706	0.0640	0.1100	0.0380
M. hiemalis	0.1046	0.3373	0.2210	0.1930	0.3060	0.1640
P. citrinum	0.4400	0.4580	0.4490	0.5255	0.5580	0.2635
B. theobromae	0.1510	0.2096	0.1803	0.0495	0.2450	0.2465
C. senegalensis	0.0636	0.0866	0.0776	0.0200	0.0840	0.1290
Control	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P_1	0.1515	0.1488	0.1501			
P_2	0.1765	0.2455	0.2110			
P_3	0.0960	0.1655	0.1307			
Mean	0.1413	0.1866				

C.D. for P = 0.0032
,, S = 0.0260
,, F = 0.0520
C.D. for combination of S and F = 0.0743
,, ,, F and P = 0.0910
,, ,, S and P = 0.0465

P_1 = Period of incubation 7 days
P_2 = ,, ,, 14 days
P_3 = ,, ,, 21 days

S_1 = Medium where peptone is used as the nitrogen source
S_2 = ,, casein ,, ,,

The metroglyph showing the enzyme activity of various fungi tested is obtained by plotting Cx activity on the X axis and PME activity on the Y axis. All other enzymes are plotted as rays diverging from the point where Cx and PME activities coincide.

The seven fungi fall into four broad clusters. A. niger and A. flavus which are similar fall into the first cluster and R. oryzae, P. citrinum and C. senegalensis fall into the second cluster, though they differ in certain aspects. R. oryzae shows macerating activity whereas the others do not. P. citrinum possess high proteolytic activity as compared to R. oryzae and C. senegalensis. M. hiemalis and B. theobromae form separate clusters being different from all other fungi.

STUDIES ON MYCOTOXINS IN COPRA

Aflatoxin

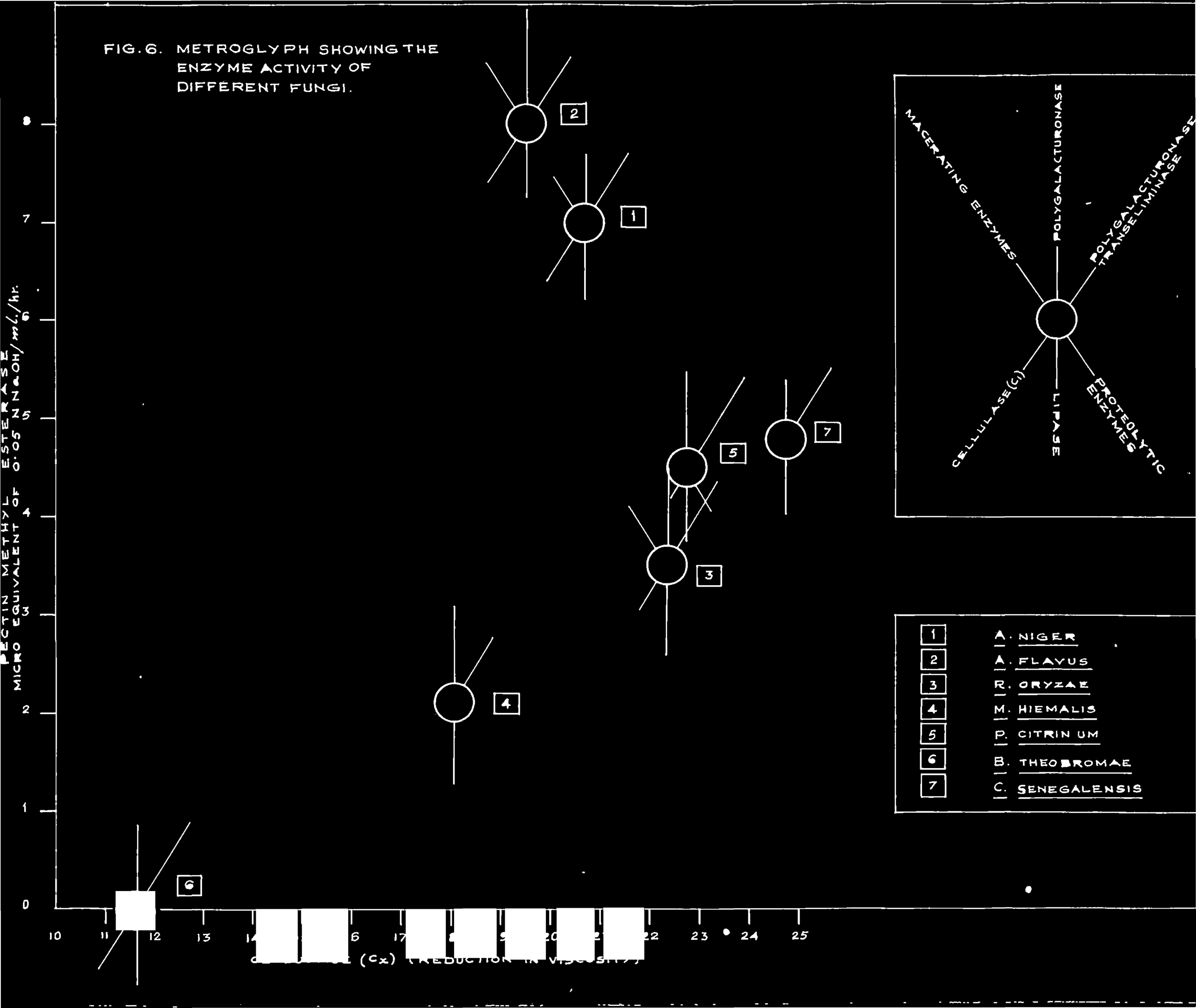
Microbiological assay of aflatoxin

The extract of copra showed an inhibition zone of 10 mm with B. megaterium as the test organism. This clearly shows the presence of toxin in the crude extract of copra.

Estimation of aflatoxin B_1 produced by fungi in copra

Aflatoxin B_1 is identified under the ultraviolet light by the fluorescence produced by it. It is estimated that the

FIG. 6. METROGLYPH SHOWING THE ENZYME ACTIVITY OF DIFFERENT FUNGI.



sample contained 2 ppm of the toxin.

Estimation of aflatoxin in copra during different periods of the year.

Results are presented in Table 32. Aflatoxin B_1 is detected in samples collected during March-April, May-June and July-August. The quantity observed is very high, viz., above one ppm. During the other periods, aflatoxin B_1 is not detected in the samples of copra analysed.

Table 32. Estimation of aflatoxin in copra during different periods of the year
(January - December 1977)

Period	Concentration of aflatoxin B_1 (ppm) Medium 0.05 - 0.25	High 0.25 - 1.0	Very high above 1.0
January-February	-	-	-
March-April	-	-	+
May-June	-	-	+
July-August	-	-	+
September-October	-	-	-
November-December	-	-	-

\- = Nil
\+ = Present

Screening of fungi for aflatoxin production

Of all the seven species of fungi commonly infecting stored copra only A. flavus showed fluorescence similar to

aflatoxin B_1 with an Rf value of 0.70 (Table 33). Under Kerala conditions, other fungi are not producing aflatoxin in copra.

Table 33. Production of aflatoxin B_1 by different fungi

Fungi tested	Aflatoxin B_1	Rf value
A. niger	-	-
A. flavus	+	0.70
A. oryzae	-	-
A. hiemalis	-	-
P. citrinum	-	-
C. theobromae	-	-
C. senegalensis	-	-

- = Nil + = Present

Biological assay

Chick embryo

The results are presented in Table 34. The embryos of all eggs which are injected with 0.10 μg of the extract of copra infected with A. flavus died on the first day after inoculation. In the group of eggs, injected with 0.05, 0.03 and 0.025 μg of the extract, the embryo of one egg each is found to be dead during the above period.

Table 34. Pattern of mortality of chick embryos inoculated with different dosages of extracts of copra infected with A. flavus

Concentration of the extract	1st day Pi		IInd day Pi		IIIrd day Pi	
	C	I	C	I	C	I
0.10 µg	0/3	3/3	0/3	-	0/3	-
0.05 ,,	0/3	1/3	0/3	2/3	0/3	-
0.03 ,,	0/3	1/3	0/3	2/3	0/3	-
0.025 ,,	0/3	1/3	0/3	1/3	0/3	1/3

C = Control I = Inoculated

Pi = Post inoculation

Numerator: Number of eggs in which embryo is dead

Denomenator: Total number of eggs

On the second day after inoculation, the embryo of the remaining eggs which received 0.05 and 0.03 µg of the extract died; while only one in the group injected with 0.025 µg is dead. The remaining one egg injected with 0.025 µg of the extract also died on the third day after inoculation. The eggs in the control group remained alive and hatched out normally.

Ducklings

In all the experimental birds the general health and vigour is found to be reduced when compared to the control

(Plate VII, VIII). The mortality of ducklings during the experimental period of 30 days is presented in Appendix XXXIII, Fig.7. Out of a total of thirty-seven ducklings used for the trial twenty-seven ducklings died within 7 days, three ducklings within 8 to 15 days and two birds within 16 to 30 days. Five ducklings which survived the experimental period of 30 days are killed. In all cases the liver and kidney of the dead as well as the killed are subjected to histopatholigical studies.

Liver

The liver of ducklings showed varying degrees of histopathological alterations compared to the control (Plate IX). Necrobiotic changes are more pronounced in those that died within 7 days, While proliferation of bileduct with or without fibrotic changes are more marked during the later stages. Those that survived for 30 days showed necrosis, bileduct proliferation and hepatic fibrosis. Hepatic cell regeneration as evidenced by large hyperchromatic cells is also present in those ducklings. Pseudolobulation is noticed in two cases.

The degenerative changes in the hepatic cells are manifested as diffuse hydropic and fatty degenerations giving a moth eaten appearance to the parenchyma (Plate X). The fat vacuoles in the hepatic cells are either single large or numerous small ones. These cells with degenerative changes

CUMULATIVE MORTALITY OF DUCKLINGS FED WITH COPRA INFECTED BY FUNG

are seen along with individual hepatic cells which had become necrotic. These degenerative and necrotic changes are more pronounced at the periphery of the lobules. Proliferation of the bileduct is seen in ducklings that died after seven days (Plate XI). However this is very pronounced in those that are killed at the end of the experimental period. They are seen either as bands of biliary epithelium without any patent lumen or as well formed ductules (Plate XII).

The onset of fibroblastic reaction is evident after seven days. Early manifestations are in the portal areas and later the fibroblasts are seen to be extending in between the hepatic cords. In those that died within fifteen days, a slight collagen deposition is seen. Later (by 29 days) thick strands of fibrous tissues are seen around individual lobules and also intralobularly (Plate XIII). The hepatic cells at this stage increased in size and the nuclei of such cells appear active.

Kidney

Kidney of ducklings that died during the first seven days showed necrosis and degeneration of tubular epithelial cells. The nephrotic changes are inconsistently seen in the ducklings that died after the first seven days.

Guinea pigs

Out of the three guinea pigs, one died on the twenty-first day, while the other two survived the experimental period.

Liver

In the animal that died and in those that survived the experimental period diffuse parenchymatous degeneration is observed (Plate XIV).

Kidney

There is necrosis of the proximal tubules in the epithelial cells. A few Bowman's capsule contained albuminous fluid devoid of any leukocytes. Mild necrosis of glomeruli is seen occasionally with a tendancy for tubular reflux.

Rats

All the animals in this group survived the experimental period.

Liver

The necrosis and degenerative changes are observed in the periportal areas. Cytomegaly is a constant feature in many sections. In some cases there are biliary hyperplasia. Mitosis is seen in many hepatic cells. Mild degenerative changes of the hepatic cells are also observed.

Kidney

There is mild nephrotic changes. Few of the tubular lumen contained cesinophilic albuminous fluid.

P. citrinum toxins

Biological effects

Chick embryo

The results are presented in Table 35.

Table 35. Pattern of mortality of chick embryos inoculated with different doses of P. citrinum infected copra

Dilution of the extract tried	Ist day Pi		IInd day Pi		IIIrd day Pi		IVthday Pi		Vth day Pi	
	C	I	C	I	C	I	C	I	C	I
1:10	0/3	0/3	1/3	1/3	0/3	2/3	0/3	-	0/3	-
1:20	0/3	0/3	0/3	0/3	0/3	3/3	0/3	-	0/3	-
1:30	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3	1/3
1:40	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	2/3

C = Control I = Inoculated Pi = Post inoculated

Numerator = Number of eggs in which embryo is dead

Denomenator = Total number of eggs

In the group in which eggs are injected with 1:10 dilution of the extract of copra infected with P. citrinum, one embryo died on the second day and two embryos died on the third day after inoculation. In the group which received 1:20 dilution all the three embryos of eggs died on the third day while two embryos died in the 1:30 dilution group on the fourth day and one embryo died on the fifth day. In the group in which

eggs are injected with 1:40 dilution, one embryo died on the fourth day followed by two embryos on the fifth day.

Ducklings

In all the experimental birds the general health and vigour is found to be reduced as in the studies with A. flavus infected copra (Plate XV).

Results on mortality of ducklings are presented in Appendix XXXIII, Fig. 7. Out of a total of thirty-seven birds, twenty-eight died within seven days, one bird within 8 to 15 days and two birds within 16 to 30 days. Six ducklings which survived the experimental period are killed. In all cases liver and kidney are subjected to histopathological studies.

Liver

Very mild hepatic degeneration is noticed in the ducklings that died within seven days. Occasional cells showed slight fatty changes. These hepatic changes are not apparent in the birds that survived the experimental dosage and which are sacrificed after the experimental period of 30 days.

Kidney

Kidney showed degeneration with and without necrosis of epithelial cells. Moderate degree of dilation of

tubules is consistently observed in the birds that are killed after 30 days (Plate XVI). The glomeruli appeared shrunken in many cases.

Mixed inoculum of A. flavus and P. citrinum infected copra

In all the experimental birds the general health and vigour is found to be reduced when compared to the control, but slightly better than the other two treatments. (Plate XVII).

Out of a total of thirty-seven ducklings fed with infected copra, twenty died within seven days, three within 7 to 15 days and one within 15 to 30 days. Thirteen ducklings which survived the experimental period are killed as in the previous study and liver and kidney are subjected to histopathological studies.

Histopathological studies.

Liver

In those ducklings that died during the first three days after feeding of infected copra, mild diffuse congestion is seen. In those that died during the later part of seven days, degenerative changes and individual cell necrosis are noted. In a few cases the periportal areas showed slight lymphoid cell infiltration (Plate XVIII). Bileduct

proliferation is very minimal and became apparent only during the later part of the experimental period of observation. Very small ductules and bands of epithelial cells are seen in those birds that survived (Plate XIX). Hepatic cell regeneration is also noticed.

