THE USE OF IONISING RADIATION FROM ^{60}CO GAMMA SOURCE IN CONTROLLING MOULDINESS IN DRIED COCOA BEANS

by

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This thesis is submitted to the University of Ghana, Legon in partial fulfilment of the requirement for the award of Ph.D degree in Crop Science.

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DECLARATION

I, the undersigned do hereby declare that the work reported in this Thesis is my original work and also to state that the work has not been presented to any other University for the award of any Degree by me.

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DEDICATION

This Thesis is dedicated to the **GLORY OF GOD**:
to Papa and Mama whose encouragement motivated me to start this work;
to my Husband Nick ,
Ekow,
Akosua
and Ekua
whose prayer support enabled me complete this work.

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ABSTRACT

Mouldiness in stored cocoa beans in Ghana and the production of aflatoxin have been studied. Based on actual weight of discarded beans, mouldy beans have been estimated to constitute 0.13 % and 0.00002 % of marketable beans at the farmers' level and the buying agents' depots respectively in the Tafo District. This is contrasted with an estimated value of 0.16 % obtained in a questionnaire type study involving farmers. Estimated mouldy beans at the Tema port was 0.69 % per year (based on the cut test) representing a financial loss of $1,688,637.19 per year at $989/T should the mouldy beans be discarded. Fifty-eight (58) internally- and externally- borne fungal species were isolated from dried cocoa beans. Of these, forty-eight (48) were internally- borne and ten (10) were superficial. Twenty-nine (29) of the internally occurring fungi have been recorded for the first time on cocoa beans in Ghana. Twenty-six (26) of the fungi isolated belong to *Aspergillus* group. They included *A. parasiticus* and *A. flavus*, which can produce aflatoxins. Five (5) belong to *Penicillium*, eight (8) to *Fusarium* and nineteen (19) to other species.

Ionising radiation effectively controlled fungi associated with mouldiness in cocoa beans in a dose – dependent manner. A radiation dose of 6 kGy completely inactivated the moulds. *A. flavus* and *A. tamarii* were the most radiation resistant moulds encountered. The moisture content of the beans before, during and after irradiation influenced the effect of radiation. The relative humidity during storage and the type of packaging also influenced the radiation effect. Conidia of *A. flavus* subjected to moist heat at temperatures 20 ^{0}C to 60 ^{0}C for 2.5, 5 and 10 min respectively were not significantly affected by heating up to 50 ^{0}C. Heating an aqueous conidial suspension at 60 ^{0}C for at least 2.5 min reduced the number of fungal colonies by at least 5 log cycles

when the suspension was assayed on agar plate media. Heating at 59 ^{0}C for 10 min completely inactivated the wet conidia of *A. flavus*. When a combination of moist heat and radiation was applied to *A. flavus*, in different media, the inactivation dose was different in each medium. *In vitro* studies on *A. flavus* in suspension showed that the fungus could be inactivated by applying a combination of moist heat at 50^{0}C for 10 min followed by 1.0 kGy radiation. A combination of moist heat at 80 ^{0}C and radiation at 3.5 kGy produced the same effect with cocoa beans. Storage of beans for 28 days at 75% RH suppressed fungal growth while storage at 90% RH resulted in mouldiness of beans previously inoculated with *A. flavus* conidia and treated with heat and radiation. No aflatoxin was detected in cocoa beans after 4 weeks following inoculation with conidia of a toxigenic *A. flavus* strain and irradiation at 10 kGy. Without irradiation, inoculated beans became mouldy and produced aflatoxin B_1. Irradiation at 10 kGy could not destroy the already formed aflatoxin. Radiation up to 6 kGy gave cocoa butter with free fatty acid, melting point, saponification value, and unsaponifiable matter levels comparable with factory standards. Exposure of beans to radiation did not