Kidney

Renal changes are confirmed mainly to the epithelium of the proximal convoluted tubules. Some of the tubules showed only mild degenerative changes while others showed degeneration and necrosis.

For facilitating comparison between the effects of different fungi, the cumulative mortalities of different periods were transformed by angular transformation. The results are presented in Table 36. The critical difference obtained for comparison between mortalities with periods indicates that the effect of fungi are equal in bringing about mortality of ducklings.

Table 36. Mortality (cumulative) of ducklings due to ingestion of copra infected by fungi (Angular transformation after adjusting for natural mortality by Abbot's formula)

Fungus tested	Period (day) 7	15	22	30
A. flavus	29.80	30.85	31.05	31.24
P. citrinum	30.53	30.20	30.33	30.53
Mixed inoculum of A. flavus and P. citrinum	24.73	25.34	25.99	25.33

C.D. for comparison between fungi within each period = 6.66

Use of food additives, antibiotics and chemicals

The results are presented in Table 37 Copra treated initially with different chemicals and dried to a moisture level of 5 per cent showed no infection when stored for 30 days.

By 60 days of storage slight infection, up to 10 per cent is noticed in samples treated with common salt, citric acid, tartaric acid, sodium metabisulphate, sodium benzoate, nebasulf, aureofungin, penicillin, calcium chloride and calcium carbonate. Copra treated with streptocycline 250,500 and 1000 ppm as well as sodium carbonate 4.0, 5.0

Table 37. Effect of food additives, antibiotics and chemicals in the prevention of deterioration of copra (Moisture content about 5 per cent).

January - June 1977

Treatment	Concen-tration	Period of storage (day) 30	60	90	180
Food additives					
Common salt	1.0 %	-	+	++	++
,,	1.5 %	-	+	++	++
,,	2.0 %	-	+	++	++
Citric acid	1.0 %	-	+	++	++
,,	1.5 %	-	+	++	++
,,	2.0 %	-	+	++	+
Tartaric acid	1.0 %	-	+	++	++
,,	1.5 %	-	+	++	++
,,	2.0 %	-	+	++	++
Sodium metabisulphate	0.5 %	-	+	++	++
,,	1.0 %	-	+	++	++
,,	1.5 %	-	+	++	++
Sodium benzoate	0.5 %	-	+	++	++
,,	1.0 %	-	+	++	++
,,	1.5 %	-	+	++	++
Antibiotics					
Streptocycline	250 ppm	-	-	++	++
,,	500 ,,	-	-	-	+
,,	1000 ,,	-	-	-	+
Nebasulf	250 ,,	-	+	++	++
,,	500 ,,	-	+	++	++
,,	1000 ,,	-	+	++	++

Table continued..

Table 27 continued

Treatment	Concen-tration	Period of storage (day) 30	60	90	180
Aureofungin	250 ppm	-	+	++	++
,,	500 ..	-	+	++	++
,,	1000 ..	-	+	++	++
Penicillin	250 ..	-	+	++	++
,,	500 ..	-	+	++	++
,,	1000 ..	-	+	++	++
Chemicals					
Calcium chloride	0.5%	-	+	++	++
,,	1.0%	-	+	++	++
,,	1.5%	-	+	++	++
Calcium carbonate	4.0%	-	+	++	++
,,	5.0%	-	+	++	++
,,	5.5%	-	+	++	++
Sodium carbonate	4.0%	-	-	-	+
,,	5.0%	-	-	-	+
,,	5.5%	-	-	-	+
Acetic acid	4.0%	-	-	-	+
,,	5.0%	-	-	-	+
,,	6.0%	-	-	-	+

\- = No fungal infection

\+ = Slight infection up to 10 per cent

++ = Infection up to 20 per cent

and 5.5 per cent and acetic acid 4.0, 5.0 and 6.0 per cent showed no fungal infection. By 90 days of storage, infection increased up to 20 per cent, in all the samples treated except streptocycline 500 and 1000 ppm, sodium carbonate 4.0, 5.0 and 5.5 per cent and acetic acid 4.0, 5.0 and 6.0 per cent. On further storage for 180 days copra treated with the above chemicals also showed slight infection.

Assay of mammalian toxicity of added chemicals

The animals fed on diets supplemented with copra and treated with streptocycline, sodium carbonate and acetic acid showed normal health and vigour (Table 38) as compared to the control. All the animals in the treated group survived the experimental period and recorded normal gain in weight as compared to the control. Post mortem examination of the animals killed after the experimental period did not show any gross lesions. Histopathological studies of the liver and kidney also showed no deviation from normal healthy tissues.

Table 38. Mean gain in body weight (g) of small animals fed on diets supplemented with copra treated with chemicals (Duration of experiment: 60 days. 3 animals per group)

Animals used		Copra treated with chemicals											
		Streptocycline				Sodium carbonate				Acetic acid			
		Initial weight	Final weight	Gain in weight	Per cent increase	Initial weight	Final weight	Gain in weight	Per cent increase	Initial weight	Final weight	Gain in weight	Per cent increase
Rabbit													
	E	604.78	736.25	131.47	21.73	600.75	731.56	130.81	21.77	589.63	718.15	128.52	21.79
	C	609.50	742.40	132.90	21.80	590.32	722.32	132.00	22.36	592.33	720.33	128.00	21.60
Guinea pig													
	E	400.58	493.72	93.14	23.25	335.67	426.82	91.15	27.15	407.55	496.75	80.22	21.83
	C	393.85	484.97	91.12	23.13	382.07	487.13	105.06	27.50	415.25	505.40	90.15	21.70
Rat													
	E	130.50	160.79	30.29	23.21	135.15	169.40	34.25	25.34	128.75	160.83	32.03	24.91
	C	132.75	163.50	30.75	23.16	138.25	174.45	36.20	26.18	128.00	160.25	32.25	25.19

E = Experimental C = Control

DISCUSSION

DISCUSSION

STUDY OF MICROFLORA ASSOCIATED WITH COPRA AND COCONUT OILCAKE

Copra

Samples of copra collected from four different oil mills in Trivandrum District over a period of one year revealed that the maximum fungal population occurred during the month of August (Table 1). The fungal population recorded during July, September and November is also very high and it is on par with that recorded for August. The fungal population of samples collected during January, February, March, April and May is significantly low. The high population recorded coincided with the two monsoon seasons that prevailed during the period, and the periods of incidence of low population coincided with the dry and hot period. Significant difference is noted between the fungal population in the samples obtained from different sources (mills). These differences have to be attributed to the differences in the processing of copra and storage conditions in these different mills.

The predominent fungi recorded include *Aspergillus niger*, *A. flavus*, *A. chevalieri*, *Rhizopus oryzae*, *R. stolonifer*, *Mucor hiemalis*, *Penicillium citrinum*, *Botrydiplodia theobromae*

and Curvularia senegalensis.

Most of the above fungi have been reported from copra by earlier workers like Fishlock (1929), Passmore (1931), Cooke (1932), Thompson (1933), Ward (1937), Subrahmanyan (1965), Unnikrisnnan (1968), Paul (1969) and Sreemulanathan and Nair (1971).

These fungi have been reported on other oilseeds also, on castor seeds (Jain and Patel, 1969), on groundnut (Lisker and Joffe, 1970), on pea nut (Diener, 1960; Garren and Porter, 1970) and on soybeans (Schneider et al., 1971).

A. oryzae, A. chevalieri, R. oryzae, M. hiemalis, P. citrinum and C. senegalensis obtained in the present study have not been reported earlier on copra.

The bacterial population fluctuated and no definite pattern of incidence is revealed (Table 2). However, the bacterial population during August, September and November are significantly higher than those recorded during the other periods. The monsoon period appears to favour bacterial population also as in the case of fungal population. The bacteria noted on copra included Bacillus subtilis, Enterobacter aerogenes, Serratia marcescens, Staphylococcus aureus, and Pseudomonas fluorescens. Subrahmanyan (1965) and Sreemulanathan and Nair (1971) have reported

Staphylococcus aureus, B. subtilis and Serratia marcescens on stored copra.

E. aerogenes and P. fluorescens observed in the present study are new reports.

The results showed that the incidence of actinomycete population is not significant on stored copra when compared to fungal and bacterial population as has been the case in other habitats like soil, rhizosphere etc. Further it is not uniformly present throughout the period of investigation (Table 3). Streptomyces sp. obtained in the present study has not been reported earlier.

Coconut oil cake.

The fungal and bacterial population of coconut oil cake are comparatively less than that of copra. There is no significant difference in the fungal population during different periods and among different sources (mills). This may be due to the lower moisture content and nutrient status of the processed material (Table 4). Rajasekharan et al. (1960) in their studies on the shelf-life of coconut poonac has also observed the feasibility of coconut poonac to mould infection under storage conditions.

Significant difference is observed in the bacterial population during different periods. Bacterial population is higher during June, July, October and November. The

monsoon period appears to favour the bacterial population as evidenced from the slightly increased moisture content observed during these months (Table 5).

Inoculation studies

All the microorganisms associated with stored copra are not found to infect copra under conditions of artificial inoculation. This indicates the possible occurrence of two types of microorganisms in copra. Some are only casual contaminents, while others are capable of infecting copra and causing deterioration. Aspergillus spp. is observed to be the predominant fungi infecting copra.

INFLUENCE OF WEATHER ELEMENTS AND MOISTURE CONTENT OF COPRA ON THE MICROBIAL POPULATION

Path coefficient studies

Fungal population

The path of influence envisaged by weather elements and moisture content of copra on fungal population is given in Fig. 8. Relative humidity, minimum temperature and moisture content of copra are taken as the first order components and rainfall as the second order component (Table 39, 40). It is observed that relative humidity of the atmosphere and moisture content of copra are the major factors determining the fungal population. The above factors

are in turn influenced by rainfall. The effect of rainfall on minimum temperature is negligible. The indirect effect of minimum temperature as influenced by relative humidity and the effect of relative humidity on minimum temperature is insignificant. But the influence of relative humidity and minimum temperature is prominent as can be observed from the small residue of 0.4626 (Fig. 3).

Table 39. Direct and indirect effects of the first order components on fungal population

Component	Relative humidity	Minimum temperature	Moisture content	Correlation coefficient
Relative humidity	(0.8195)*	0.0091	-	0.8286*
Minimum temperature	0.0238	(0.3152)	-	0.3390
Moisture content of copra	-	-	(0.8018)*	0.8018*

Direct effects are given within parenthesis

*Significant at 0.05 level

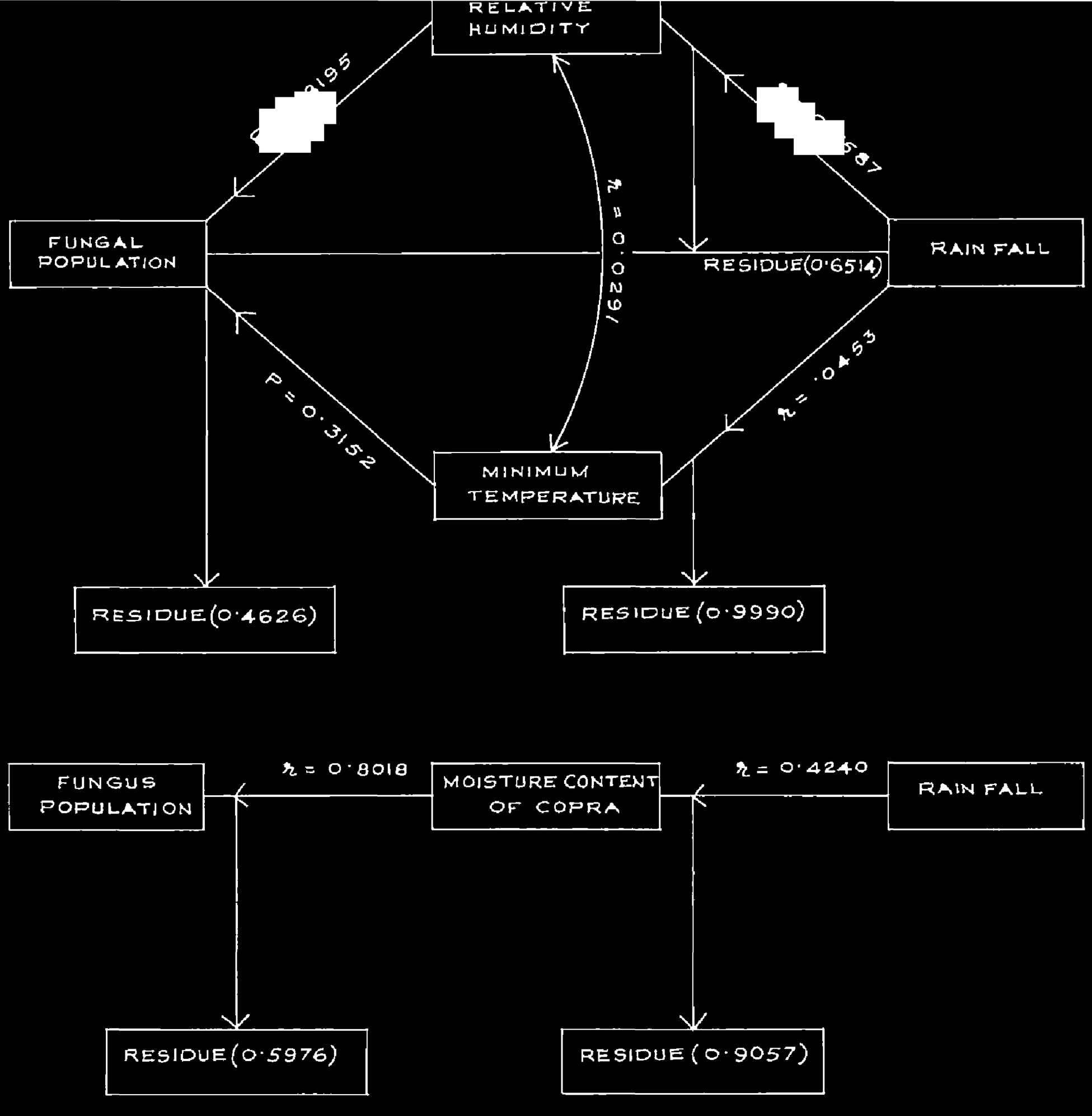

RELATIVE HUMIDITY
FUNGAL POPULATION
RAIN FALL
RESIDUE(0·6514)
r = 0·0291
P = 0·3152
r = ·0453
MINIMUM TEMPERATURE
RESIDUE(0·4626)
RESIDUE(0·9990)
FUNGUS POPULATION
r = 0·8018
MOISTURE CONTENT OF COPRA
r = 0·4240
RAIN FALL
RESIDUE(0·5976)
RESIDUE(0·9057)

Table 40. Correlation coefficients between fungal population, weather elements and moisture percentage of copra

	Fungal popu-lation	Relative humidity	Minimum tempe-rature	Moisture	Rainfall
Fungal population	1.0000	0.8287*	0.3390	0.8016*	0.6296*
Relative humidity	0.8286	1.0000	0.0291	-	0.7587*
Minimum temperature	0.3390	0.0291	1.0000	-	0.0453
Moisture	0.8018*	-	-	1.0000	0.4240
Rainfall	0.6296*	0.7587*	0.0453	0.4240	1.0000

*Significant at 0.05 level

Bacterial population

In the case of bacterial population also the first order components are taken as relative humidity, minimum temperature and moisture content of copra and rainfall as the second order component (Table 41, 42). The path of influence envisaged is given in Fig.9.

Table 41. Direct and indirect effects of the first order components on bacterial population

Component	Relative humidity	Minimum temperature	Moisture content of copra	Correlation coefficient
Relative humidity	(0.7182)*	0.0120	-	0.7302*
Minimum temperature	0.0209	(0.4138)	-	0.4347
Moisture	-	-	(0.7472)*	0.7472*

Direct effects are given in parenthesis

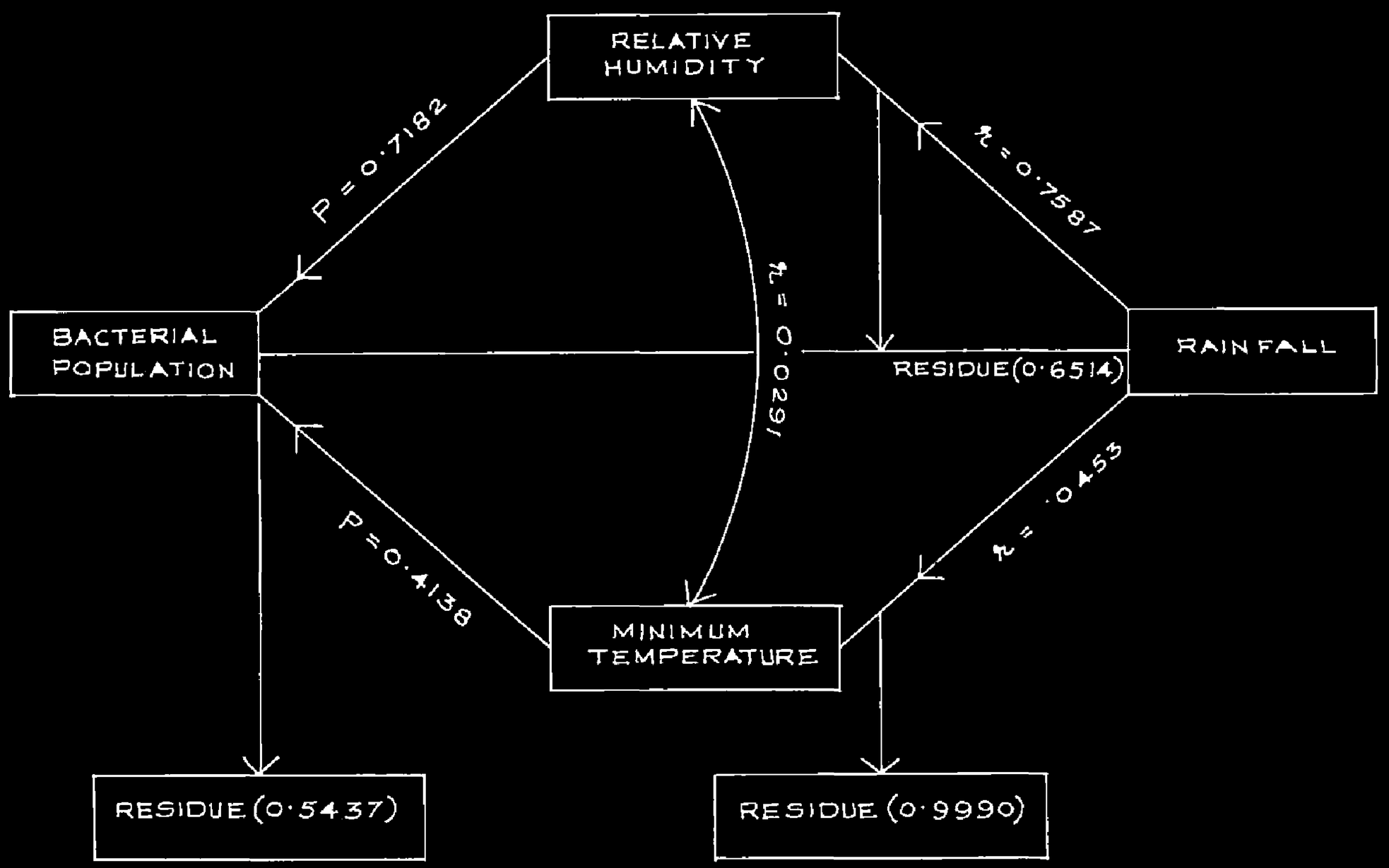
*Significant at 0.05 level

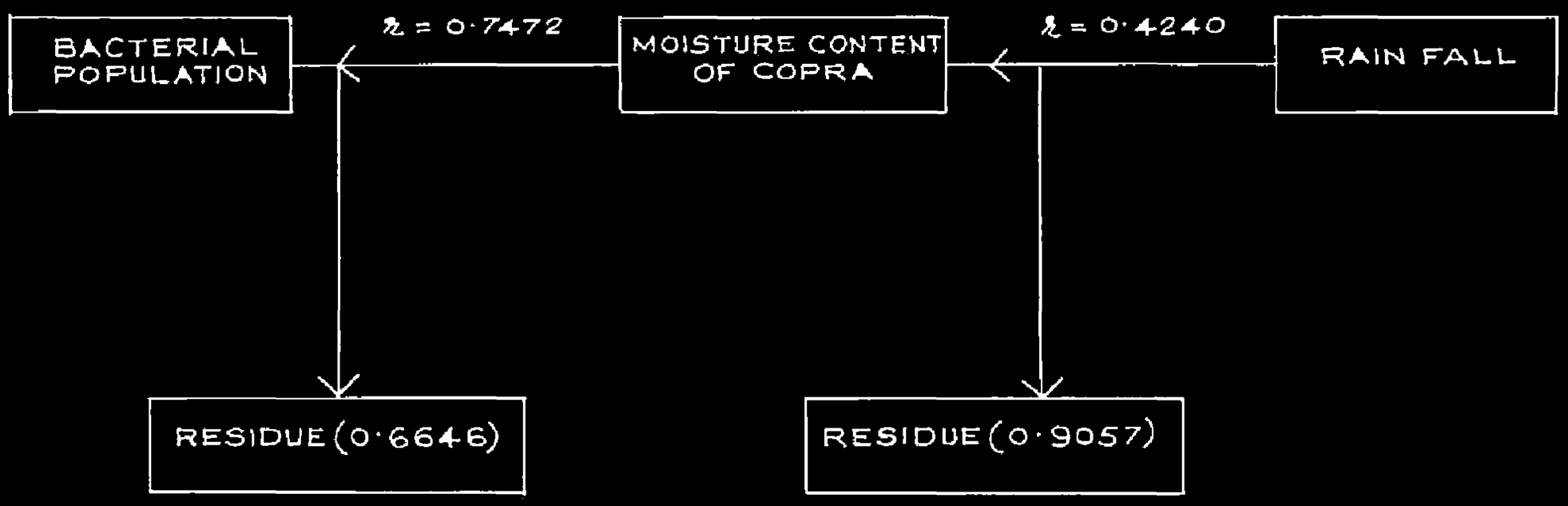
Table 42. Correlation coefficients between bacterial population, weather elements and moisture content of copra

Component	Bacterial population	Relative humidity	Minimum temperature	Moisture	Rainfall
Bacterial population	1.0000	0.7300*	0.4347	0.7472*	0.5352
Relative humidity	0.7302	1.0000	0.0291	-	0.7587*
Minimum temperature	0.4347	0.0291	1.0000	-	0.0453
Moisture	0.7472*	-	-	1.0000	0.4240
Rainfall	0.5332	0.7587*	0.4530	0.4240	1.0000

*Significant at 0.05 level

FIG. 9. PATH ANALYSIS OF WEATHER ELEMENTS AND MOISTURE CONTENT OF COPRA ON THE BACTERIAL POPULATION.





P = PATH COEFFICIENT

r = CORRELATION COEFFICIENT

It is observed that moisture content and relative humidity considerably influenced the bacterial population; the minimum temperature also influenced the same, though to a lesser magnitude. Rainfall exercised its influence on relative humidity of the atmosphere and moisture content of copra. But the influence of rainfall on minimum temperature is negligible. The influence of relative humidity and minimum temperature is evident here also as can be observed from the small residue of 0.5437 (Fig.9).

Moisture content and succession of microflora during copra making

The results revealed that microbial population followed a definite pattern (Table 7). At the time of opening the coconuts the kernels are sterile. When the split nuts are exposed for drying, microorganisms present in the atmosphere gain entry into it. Bacteria are the primary contaminants immediately after splitting the nuts and these organisms multiply and form a slimmy layer within the first 24 hours of drying. This is followed by fungus attack. On further drying, the moisture level decreases and a fungal population consisting of Rhizopus spp. and M. hiemalis dominates till the copra attains a moisture level of 20 per cent. With further decrease in the moisture level, 18 to 13 per cent, Aspergillus spp. make their appearance. At 17.50 per cent

moisture level A. niger and A. flavus are observed which are followed by A. oryzae, A. tamarii, A. ochraceous and A. chevalieri. As the moisture is further decreased to 10.75 per cent, B. theobromae and C. senegalensis appeared. With fully dried copra having a moisture content of 7.5 to 5.0 per cent, P. citrinum is the only fungus which is present superficially. The above findings are in accordance with the work of Subrahmanyan (1965) on copra. Christensen and Kaufmann (1965) have made extensive studies on the fungal population of stored grains in relation to their deterioration. It is significant to note that they have also observed a succession of different species of Aspergillus with decreasing moisture content.

KEEPING QUALITY OF COPRA UNDER DIFFERENT STORAGE CONDITIONS

Keeping quality of copra under different storage conditions revealed that the fungal infection is minimum at low temperature, low relative humidity, low moisture level of copra and with proper storage conditions, i.e., gunny bags lined with polythene sheet (Table 8, 9, 10 and 11).

Storage temperature, relative humidity, moisture content of the substrate, viz., copra and storage practices have great influence on the colonization by microbes resulting

in deterioration. This has been elucidated in different types of stored grains by several workers like Fields and King (1962), Harman and Nash (1972) and by Harman (1972) on stored pea seeds.

Estimation of the final moisture content of copra stored under different conditions revealed (Table 8, 9, 10 and 11) that it has absorbed moisture from the atmosphere. The moisture level increased from five to about eight per cent in almost all the samples. Copra stored in ordinary gunny bags becomes moist and completely mouldy along with insect attack while copra preserved in polythene lined gunny bags remains in good condition, being crisp and free from much fungal infection and insect attack. The observations recorded are in agreement with the work of Marar and Padmanabhan (1960) who also obtained similar results with polythene lined gunny bags.

It is significant to note that the succession of fungal flora in samples of five per cent moisture status and kept under different storage conditions is exactly the reverse of the succession of fungi during the process of copra making (Table 7). Penicillium citrinum known to be a xerophytic fungus (Nair and Sreemulanathan, 1970) is not found at moisture levels above 7.5 per cent in both experiments. When moisture status increases from 5 to 8 per cent Aspergillus spp., B. theobromae and C. senegalensis make their

appearance. Thus the absorption of moisture probably suppresses the growth of P. citrinum and favours the succession of other flora. It may also be that the products of degradation due to initial infection by P. citrinum stimulates infection by the succeeding fungi.

From the results it can be inferred that even if copra is dried to a moisture level below the equilibrium moisture level it will absorb and gain moisture while if it is dried above the equilibrium level it will give up moisture and will attain hygroscopic equilibrium with humidity. The results of the present investigation confirms that moisture is the important single factor governing the initiation and subsequent deterioration of copra. Rajasekharan and Pandalai (1961) regarded eight per cent moisture in copra as the critical moulding moisture level for the coastal areas of Kerala where high relative humidities prevail during the greater part of the year.

BIOCHEMICAL CHANGES IN COPRA AND COCONUT OIL DUE TO FUNGAL INFECTION

Copra

Changes in total sugars due to fungal infection.

The total sugar content of copra decreases with progressive infection by fungi, as is revealed by significant decrease with increasing period of incubation (Table 12).

The decrease in sugar content may evidently be due to utilisation of sugars by fungi for their growth and reproduction. Decrease in sugar content is indicative of certain metabolic changes which occur during the infection of copra by fungi. Though not in stored products, similar observations due to fungal diseases of several plants and fruits have been reported (McCombs and Winstead, 1964; Dayal and Joshi, 1968; Singh and Tandon, 1970 and Prasad, 1974).

Effect of fungal infection on protein content

The protein content of copra showed no significant change due to infection by different fungi. But significant increase in the protein content is observed for different periods.

Nagel and Semeniuk (1947) observed a decrease in protein nitrogen of corn infected by fungi. However Milner (1950) and Ward and Diener (1961) could not record any change in protein nitrogen due to mould infection of soybeans and pea nuts respectively, which correlates with the results of the present investigation.

Fungi in general are known to have a nutritional requirement with a wide C:N ratio (Alexander, 1977). This leads to a greater utilisation of non-protein carbon sources as well as a lower utilisation of protein. The trend to increase the percentage of protein observed is obviously

due to the utilisation of non-protein carbon source by the fungi leading to an increase in the percentage of protein. All the species of fungi tried behave more or less in a similar manner.

Effect of fungal infection on the amino acid content

Infection of copra with different fungi produces variable effects on the amino acid pattern of copra samples (Table 14 and 15). C. theobromae infected sample does not show much variation from the control samples. I. hiemalis and C. senegalensis infected samples show lesser content of all amino acids while A. flavus infected material show a definite increase in its leucine content. Slight increase is noticed in hydroxy proline and threonine and glycine and valine. A definite decrease is noticed in the case of isoleucine, tyrosine, phenyl alanine, lysine, histidine and arginine and slight decrease in aspartic acid and glutamic acid, and serine and proline. R. oryzae infected samples in general shows an increase in almost all the amino acids and decrease in histidine content. P. citrinum infection increases hydroxy proline and threonine, serine and proline, glycine and valine, isoleucine and leucine and decreases tyrosine, lysine, and arginine. Similar quantitative changes in the amino acid pattern of peanuts infected by Neurospora sitophila and Rhizopus oligosporus have been observed by Cherry and Beuchat (1976).

In the copra material with a uniform pattern of distribution of amino acids, infection by different fungi thus causes increase in the content of certain amino acids and decrease in the content of certain others. This can be attributed to two reasons. Firstly it may be due to the utilization of certain amino acids, as a consequence of which their contents get decreased. Depletion of amino acids due to their utilisation by pathogens has been reported (Aulakh and Sandhu, 1970). This decrease itself causes, an increase in the content of the unutilised amino acids. Secondly it could be that some of the amino acids whose contents have got increased consequent to infection might have been contributed by the pathogens metabolism. No attempt was made to differentiate these two.

Similar changes in amino acid pattern of infected fruits and host plants have been recorded. Thus Srivastava and Tandon (1966) working on the free amino acid spectrum of mosambi fruits infected by *B. theobromae* showed that glutamic acid, threonine and proline were absent, while asparagine, r-aminobutyric acid and arginine were markedly reduced. An increase in serine and glycine was observed. Bisen (1975) recorded a decrease in asparagine, aspartic acid, glutamic acid and threonine and an increase in alanine, proline and leucine during pathogenesis of apple tissues by *A. niger*.

Plant pathogens cause remarkable changes in the nitrogen metabolism of host plants. Compounds like free and bound amino acids have been reported to disturb the degree of virulence of pathogens. Consequently the disease inciting agents change the qualitative and quantitative content of amino acids and other compounds as a result of infection (Rangaswami and Natarajan, 1960; Raghunathan et al., 1966; Naik et al., 1970 and Chahal and Grover, 1972a).

Coconut oil

Physical and chemical constants

Colour

As a result of fungus infection on copra the quality of oil is impaired. Considerable variation in the colour of the oil is noticed due to infection (Table 16). Among the fungi tested A. flavus imparted the maximum deepening of colour. The colour increases with the period of incubation up to 15 days and thereafter it remains constant. The changes in the colour of the oil due to fungal infection of copra are in agreement with the findings of Ward and Diener (1961) on peanut and Lalithakumari et al. (1971a) on groundnut.

Both tyrosine and phenyl alanine are known to be precursors for the synthesis of melanin. The decreased content of phenyl alanine and tyrosine observed in all the

samples (Table 14) corroborates with the intensity of colour observed due to infection which is mainly due to melanin like pigments (White et al., 1973).

Odour

The odour of oil extracted from infected samples of copra varied from slight to highly rancid (Table 17). The rancidity brought about by the infection of fungi may be attributed to the presence of methylamyl, methyl heptyl and methylnonyl ketones (Stoke, 1923). The peculiar odour and taste being due to the liberation of free fatty acids or due to the production of odoriferous aldehydes or ketones formed as a result of oxidation (Thorpe, 1960).

The role of seed borne fungi in the development of rancid odour of oils has been reported by Wilson (1947) and Lalithakumari et al. (1971 a). The odour of oil extracted from samples of copra infected with *L. hiemalis* is markedly different from that of other samples, the smell being reminiscent of acetic acid. This can be attributed to the presence of acetic acid produced as a result of the break down of fatty acids resulting from the hydrolysis of fat by microorganisms (Buchanan and Buchanan, 1967).

Refractive index

Refractive index of the oil (Table 18) did not show

any change from the standard specifications (Jamieson, 1943). Increase in refractive index is generally considered to be indicative of the presence of more saturated acids in the oil. Infection did not affect the refractive index suggesting thereby that both saturated and unsaturated fatty acids are being utilised fairly in equal proportions by the fungi.

Acid value

The acid value of the oil obtained from copra samples infected by different fungi is found to increase with the period of incubation of the samples after infection (Table 19).

The acid value is a measure of hydrolytic rancidity. Fungus infection hydrolyses the glycerides of fatty acids to free fatty acids and glycerol. Oil expelled from samples infected by A. *flavus* showed the maximum acid value.

An increase in fatty acids due to infection by fungi on stored soybean was correlated by Milner and Geddes (1946) with moisture content. Naturally occurring mixtures of glycerides in oils contain only a small amount of free fatty acids produced by hydrolysis. Increase in fatty acids shows that these fungi have high lipase activity (Table 30). Increase in fatty acids due to fungal infection have been reported by Milner and Geddes (1946) on soybean, Stansbury (1947), Ward (1955) and Ward and Diener (1961) on pea nuts, Lalitha kumari *et al.* (1971 a) on groundnut and Lalitha kumari

et al. (1971 b) on castor seeds.

Among the fungi tested P. citrinum recorded minimum acid value of oil. This is in agreement with the work of Ward and Diener (1961) who also recorded only slight changes due to P. citrinum infection of peanuts.

Wilson (1947) showed that the strong rancid taste of oil due to fungal invasion is due to the increase in free fatty acid content.

Subramanian (1956) observed a high acid value of 76 per cent in a sample of coconut oil extracted from mould infected copra. Jayanathan (1970) also obtained a high acid value of oil from coconut refuse.

Saponification value

The saponification value of oil is not found to vary due to infection of copra by different fungi (Table 20) from the standard specifications (Jamieson, 1943). A high saponification value generally indicates a higher percentage of glycerides with fatty acids of lower molecular weight and a low saponification value indicates a higher percentage of fatty acids of higher molecular weight. Since infection by different fungi does not lead to an alteration in the saponification value it evidently indicates that the fatty acids are not being broken into smaller fragments. This finding is further confirmed by the observations on the refractive index of the

oil (Table 19) which also remained fairly constant in spite of infection by different fungi.

Iodine value

An increase in the iodine value of oil is noted in almost all the samples of oil extracted from copra infected by different fungi (Table 21). This increase in iodine value is due to the increased unsaturation created by the metabolic activity of the fungi. Ninety per cent of the fatty acids in coconut oil are known to be of the saturated type and only nine per cent are of the unsaturated type, the unsaturated acids being mainly oleic and linoleic acids. The corresponding saturated acid, viz., steric acid is present to the extent of only 2.3 per cent in coconut oil. Due to the metabolic activity of the fungi the majority of saturated acids of the oil is being changed into unsaturated oleic and linoleic acids. As a consequence significant variation is observed in the iodine values of oil extracted from the fungus affected coconut kernels. Increase in iodine value therefore suggests significant dehydrogenation of the fatty acids. Similar results have been obtained by Ward and Diener (1961) in peanuts infected by fungi, while contradictory results have been obtained by Milner (1950) on soybeans and Lalitha kumari et al. (1971) on groundnut kernels infected by seed borne fungi.

Changes in the properties of oil on storage

Oil stored for a period of three months immediately after extraction remained normal without any changes in its properties. After six months of storage there is a slight rancid smell. Acid value and iodine value showed slight increase (Table 22). Besides hydrolytic changes causing rancidity, it is known that the oxidative changes can also cause rancidity by absorption of oxygen at the double bond with the formation of peroxides, oxyacids and aldehydes (White et al., 1973).

Effect of infection by fungi on the oil content of copra

Considerable decrease in the oil content of copra due to fungal infection has been noticed (Table 23). About 50 per cent decrease in oil is recorded in samples infected by R. oryzae and M. hiemalis. This is in agreement with the work of Ward and Diener (1961) who also obtained a reduction in oil content of stored peanuts due to infection by Aspergillus spp. and P. citrinum. Lalitha kumari et al. (1971 a), (1971 b) also observed reduction in the oil content of groundnut seeds and castor seeds due to infection by seed-borne fungi. All the fungi observed on copra during storage are capable of utilising oil as a source of carbon for their growth (Ayre, 1932; Reese et al., 1955; Paul, 1969 and Sreemulanathan and Nair, 1971). The ability of the fungi to utilise oil as carbon source makes copra a suitable substrate for their growth

under humid conditions.

ROLE OF FUNGAL ENZYMES IN THE DETERIORATION OF COPRA

Pectic enzymes elaborated by pathogens *in vitro* and *in vivo*.

Macerating enzymes

Most pathogens establish on host material by invading tissues composed of undifferentiated cells. The walls of such cells consist largely of cellulose and pectic substances and the degradation of these materials are important in the progress of the infection and further colonization. The significance of pectic enzymes in pathogenesis has long been recognised by Brown (1915). In the present investigation among the fungi tested, only *A. niger*, *A. flavus* and *A. oryzae* showed macerating enzyme activity (Table 24). Maceration of plant tissue is an important phenomenon in the disease syndrome. It is caused by the dissolution of the middle lamella composed largely of pectic substances resulting in the loss of coherence of plant tissues. The identity of cells are lost and the cell wall disintegrates through the continued action of the enzymes produced by the parasite (Wood, 1967). Production of protopectinases or macerating enzymes by *R. stolonifer* have been reported by Gupta and Pandey (1959) in plum rot and Srivastava *et al.* (1959) on

soft rot of sweet potato. Vidhyasekharan et al. (1966) observed the production of protopectinase by A. flavus associated with paddy seed spoilage. Ishii (1976) and Ishii and Kiho (1976) isolated a factor that stimulated tissue maceration from the culture filtrate of A. japonicum. Chakrabarti and Nandi (1976) recorded maceration activity of B. theobromae isolated from decayed banana fruit.

Pectin Methylesterase (PME)

These are highly specific enzymes which saponify methyl ester groups of pectinic acids to give methyl alcohol and pectinic acids of lower methoxyl content. PME brings about demethylation of pectic substances, middle lamella and primary cell walls, further predisposing them to the action of other pectolytic enzymes.

Provided cultural conditions are suitable, PME secretion is the rule rather than exception among microorganisms and it is produced much more abundantly in the presence of specific substrates (Wood, 1967). In the present investigation also PME activity is, detected both in vitro and in vivo studies. Comparing in vitro preparations with sodium nitrate and ammonium sulphate as nitrogen source it is seen that higher activity is shown in preparations where sodium nitrate is used. Comparing PME activity in vitro with in vivo preparations it is seen that the level of activity is

significantly lower *in vivo* preparations. From the results (Table 25) it appears that during early stages of incubation the fungi have greater capacity to produce this enzyme. Similarly sodium nitrate as nitrogen source might have supported better growth of the fungi. *A. flavus* showed maximum PL activity which is on par with that of *A. niger*. *Botryodiplodia theobromae* did not show any activity.

PML production was reported by Srivastava *et al.* (1959) in *Rhizopus stolonifer* causing soft rot of sweet potatoes and Collins and Kledjoski (1960) in *Penicillium italicum*. Among other fungi, Benandi and Walker (1957) and Hancock (1966) recorded PL production in *Sclerotinia sclerotiorum* while Papavizas and Ayers (1965) observed in *Rhizoctonia praticola* and *R. solani* and Chahal and Grover (1972*b*) in *Choanephora cucurbitarum*.

Polygalacturonase (PG)

The enzyme polygalacturonase break the polygalacturonide chains by hydrolysis at the glycosidic linkages to give shorter chains and as a result simultaneously liberates reducing groups with progress in the enzymatic hydrolysis. Demain and Phaff (1957) distinguished between 'endo' types of PG which attack the glycosidic linkages at random and 'exo' types which preferentially or only attack end linkages. Attempt was made to study only exo-PG.

Exo-Polygalacturonase activity is detected in the culture filtrates of different fungi tested (Table 26). Maximum activity is noticed on the eighth day of incubation. The enzyme activity is maximum in *in vivo* preparations. The role of Exo-PG in pathogenesis has been reported by Ayers *et al.* (1966) in *Rhizoctonia solani* and Bateman (1972) in *Sclerotium rolfsii*.

Polygalacturonase trans-eliminase (PGTE)

The enzyme splits the galacturonic acid chain at the 1-4 linkages and removes the hydrogen atom at C-5 of one unit to C-1 of the adjacent unit and then cleavages the glycosidic linkage with the formation of a double bond between C-4 and C-5 of the unit (Albersheim *et al.*, 1960).

In the present study also PGTE is detected by the absorption of TBA products (Table 27). The enzyme activity varied among different fungi. Maximum enzyme activity is showed by *in vivo* preparations and in 16 day old cultures. Among the different fungi tested *F. citrinum* showed the highest activity. Production of PGTE *in vitro* and *in vivo* has been reported by Ayers *et al.* (1966) and Sherwood (1966) in *Rhizoctonia solani*; Papavizas and Ayers (1966) in *Fusarium oxysporum* and *F. solani* and Kathirvelu and Mahadevan (1967) in *Fusarium moniliforme* and *Cephalosporium sacchari*.

Cellulolytic enzymes

The primary and secondary cell walls consist of a matrix in which a large number of microfibrils consisting of aggregations of glucose units are embedded. The cellulolytic enzyme C1 acts upon native insoluble cellulose to produce linear chains susceptible to attack by a second enzyme Cx which acts and degrades reprecipitated or soluble celluloses (Wood, 1967).

The enzyme cellulase (C1) is produced by all the fungi tested *in vitro* and *in vivo* (Table 28). Maximum activity is noticed in the 16th day of incubation showing that the fungus produced enzymes only at the later stage of its growth. Ramasami and Shanmugam (1976) also obtained similar results with *Rhizoctonia bataticola* while Kannaiyan *et al.* (1975a) observed maximum enzyme activity on the eighth day by *Claviceps microcephala* in *in vitro* studies, *in vivo* preparations showed the minimum activity.

Cellulase (Cx)

All the fungi tested showed high cellulase (Cx) activity (Table 29). The enzyme activity is maximum on the sixteenth day of incubation. Rao (1977) obtained maximum enzyme activity around twelfth day of incubation with an *Aspergillus* strain isolated from soil. Kannaiyan *et al.* (1975a) could not detect any activity in the *in vivo*

preparations by Clavicops microcephala but obtained considerable in vitro production. In the present investigation also in vivo preparations showed the least activity.

White et al. (1943) assayed the cellulolytic activity of moulds isolated from fabrics and observed that Mucorales are devoid of any activity while Aspergillus spp. demonstrated a weak cellulolytic activity. Marsh et al. (1949) also observed Mucor spp. to be devoid of cellulose decomposing property. Olutiola (1976,a,b) studied the cellulase enzyme (C1) in the culture filtrate of P. citrinum and A. flavus.

The increased cellulolytic activity with increase in the period of incubation coincides with the decrease in oil content of copra (Table 23). In the present investigation also P. hiemalis showed comparatively lower C1 and Cx enzyme activity.

Lipase

All the fungi are found to produce lipolytic enzymes and maximum enzyme production is obtained on the eighth day of incubation (Table 30). Lyre (1932) conducted studies on the lipolytic activity of various Aspergillus spp. Mucor racemosus, Syncephalastrum sp. and Penicillium sp. isolated from stored copra. All of them produced extra-cellular lipases in the in vitro studies. Horowitz Vlassoma and

Livschitz (1936) observed that fungi of the genera Penicillium, Aspergillus and Sterigmatocystis as well as bacteria were capable of splitting fats and oils. The lipolysis induced by fungal activity is characterised by marked rise in acid number and reduction in oil content (Lalitha kumari et al. (1971 a; 1971 b).

All the fungi tested are capable of hydrolysing fats with the production of free fatty acids. This is confirmed by the production of a rancid odour (Table 17) and high acid value (Table 10) with increasing period of inoubation of copra after infection with fungi.

Goodman (1950) investigated the adaptive production of amylase and lipase by A. flavus, A. terreus and Penicillium notatum. Ramakrishnan and Banerjee (1951) and Sharma and Chauhan (1976) studied the lipases production by storage microorganisms on oil seeds and observed high lipolytic activity. Coursey (1960) observed the importance of fungi like A. niger, A. terreus and A. giganteus in the lipolysis of palm oil. Hiscocks (1964) made extensive studies on the importance of lipolysis by various Aspergillus spp. in the deterioration of tropical foods and feed stuffs and recorded A. flavus, A. niger, A. fumigatus and A. awamori to be actively lipolytic and A. nidulans, A. sulphureus and A. tamarii to be slightly lipolytic.

Proteolytic enzymes

The proteolytic enzymes hydrolyze the peptide links of proteins or peptides. Their role in pathogenesis has been investigated only to a limited extent.

Rao et al. (1971) and Rao and Sreekantiah (1976) studied the protease activity of J. theobromae isolated from coconut eye. Chakrabarti and Nandi (1976) also showed protease activity by J. theobromae isolated from decayed banana fruits. The results of the present investigation also showed significant enzyme activity by different fungi (Table 31) which are in agreement with above workers.

It is significant to note that in in vitro studies on proteolytic enzymes J. hienalis is found to have a high proteolytic activity. Relating this result with the in vivo studies on the amino acid pattern of infected copra samples (Table 14 and 15) it is observed that infection by J. hienalis and C. senegalensis showed a decrease in all the amino acids with respect to control. These results thus confirm that proteolysis leads to the utilisation of amino acids so produced.

STUDIES ON MYCOTOXINS IN COPRA

Aflatoxin

Microbiological assay of aflatoxin in copra.

Microbiological assay method for antibiotics based on

growth inhibition of bacteria is used to determine the production of aflatoxin in copra samples consequent to infection by different fungi.

From the well defined zone of inhibition noticed it is clear that aflatoxin is present in the extracted sap. The results are in conformity with the work of Clements (1969) where a zone of inhibition with 1/μg of aflatoxin B_1 was noticed on incubation with B. megaterium and confirmed that the test is sensitive to as little as 1/μg of aflatoxin.

Estimation of aflatoxin B_1 produced by fungi in copra

A concentration of two ppm of aflatoxin B_1 is estimated in one sample of copra tested. Aflatoxin production in coconut is already reported (Schindler and Eisenberg, 1968; Maur and Armstrong, 1971; Arseculeratne and De Silva 1971 and Samarjeewa, 1972).

Studies on the production of aflatoxin during different periods, of the year (Table 32) revealed its presence in the samples of copra collected from March to August, the concentration being above one ppm. This may be attributed to the abundant growth and sporulation of A. flavus on the substratum facilitated by the congenial atmospheric conditions.

Arseculeratne and De Silva (1971) observed medium (0.05 - 0.25 ppm) or high (0.25 - 1 ppm) aflatoxin levels in samples of poonac, coconut oil and copra. Subrahmanyan and

Rao (1974) also observed high aflatoxin content in peanut, castor seed and copra samples.

Aflatoxin producing strains of A. flavus or A. parasiticus are widely distributed in nature. Under tropical conditions they get sufficient chance to contaminate and grow on food materials which are improperly handled or stored (Hesseltine et al., 1966). High moisture content of the substratum coupled with an atmospheric humidity of 70 per cent and a temperature of 28 - 30°C favours the growth of A. flavus (Diener and Davis, 1967; Schindler et al., 1967; Krenk and Hartman, 1970 and Oke, 1970). These conditions are prevalent in Kerala (Table 6) and hence can be accounted for the high concentration of aflatoxin B_1 in copra.

Goldblatt (1971) summarised the researches of several investigators on the quantitative production of aflatoxin on numerous substrates by potent aflatoxin producing strains of A. flavus. The data indicated that high carbohydrate substrates generally supports larger yields of aflatoxin than those containing large percentage of oil that are not immediately metabolized by A. flavus. But in the present study it is found that oil is well utilised by A. flavus (Table 23). Naik et al. (1970) observed that methonine, proline and tryptophan present in peanut and coconut supported aflatoxin production by A. flavus.

Besides A. flavus several fungi are known to produce aflatoxins. Kulik and Holady (1967) has obtained aflatoxin production by A. niger, A. wentii, A. ruber, P. puberulum, P. variable, P. frequentans and P. citrinum. Basappa et al. (1967) observed aflatoxin B_1 production by A. oryzae. But in the present study (Table 33) aflatoxin production is noticed only in the case of A. flavus.

Biological assay

Chick embryo inoculated with different levels of aflatoxin did not survive for more than three days, after inoculation. Platt et al. (1962) reported five day old embryos to be sensitive to aflatoxin injury and as little as 0.3 µg of a crude groundnut preparation caused death in two days. In the present study death of embryo is noticed with 0.1, 0.05, 0.033 and 0.025 µg samples (Table 34).

At a maximum concentration of 0.1 µg which is only one-third of that tried by Platt et al. (1962) the death of embryo occurred on the first day after inoculation in all the three embryos tried. At the lowest concentration of 0.025 µg also one embryo was dead on the first day after inoculation. The toxicity observed in young embryos as evidenced by mortality pattern is in agreement with the observation of Choudhury and Manjrekar (1967).

Ducklings

The fact that 27 out of 37 ducklings fed with copra infected by A. flavus died within the first seven days (Appendix XXXIII, Fig.7) while only three out of 37 died in the control group indicates that there is a high level of toxicity in the copra administered.

The LD_{50} of aflatoxin has been estimated by various workers with considerable agreement among them. For aflatoxin B_1 it varied from 17.5 μg to 28.2 μg for a duckling weighing 50 g (Nesbitt et al., 1962, Asao et al., 1965, Carnaghan et al., 1963 and Lijinsky and Butler, 1966). In the present study also one g of the copra infected by A. flavus produced very high mortality among ducklings and produced histopathological changes in the liver and kidney similar to that reported due to the administration of purified preparations at LD_{50} levels. Hence the present observation clearly shows that one g of infected copra contains lethal levels of toxin corresponding to the reported LD_{50} values mentioned above.

The average weight gain of the birds in the treatment groups are found to be significantly lower than those of the control group (Table 43, Fig. 10, Plate XX).

The poor weight gain in aflatoxin fed birds may be due to impaired protein synthesis. Changes in RNA synthesis following aflatoxin treatment has been shown by Spron et al. (1966). Because of the effects of aflatoxin on nuclear RNA

synthesis it could be conjectured that cytoplasmic RNA is also altered.

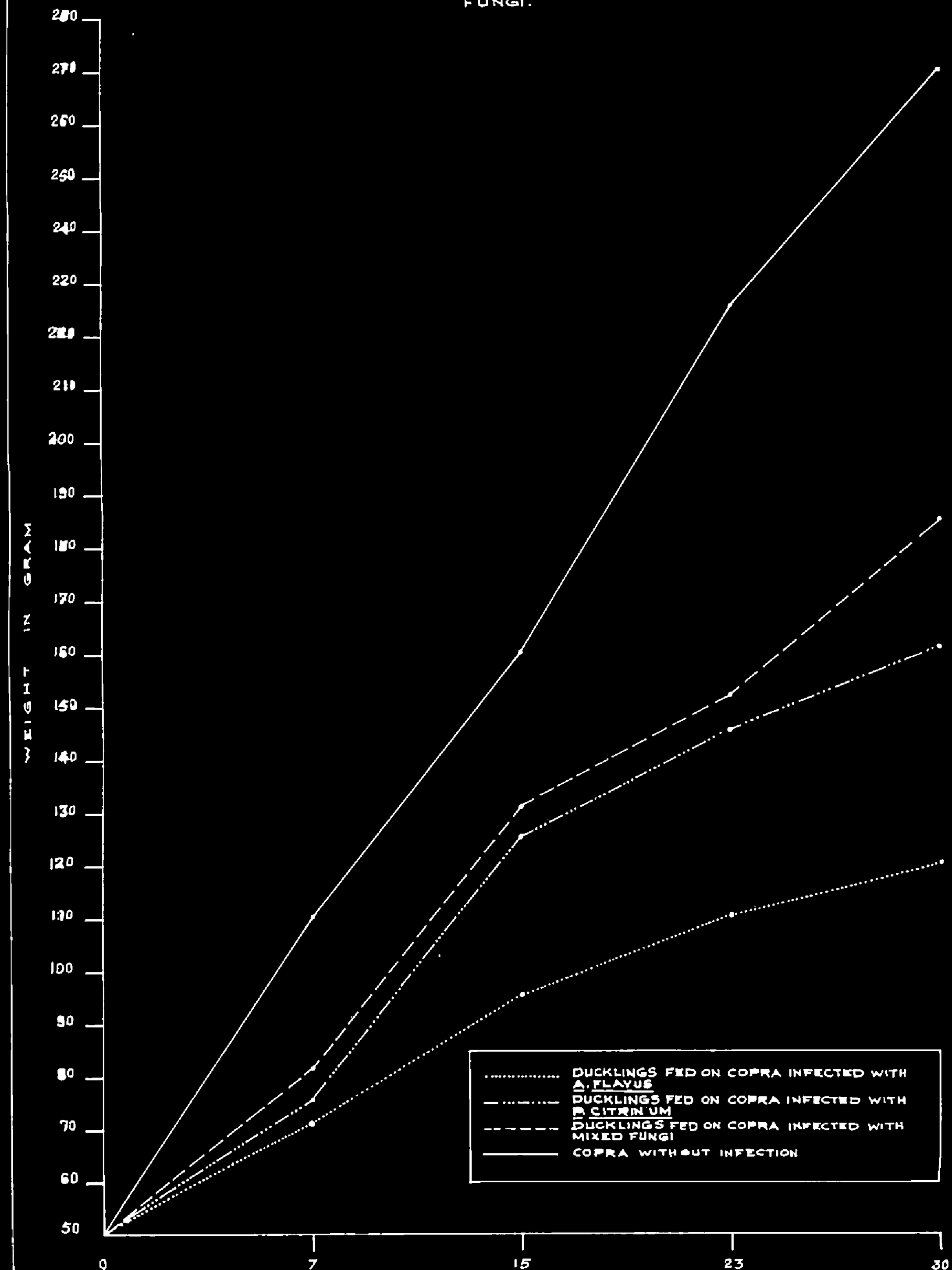
Table 43. Mean body weight (g) of ducklings fed on diets supplemented with copra infected by different fungi

Fungus tested	Period of observation (day)				
	0	7	15	21	30
A. flavus	50.75	70.58	95.70	110.45	120.80
P. citrinum	51.83	75.07	125.85	145.72	160.75
Mixed inoculum of *A. flavus* and *P. citrinum*	50.06	82.87	131.20	152.36	185.32
Control	51.15	110.50	160.25	225.55	270.45

Roy (1968) showed that DNA - dependent RNA synthesis is affected and there is induction of polysomal disaggregation. Aflatoxins have been found to displace ribosomes from endoplasmic reticulum (Williams and Rabin, 1969). The interaction of aflatoxin B_1 with a number of biologically important substrates was investigated by spectrophotometric and fluorimetric techniques (Prasanna *et al.* 1976) and observed alterations in RNA and DNA by the addition of aflatoxin B_1.

Various studies were conducted to investigate the

FIG.10. BODY WEIGHT OF DUCKLINGS FED WITH COPRA INFECTED BY FUNGI.



effects of aflatoxin on the rate of incorporation of the various amino acids (Smith; 1963; 1965; Clifford and Rees, 1966; 1967). Almost complete or partial suppression of incorporation was seen in many cases. Hence it can be assumed that the reduction in weight might be due to a general failure of protein synthesis as a result of the action of aflatoxins.

The acute toxicity of aflatoxin has been attributed to its effect on the nucleøus. Ultra structural lesions in liver cell nucleoli consist of segregated granular or fibrillar components with the formation of scattered nucleolar caps. Light and electron microscopic studies of cirrhotic liver of aflatoxin fed ducklings showed regressive changes in the mitochandria (Kohler and Schumacher, 1967).

It is well established that ducklings could be employed for biological assay of aflatoxin, mainly for semiquantitative evaluation. Butler (1964) in his studies on acute liver injury to one day old ducklings reported that acute bile duct proliferation reached a maximum in three days and then regressed with subsequent repair of their parenchyma. The minimum concentration of aflatoxin B_1 required to produce bile duct proliferation is reported as 0.04 mg/kg based on administration of five equal doses. It was also pointed out that it would not be possible to distinguish a two-fold

difference in the quantity of aflatoxin administered on the basis of the measurement of bile duct proliferation. The pattern of mortality and the nature of bile duct proliferation corroborate the analytical results of high aflatoxin content in copra due to infection by A. flavus.

Carnaghan (1965) observed ducklings developed tumours only after 14 months, after being fed 0.03 ppm aflatoxin in the diet. Because of the short term experiments, the oncogenic effect of aflatoxin could not be verified in the present investigation.

The pathological effects and tissue alterations observed in the present investigation are in agreement with the results obtained by many earlier workers (Blount, 1961; Carnaghan and Sargeant, 1961; Asplin and Carnaghan, 1961; Butler, 1964 and Theron et al. 1965).

The short term studies conducted on guinea pigs and rats are more for confirming the presence of toxins and as a corroborative study than an attempt for detailed investigation to elucidate the various pathological aspects of aflatoxin.

Histopathological studies of the liver and kidney of guineapigs and rats also showed similar changes as in ducklings. Paterson et al., (1962) and Butler (1966; 1969) observed the acute toxicity of aflatoxin B_1 in guinea pigs

while Hogan and Newberne (1967) and Butler (1969) studied the pathological changes in rats.

Though aflatoxin has been reported from copra, this is the first study on biological assay using infected samples of copra.

P. citrinum toxins

The fact that 28 out of 37 ducklings which were fed with copra infected with *P. citrinum* died while only three out of 37 died in the control group (Appendix XXXIII, Fig. 7) indicate that a high level of toxin is present in this case also. *P. citrinum* is known to produce the toxic metabolite citrinin on several moistened food materials (Wilson, 1964; Scott *et al.*, 1972 and Butler, 1974). The average weight of the birds in the treatment group is also found to be significantly lower than those within the control group (Table 43, Fig.10). The poor weight gain may be due to impaired protein synthesis as in the case of aflatoxin poisoning. The significant growth retardation observed in ducklings fed with *P. citrinum* infected copra is in agreement with that of Sakai (1955) who obtained a slight but definite growth retardation in experimental rats fed with mouldy rice containing *P. citrinum*.

The pathological changes in the liver of experimental ducklings were not as severe as in those with aflatoxicosis.

The target tissue appeared to be renal parenchyma as evidenced by marked degeneration and necrotic changes. Citrinin isolated from P. citrinum was found to cause renal damage in mice (Sakai, 1955) and was implicated in swine nephropathy (Korgh, 1973) and in yellowed rice toxicoses (Miyake and Saito, 1965 and Mirocha and Christensen, 1974).

It is significant to point out that this is the first study on the toxins of P. citrinum from infected samples of copra.

Mixed inoculum of A. flavus and P. citrinum

Death of 20 ducklings out of a total of 37 ducklings observed during the first seven days (Appendix XXXIII, Fig. 7) indicates a high level of toxin in the copra samples administered to the ducklings. The reduction in weight (Table 45) is not so marked as A. flavus group and P. citrinum group. This suggests that aflatoxin and P. citrinum toxin do not exert a synergistic action and the quantum of each of the incorporated toxins is not sufficient to have definite deleterious effect as in the other treatments.

Studies on the synergetic action of A. flavus and P. citrinum infected copra on ducklings is a new line of study.

The presence of toxin producing strains like A. flavus and P. citrinum as well as other toxin producing fungi like A.ochraceous in copra is of great practical significance

since their presence beyond a certain level will definitely inflict injury to human beings and animals which are fed with the products of copra.

The report of de Iongh et al. (1964) that milk of cows fed with aflatoxin contained toxic principles (a hydroxylated aflatoxin derivative),focusses the great importance of providing aflatoxin free feed to animals, lest the milk which human beings consume might be contaminated.

From the available literature it seems that the deleterious effect of P. citrinum toxin has not been studied in different species of domestic animals and birds. It is not known whether there is any species difference in its action. Further detailed studies are warranted both in experimental small animals and also in domestic animals to elucidate the effects of different levels of this toxin either alone or in combination with other toxins.

The recognition of the carcinogenic activity of aflatoxins has raised many questions concerning the public health hazard presented by fungal contamination of food. Direct evidences that aflatoxin causes human liver diseases is difficult to obtain. However there is increasing evidence that in those areas of the world where there is increased incidence of hepatic carcinoma, the population is exposed toaflatoxin (Alpert et al. 1968; Shank, 1971; Campbell and Salamat, 1971).

As a result of the experimental data on the carcinogenicity of aflatoxins the WHO have recommended a maximum level of 0.3 ppm aflatoxin in supplemented food for young children in regions where protein mal-nutrition is common (Butler, 1974).

Since copra has been identified as a good source of fungal toxins, and de-oiled cake being a major component in animal and poultry feeds, detailed investigations are required on the possible ways by which these toxins reaches both human and animal population.

Use of food additives, antibiotics and chemicals

A number of chemicals with bacteriostatic and fungistatic action as well as preservatives were tried as surface protectants. Only three of the chemicals viz., streptocycline (500 ppm and 1000 ppm), sodium carbonate and acetic acid at all levels tried are found effective in preventing deterioration (Table 38) under storage conditions upto 60 days. The effectiveness of acetic acid treatment as a method of controlling infection during sun drying of coconut kernel is already reported (Subrahmanyan et al. 1966 and Sreemulanathan and Nair, 1971).

The fact that other chemicals tried could not offer protection may be due to their lack of penetration.

Subrahmonian et al.(1978) observed that coconut kernel possessed a unique property that would prevent the penetration of chemicals beyond half a millimetre. However the effect of the above mentioned chemicals beyond a period of 60 days was not observed. Acetic acid being volatile might have been gradually lost after exposure. This coupled with the humidity conditions of the atmosphere might have been the contributing factors for the slight infection noticed beyond 60 days of storage.

ASSAY OF MAMMALIAN TOXICITY OF ADDED PRESERVATIVES

Copra treated with streptocycline, sodium carbonate and acetic acid showed no deleterious effect on the health of animals as evidenced by the increase in body weight of the animals compared to the control (Table 38). The liver and kidney of the animals were normal and showed no gross lesions or pathologic lesions on histopathologic studies. This clearly shows that all these chemicals can be used without any toxic hazards.

From the above results it can be confirmed that treating copra initially with streptocycline, sodium carbonate or acetic acid and then drying to a moisture level of about five per cent will result in good quality copra which will remain free of fungus infection up to a period of 60 days.

SUMMARY

SUMMARY

The microbial population of copra samples collected from four different oil mills in Trivandrum District for a continuous period of one year was studied. Copra samples with a surface area of one sq.cm and weighing one g were used for assessing the population by the modified soil dilution technique. The results revealed that the maximum fungal and bacterial population coincided with the two monsoon seasons. Actinomycetes were not found to be uniformly present.

The predominant fungal flora included Aspergillus niger, A. flavus, Mucor hiemalis, Rhizopus oryzae, R. stolonifer, Penicillium citrinum, Botryodiplodia theobromae and Curvularia senegalensis. The important bacteria recorded were Staphylococcous aureus, Bacillus subtilis, Enterobacter aerogenes and Pseudomonas fluorescens. Species of Streptomyces was found to be associated with copra, but not found to infect copra on artificial inoculation.

Among the microbes observed on copra A. oryzae, A. chevalieri, R. stolonifer, R. oryzae, M. hiemalis, P. citrinum, C. senegalensis, E. aerogenes and P. fluoresoens were new records.

Samples of coconut oil cake collected from the same locations showed no significant changes in the fungal population. But bacterial population was found to vary significantly. The maximum population coincided with the two monsoon seasons as in the bacterial population on copra. The microorganisms included A. niger, A. flavus, R. stolonifer, P. citrinum, Bacillus subtilis and Staphylococcus aureus.

Inoculation studies with the fungi isolated from samples of copra showed that all of them were not capable of infecting copra under artificial conditions.

The correlation coefficient and path coefficient analysis of the weather elements viz., relative humidity of the atmosphere, minimum temperature and rainfall for ten days prior to the collection of samples, and the moisture content of copra revealed significant correlation with the microbial population. It was observed that relative humidity of the atmosphere and moisture content of copra were the important factors influencing the microbial population. These two factors were in turn influenced by the rainfall.

The sequance of occurrence of the microflora on copra during the process of drying showed that the microorganisms were specific to particular moisture limits beyond which they could not survive.

Keeping quality of well dried copra under different conditions revealed that fungal infection was minimum at low temperature, low relative humidity, and under proper storage conditions.

Total sugar content of copra was found to be reduced while there was no significant difference in the protein content due to infection.

The amino acid pattern of copra samples was found to be altered due to infection. Samples of copra infected by B. theobromae did not record much variation compared to the control, while samples infected by A. hiemalis and C. senegalensis recorded lower quantities. R. oryzae infected samples recorded higher amino acid pattern.

Coconut oil extracted from samples of copra infected by fungi and incubated for different periods showed considerable changes in their physical and chemical constants. The colour of the oil was deepened and a rancid odour was noted due to fungal infection. A. flavus produced the maximum change in the colour and odour of the oil. No change was noticed in the refractive index of oil while acid value and iodine value increased in all cases. Maximum increase was caused by A. hiemalis and A. flavus. The acid value and iodine value increased with the period of incubation of infected copra. The saponification value was not altered

due to infection.

Oil stored for one year remained without changes for the first three months of storage after which it showed increase in acid value and iodine value.

Infection by fungi considerably reduced the oil content. R. oryzae and I. hiemalis infected samples caused maximum reduction in oil content while P. citrinum caused the minimum reduction. The oil content decreased considerably with the period of incubation.

Elaboration of pectic and cellulolytic enzymes (in vitro and in vivo) and lipase and proteolytic enzymes (in vitro) in the cell free extracts of different fungi were studied.

Among the pectolytic enzymes studied, A. niger, A. flavus and R. oryzae were alone found to produce macerating enzymes. Pectin methyl esterase (PME) activity was shown by all fungi except B. theobromae. A. flavus and A. niger showed maximum PME activity. PME activity was more in in vitro preparations while polygalacturonase (PG) and polygalacturonase transeliminase (PGTE) activities were maximum in in vivo preparations. A. flavus and R. oryzae, P. citrinum and I. hiemalis showed maximum PG activity, while maximum PGTE activity was shown by P. citrinum and R. oryzae. The maximum activity was noticed on the eighth day of

incubation in the case of PG and sixteenth day in the case of PGTE.

Among the cellulolytic enzymes studied, maximum C1 activity was shown by B. theobromae, A. flavus and A. niger. But maximum Cx activity was recorded in the case of C. senegalensis, P. citrinum, R. oryzae and A. niger. The enzyme activity increased with the period of incubation in both in vitro and in vivo preparations. Maximum activity was noticed on the 16th day of incubation.

All the fungi were found to be lipolytic and maximum activity was shown by R. oryzae and A. niger, the activity being maximum on the twelfth day of incubation.

Proteolytic activity was shown by all fungi. P. citrinum recorded the maximum activity followed by A. flavus and M. hiemalis. Maximum activity was observed on the fourteenth day of incubation.

Production of mycotoxins in deteriorated samples of copra were studied. Presence of aflatoxin in infected samples of copra were assessed using B. megaterium as the test organism. An inhibition zone of 10 mm was obtained.

Aflatoxin B_1 content of copra samples were identified and estimated. One sample contained two ppm of aflatoxin B_1 which is far above the normal admissible level. Aflatoxin B_1

was detected in samples collected from March to August, the concentration being very high, above one ppm.

The fungi associated with the deterioration of copra were tested for aflatoxin B_1 production. But A. flavus alone was found to produce this toxin.

The aflatoxin produced in copra was assayed biologically employing chick embryo, ducklings, guineapigs and rats. All the chick embryos were dead by the third day of inoculation of the toxin. Heavy mortality of ducklings fed daily with one g of A. flavus infected copra, was observed during a period of thirty days. Histopathologic studies revealed degenerative and necrotic changes in the hepatic cells with proliferation of the bile duct. Kidney of the ducklings also showed degeneration of tubular epithelial cells. The liver and kidney of guineapigs and rats also showed similar changes.

Chick embryo inoculated with the extract of copra infected with P. citrinum did not survive for more than five days. Ingestion of one g of P. citrinum infected copra also resulted in high mortality of ducklings. Liver showed very mild hepatic degenerations but kidney showed degeneration with or without necrosis of epithelial cells and the glomeruli appeared shrunken in many cases. Such a bioassay of A. flavus and P. citrinum infected copra using ducklings

is a new aspect of study.

A combination of A. flavus and P. citrinum infected copra in equal quantities also caused a high mortality in ducklings.

Food additives, antibiotics and chemicals were tried for the prevention of deterioration of copra. Treatment of coconut kernels in the initial stages of copra making with any one of the chemicals (streptocycline 500 or 1000 ppm, sodium carbonate at 4.0 or 5.0 or 5.5 per cent, acetic acid at 4.0 or 5.0 or 6.0 per cent) completely inhibited fungal growth up to 60 days during storage.

Mammalian toxicity of copra treated with streptocycline, sodium carbonate and acetic acid was tested by feeding it to small animals like rabbits, guinea pigs and rats. The chemicals showed no deleterious effect on the health of the animals. Post mortem examination as well as histopathological studies of liver and kidney showed no abnormality from healthy tissues.

Plate No. I & II. Copra infected by fungi

Plate No.III. Copra infected by M. hiemalis and Rhizopus spp.

Plate No.IV. Copra infected by Aspergillus spp.

PLATE III

Plate No.V. Copra infected by Botryodiplodia theobromae and Curvularia senegalensis

Plate No.VI. Copra infected by Penicillium citrinum

Plate No.V. Copra infected by Botryodiplodia theobromae and Curvularia senegalensis

Plate No.VI. Copra infected by Penicillium citrinum

PLATE VI

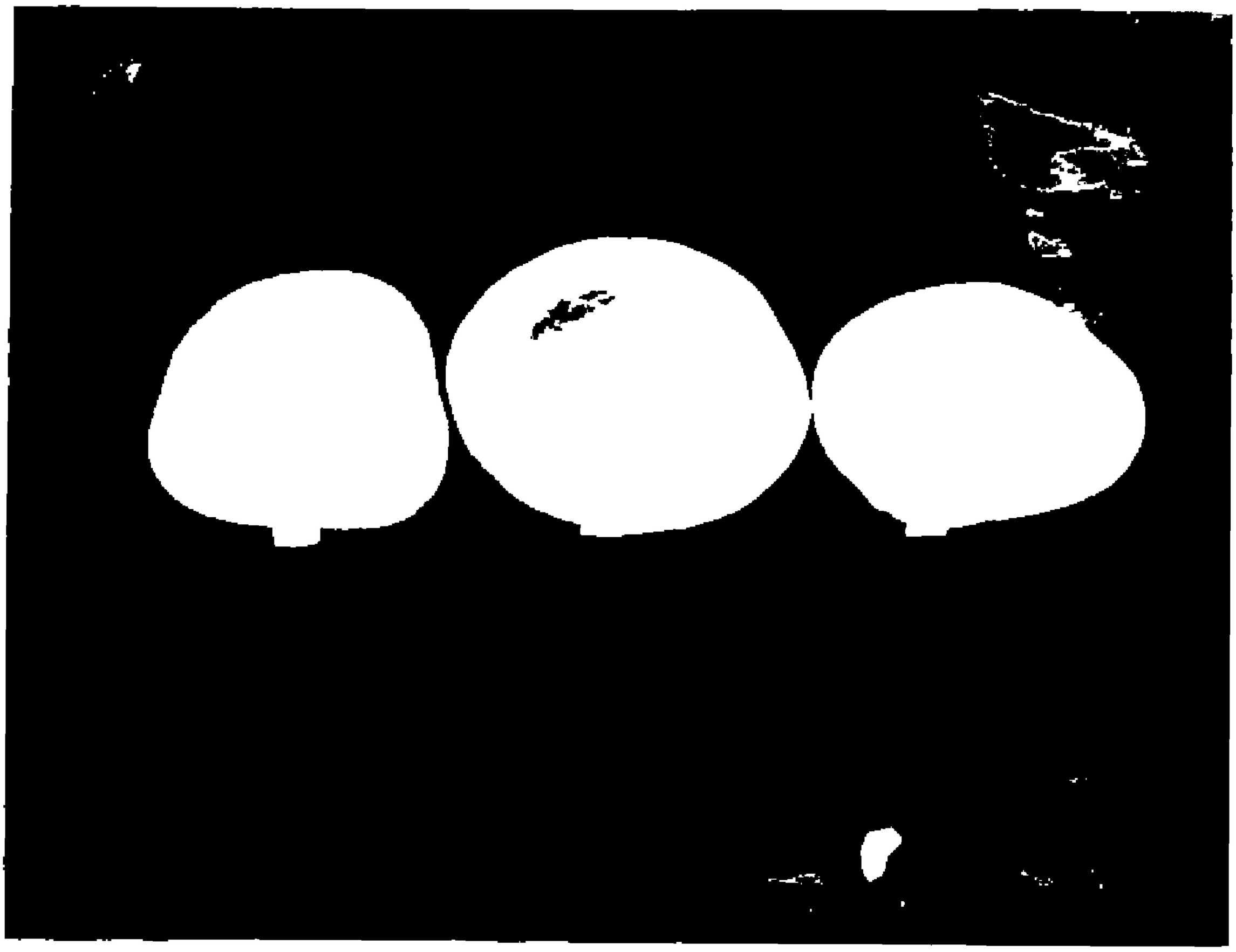


Plate No. VII. Duckling fed with copra free of fungal infection

Plate No.VIII. Duckling fed with copra infected by A. flavus

PLATE VIII

Plate No. IX. Liver section of duckling (control) showing normal hepatic architecture (H & E 200x).

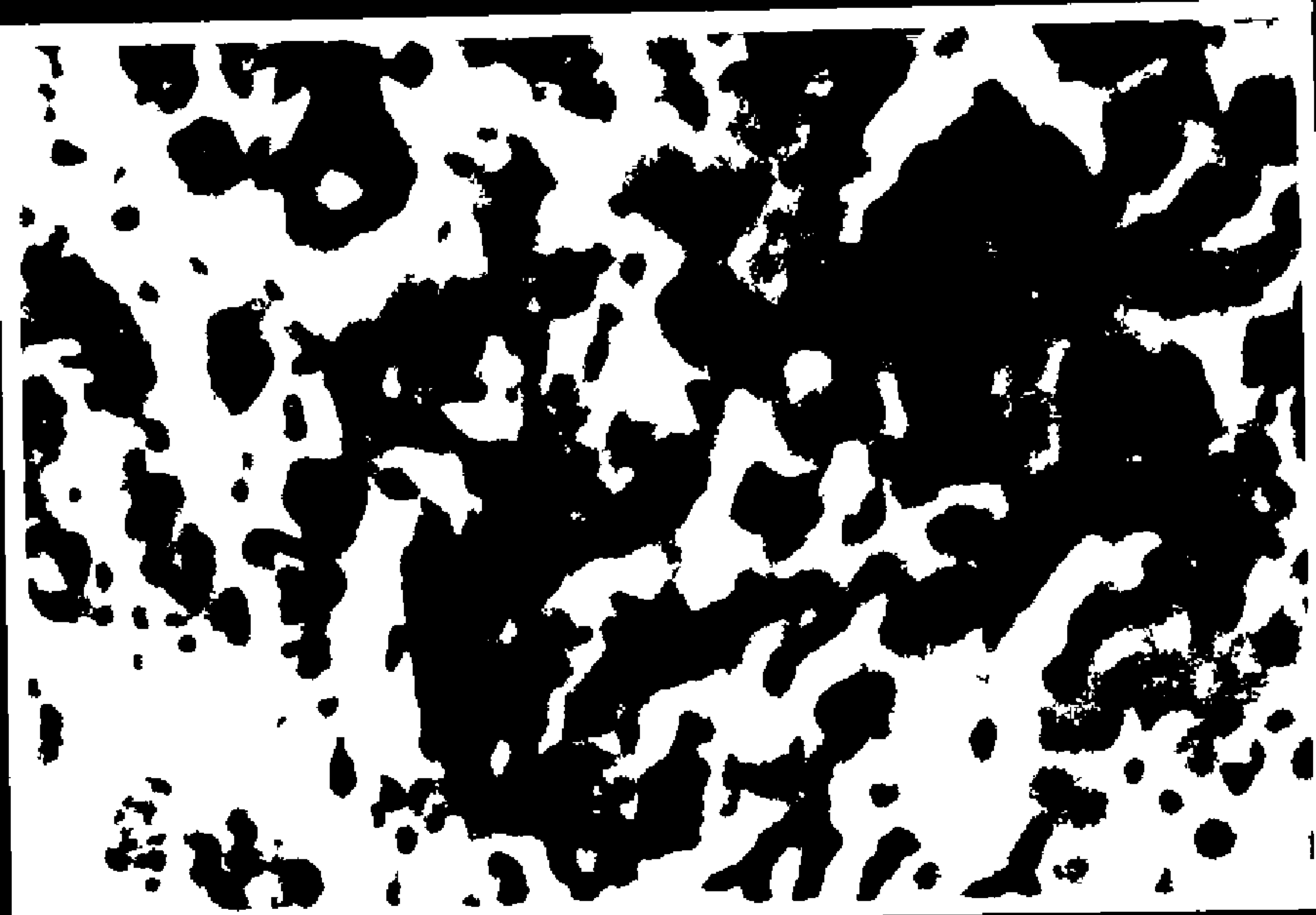
Plate No. X. Liver section of duokling fed on aflatoxin containing copra (died within seven days) showing marked fatty degeneration and necrosis of hepatic cells (H & E 600x)

PLATE X

PLATE X

Plate No. XI. Liver section of duckling fed on aflatoxin containing copra (died 7-15 days) showing early bile duct proliferation along with necrobiotic changes (H & E 600 x)

Plate No.XII. Liver section of duckling fed on aflatoxin containing copra (killed after 30 days) showing extensive bile duct proliferation and moderate fibroblastic reaction (H & E 600x)



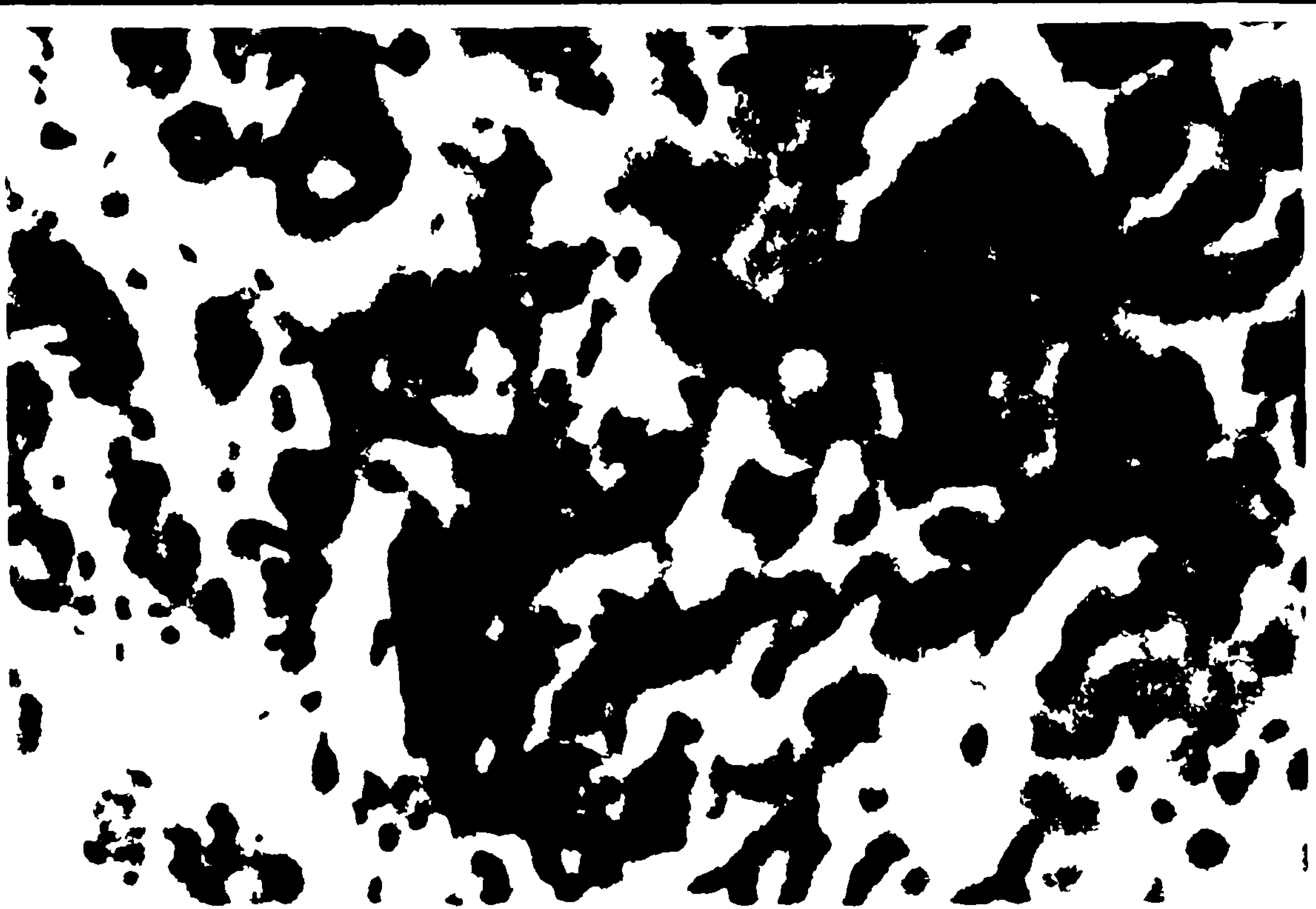


Plate No. XIII. Liver section of duckling fed on aflatoxin containing copra (died on 29th day) showing marked perilobular fibrosis with slight bile duct proliferation (H & E 200x)

Plate No. XIV. Liver section of guinea pig fed on aflatoxin containing copra (killed after 30 days) showing varying grades of hepatic parenchymatous degeneration (H & E 200x)

PLATE XIV

Plate No. XV. Duckling fed on copra infected with Penicillium citrinum

Plate No. XVI. Kidney section of duckling fed on copra infected with Penicillium citrinum (killed after 30 days) showing slight degeneration of tubular epithelial cells (H & E 200x)

PLATE XVI

Plate No.XVII. Duckling fed with copra infected with a mixed inoculum of *A. flavus* and *P. citrinum*

Plate No.XVIII. Liver section of duckling fed on mixed inoculum of *A. flavus* and *P. citrinum* (died on 13th day) showing necrosis of individual hepatic cells with mild lymphoid cell infiltration and biliary hyperplasia (H & E 200x)

PLATE XVIII

Plate No.XIX. Liver section of duckling fed on mixed inoculum of A. flavus and P. citrinum (killed after 30 days) showing fibrosis, bile duct proliferation and hepatic cell regeneration (H & E 200x)

Plate No.XX. Effect of ingestion of A. flavus infected copra on duckling

A. Duckling fed with copra free of fungal infection

B. Duckling fed with copra infected with A. flavus.

PLATE XX

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*Original not seen

APPENDICES

APPENDIX I

Peptone dextrose agar with rose bengal and strpetomycin (Martin, 1950)

Peptone	-	5.0 g
Dextrose	-	10.0 g
KH_2PO_4	-	1.0 g
$MgSO_4 \cdot 7\ H_2O$	-	0.5 g
Agar	-	15.0 g
Rose bengal	-	1 part in 30,000 parts of the medium
Distilled water	-	1000 ml
Streptomycin	-	30 µg per ml of the cooled medium added at the time of pouring the plate
pH	-	6.2

The medium was sterilized by autoclaving at 15 lb pressure for 20 minutes.

APPENDIX II

Nutrient agar (NA) (as quoted by Rangaswami, 1966)

Peptone	-	5.0 g
Beef extract	-	3.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.3 - 7.2

The medium was sterilised by autoclaving at 15 lb pressure for 20 minutes

APPENDIX III

Ken Knight's medium (Anon. 1967)

Glucose	-	1.0 g
KH_2PO_4	-	0.1 g
$MgSO_4 \cdot 7 H_2O$	-	0.1 g
$NaNO_3$	-	0.1 g
KCl	-	0.1 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	6.8

The medium was sterilized by autoclaving at 15 lb pressure for 20 minutes

APPENDIX IV

Potato dextrose agar (PDA)

Peeled potato	-	200.0 g
Dextrose	-	20.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	6.0 - 6.5

The medium was sterilized by autoclaving at 15 lb pressure for 20 minutes

APPENDIX V

Media for pectic enzyme production (Kannaiyan et al. 1975a)

KH_2PO_4	-	1.0 g
$MgSO_4$	-	200 mg
KNO_3	-	1.0 g
Pectin	-	15.0 g
Distilled water	-	1000 ml

The medium was sterilized by autoclaving at 15 lb pressure for 30 minutes

APPENDIX VI

Czapek's solution
(Czapek, 1902)

Sucrose	-	30.00 g
$NaNO_3$	-	2.00 g
KH_2PO_4	-	1.00 g
$MgSO_4$	-	0.50 g
KCl	-	0.50 g
$FeSO_4$	-	0.01 g
Distilled water	-	1000 ml

The medium was sterilized by autoclaving at 15 lb pressure for 30 minutes.

APPENDIX VII

Media for cellulolytic enzyme production (Kannaiyan *et al.* 1975a)

$CaNO_3$	-	10.0 g
KH_2PO_4	-	5.0 g
$MgSO_4$	-	2.5 g
$FeCl_3$	-	10.0 g
Sucrose	-	40.0 g
Distilled water	-	1000 ml

The medium was sterilized by autoclaving at 15 lb pressure for 20 minutes

APPENDIX VIII

Richard's solution (as quoted by Rangaswami, 1966)

KNO_3	-	10.0 g
KH_2PO_4	-	5.0 g
$MgSO_4$	-	2.5 g
$FeCl_3$	-	0.02 g
Sucrose	-	50.0 g
Distilled water	-	1000 ml
pH	-	6.6 - 7.2

The medium was sterilized by autoclaving at 15 lb pressure for 20 minutes

APPENDIX IX

Media for proteolytic enzyme production (Kirchoff, 1929)

KH_2PO_4	-	1.0 g
$MgSO_4$	-	0.2 g
Asparagine	-	1.0 g
Sucrose	-	100 g
Distilled water	-	1000 ml

The medium was sterilized by autoclaving at 15 pressure for 20 minutes

APPENDIX X

Starter ration for ducklings (ISI specifications)
(ingredients in per cent)

Maize	-	30.0
Rice polish	-	22.5
Tapioca	-	10.0
G.N.C.	-	10.0
Fishmeal	-	10.0
C.O.C.	-	15.0
Mineral mixture	-	2.5
Vitamin A, B, D	-	Added 0.25 g

APPENDIX XI

Formaldehyde-saline mixture

Formalin 40%	-	100.0 ml
NaCl	-	9.0 g
Tap water	-	900.0 ml

APPENDIX XII

Diet mixture for Guinea pigs/Rabbits (Hind lever concentrate)
Chemical composition per cent

Crude protein Min.	-	20.0
Ether extract, Min	-	2.5
Crude fibre, Max.	-	12.0
Ash, Max.	-	8.0
Calcium, Min.	-	1.5
Phosphorus	-	0.6
Nitrogen free extract	-	48.0
Metabolizable energy cal/kg		3000

APPENDIX XIII

Diet mixture for rats (Hind lever concentrate)

Chemical composition per cent

Crude protein, Min.	-	24.0
Ether extract, Min.	-	4.0
Crude fibre, Max.	-	4.0
Ash, Max.	-	8.0
Calcium, Min.	-	1.0
Phosphorus, Min.	-	0.6
Nitrogen free extract	-	50.0
Metabolizable energy Cal/kg	-	3200

APPENDIX XIV

ANOVA for fungal population (log count x 10^{-6} per g of copra)
According to periods and Mills

Source	S.S.	df	Variance	F
Total	9.8976	143		
Mill	0.1810	3	0.0603	9.2769**
Period	8.4344	11	0.7667	117.9500**
M x P	0.6528	33	0.0197	3.0307**
Error	0.6292	96	0.0065	

**Significant at 0.01 level

APPENDIX XV

ANOVA for bacterial population (log count x 10^{-6} per g of copra)

Source	S.S.	df	Variance	F
Total	9.0018	143		
Mill	0.1251	3	0.0417	2.320
Period	8.0699	11	0.7336	40.760**
M x P	0.5954	33	0.0180	8.180**
Error	0.2114	96	0.0022	

**Significant at 0.01 level

APPENDIX XVI

ANOVA for fungal population (log count x 10^{-4} per g of coconut oil cake

Source	S.S.	df	Variance	F
Total	7.9098	143		
Mill	0.0011	3	0.00036	Less than 1
Period	0.2411	11	0.00740	Less than 1
M x P	0.0780	33	0.00240	Less than 1
Error	7.5866	96	0.07900	

APPENDIX XVII

ANOVA for bacterial population (log count x 10^{-4} per g of coconut oil cake

Source	S.S.	df	Variance	F
Total	3.7314	143		
Mill	0.0646	3	0.0315	1.6
Period	1.5555	11	0.1414	7.4**
M x P	0.2884	33	0.0087	Less than 1
Error	1.8229	96	0.0190	

**Significant at 0.01 level

APPENDIX XVIII

ANOVA for total sugars of copra as affected by various periods and fungi causing deterioration

Source	S.S.	df	Variance	F
Total	0.15017	23		
Period (P)	0.02710	2	0.01235	8.4375**
Fungi (F)	0.10220	7	0.01460	9.1250**
Error	0.02360	14	0.00160	

**Significant at 0.01 level

APPENDIX XIX

ANOVA for protein content of copra as affected by various fungi causing deterioration

Source	S.S.	df	Variance	F
Total	2.0439	31		
Period (P)	1.1263	3	0.3754	5.2720**
Fungi (F)	0.3704	7	0.0529	0.7429
Error	0.5472	21	0.0712	

**Significant at 0.01 level

APPENDIX XX

ANOVA for colour of coconut oil on Lovibond tintometer as affected by various fungi causing deterioration of copra

Source	S.S.	df	Variance	F
Total	780.9100	31		
Period (P)	19.2800	3	6.4260	10.9990**
Fungi (F)	749.3600	7	107.0500	183.2400**
Error	12.2700	21	0.5842	

**Significant at 0.01 level

APPENDIX XXI

ANOVA for acid value (in per cent) of coconut oil as affected by various fungi causing deterioration of copra

Source	S.S.	df	Variance	F
Total	1664.82	31		
Period (P)	342.91	3	114.30	7.40**
Fungi (F)	997.71	7	142.53	9.23**
Error	324.20	21	15.43	

**Significant at 0.01 level

APPENDIX XXII

ANOVA for iodine value (in per cent) of coconut oil as affected by various fungi causing deterioration of copra

Source	S.S.	df	Variance	F
Total	287.4216	31		
Period (P)	79.0092	3	26.3364	10.7113**
Fungi (F)	157.0709	7	22.4387	9.1773**
Error	51.3415	21	2.4450	

**Significant at 0.01 level

APPENDIX XXIII

ANOVA for oil content of copra (in per cent) after different periods of incubation with fungi associated with deterioration

Source	S.S.	df	Variance	F
Total	6963.93	53		
Fungi (F)	4426.18	8	553.25	35.579**
Period (P)	1915.41	5	383.08	24.64**
Error	622.34	40	15.55	

**Significant at 0.01 level

APPENDIX XXIV

ANOVA for macerating enzymes as influenced by different periods, treatments and fungi

Source	S.S.	df	Variance	F
Total	468.8321	26		
Periods (P)	418.8261	2	209.41300	418.74**
Treatment (S)	13.7205	2	6.86025	13.71**
Fungi (F)	6.4695	2	3.23475	6.47*
P x S	11.1713	4	2.79280	5.58*
P x F	12.4274	4	3.10680	6.21*
S x F	2.2165	4	0.55410	1.11
Error	4.0008	8	0.50010	

*Significant at 0.05 level
**Significant at 0.01 level

APPENDIX XXV

ANOVA for pectin methyl esterase as influenced by different periods, treatments and fungi

Source	S.S	df	Variance	F
Total	1103.9348	71		
Period (P)	177.4382	2	88.7191	71.8373**
Treatment (S)	131.5272	2	65.7636	53.2498**
Fungi (F)	553.0804	7	79.0114	63.9768**
P x S	25.5094	3	6.3773	5.1638**
P x F	89.6210	14	6.4015	5.1834**
S x F	92.1760	14	6.5840	5.3311**
Error	34.5826	28	1.2350	

**Significant at 0.01 level

APPENDIX XXVI

ANOVA for polygalacturonase as influenced by different periods, treatments and fungi, absorbance of TBA products at 515 mμ

Source	S.S.	df	Variance	F
Total	14.0235	71		
Period (P)	1.2996	2	0.6493	7.5911**
Treatment(S)	1.6541	2	0.8270	9.6617**
Fungi (F)	5.7337	7	0.8191	9.5690**
P x S	0.6776	4	0.1694	1.9790
P x F	0.8720	14	0.0623	0.7278
S x F	1.3907	14	0.0993	1.1600
Error	2.3958	28	0.0856	

**Significant at 0.01 level

APPENDIX XXVII

ANOVA for polygalacturonase transeliminase (PGTE)(Absorbance of TBA products at 550 mμ)

Source	S.S.	df	Variance	F
Total	10.0608	71		
Period (P)	1.1304	2	0.5652	64.9655**
Treatment (S)	1.5455	2	0.7727	88.8160**
Fungi (F)	5.3410	7	0.7630	87.7011**
P x S	0.0553	4	0.0138	1.5862
P x F	0.3693	14	0.0263	3.0229**
S x F	1.4749	14	0.1053	12.1034**
Error	0.2444	28	0.0087	

**Significant at 0.01 level

APPENDIX XXVIII

ANOVA for cellulase (c1) as influenced by different periods, treatments and fungi (Absorbance at 610 mμ)

Source	S.S.	df	Variance	F
Total	9.30789	71		
Period (P)	0.78920	2	0.39460	21.9220**
Treatment (S)	2.49290	2	1.24640	66.3005**
Fungi (F)	3.52950	7	0.50420	26.8191**
P x S	0.14490	4	0.03622	1.9265
P x F	0.4566	14	0.03260	1.7340
S x F	1.3683	14	0.09770	5.1968**
Error	0.5264	28	0.01880	

**Significant at 0.01 level

APPENDIX XXIX

ANOVA for cellulose (cx) as influenced by different periods, treatments and fungi

Source	S.S.	df	Variance	F
Total	17547.3960	71		
Period (P)	6464.4140	2	3232.2070	168.8900**
Treatment (S)	3399.3110	2	1699.6555	88.8100**
Fungi (F)	4142.1770	7	591.7395	30.9196**
P x S	635.6180	4	158.9045	8.3030**
P x F	1367.3690	14	97.6692	5.1034**
S x F	1002.6420	14	71.6172	3.7421**
Error	535.8650	28	19.1380	

**Significant at 0.01 level

APPENDIX XXX

ANOVA for lipases as influenced by different periods, treatments and fungi

Source	S.S.	df	Variance	F
Total	47.4792	47		
Period (P)	22.3929	2	11.1964	191.7191**
Treatment (S)	0.2408	1	0.2408	4.1232
Fungi (F)	15.4292	7	2.2041	37.7414**
P,x S	0.1905	2	0.0952	1.6301
P x F	4.1171	14	0.2940	5.0342**
S x F	4.2900	7	0.6128	10.4931**
Error	0.8187	14	0.0584	

**Significant at 0.01 level

APPENDIX XXXI

ANOVA for proteolytic enzymes influenced by different periods, treatments and fungi

Source	S.S.	df	Variance	F
Total	1.26128	47		
Period (P)	0.05608	2	0.02804	15.5777**
Treatment (S)	0.02461	1	0.02461	13.6722**
Fungi (F)	0.90592	7	0.12941	71.9444**
P x S	0.05390	2	0.02695	14.9722**
P x F	0.16378	14	0.01169	6.4944**
S x F	0.03199	7	0.00450	2.5000
Error	0.02500	14	0.0018	

**Significant at 0.01 level

APPENDIX XXXII

Enzyme production by fungi

Fungus tested	Macerating enzymes	PME	Polygalacturonase	PGTE	Cellulase (C1)	Cellulase (Cx)	Lipases	Proteolytic enzymes
A. niger	4.8456	7.0377	0.5810	0.6329	0.5517	20.8888	1.7666	0.0491
A. flavus	6.0420	8.0248	0.9565	0.6603	0.5665	19.6933	1.6666	0.2640
R. oryzae	5.3753	3.5405	0.8973	0.8933	0.4353	22.4000	1.9000	0.0706
M. hiemalis	0.0000	2.1720	0.7546	0.6193	0.0676	18.1200	1.6166	0.2210
P. citrinum	0.0000	4.5708	0.7996	0.9086	0.3177	22.7822	1.6333	0.4490
B. theobromae	0.0000	0.0000	0.6962	0.8523	0.5916	11.7900	1.6666	0.1803
C. senegalensis	0.0000	4.7684	0.5115	0.7040	0.1569	24.7644	1.5833	0.7760

APPENDIX XXXIII

Mortality of ducklings due to the ingestion of mycotoxins

Source of copra	No. of ducklings	Period of incubation (day)																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A. flavus Aflatoxin (20/g)	37	-	2	5	8	5	4	3	1	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	5
P. citrinum Citrinin not known	37	-	2	3	4	5	8	6	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	6
Mixed infection	37	-	1	3	5	4	5	2	2	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	13
Control	37	-	1	1	-	-	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-	30

INVESTIGATIONS ON THE MICROBIAL DETERIORATION OF COPRA

BY

SUSAMMA PHILIP

ABSTRACT OF A THESIS
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Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF AGRICULTURE
Vellayani - Trivandrum

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ABSTRACT

Copra, the dried endosperm of coconut, is the major source of oil and oilcakes in Kerala. As no serious attempt has been made in this State to study the problem of spoilage due to microorganisms and to minimise the loss due to microbial deterioration, this investigation was taken up. It included studies on the microorganisms associated with deterioration of copra, the biochemical changes in copra and coconut oil due to microbial infection, mycotoxins produced by them as well as preventive methods for controlling deterioration.

Studies on the microbial population of copra samples collected from four different oil mills in Trivandrum District, Kerala, for a continuous period of one year revealed that the maximum fungal and bacterial population coincided with the monsoon period. The predominant fungi included Aspergillus niger, A. flavus, Rhizopus oryzae, R. stolonifer, Mucor hiemalis, Penicillium citrinum, Botryodiplodia theobromae and Curvularia senegalensis. The bacteria included Bacillus subtilis, Enterobacter aerogenes, Serratia marcescens, Staphylococcus aureus and Pseudomonas fluorescens. An unidentified species of Streptomyces was also found to be associated.

The oil extracted from copra infected by fungi showed variations from its normal properties. Colour was deepened and odour was changed. The acid value and iodine value increased while there was no change in the refractive index and saponification value of oil.

Studies on the storage of oil under laboratory conditions showed that it remained without any change for three months after which there was an increase in acid value and iodine value.

Considerable reduction in oil content of copra was observed due to infection by fungi individually as well as by mixed infection.

Enzymes elaborated by fungi were studied *in vitro* and *in vivo*. Among the enzymes studied macerating enzymes were found to be produced only by *A. niger*, *A. flavus* and *R. oryzae*. Pectin methyl esterase activity was noted in all fungi except *B. theobromae*. All the fungi tested produced polygalacturonase, polygalacturonase transeliminase and cellulases (Cl and Cx) *in vitro* and *in vivo*. Lipase and proteolytic enzymes were studied only *in vitro* and all the fungi elaborated these enzymes.

Aflatoxin was found to be produced in copra samples infected by a mixture of fungi including *A. flavus*, the

concentration being above one ppm in the samples collected from March to August.

Presence of aflatoxin in the crude extract of naturally infected copra was assayed microbiologically using Bacillus megaterium.

Biological assay of the effects of aflatoxin, P. citrinum toxin and combination of both were studied by feeding one g each of infected copra to one day old ducklings for a continuous period of 30 days. Heavy mortality was observed in all cases. Histopathological studies of the liver and kidney of the surviving birds revealed degenerative and necrobiotic changes.

Bioassay of toxins produced in copra due to infection by A. flavus and P. citrinum as well as due to mixed infection by both fungi is a new line of study.

Treating of copra with streptocycline (500 and 1000 ppm), sodium carbonate (4.0, 5.0 and 5.5 per cent) and acetic acid (4.0, 5.0 and 6.0 per cent) in the initial stages of sun-drying was found effective in checking microbial infection upto 60 days of storage. Post-mortem and histopathological studies of the liver and kidney of rabbits, guinea pigs and rats fed with the above treated copra showed no mammalian toxicity.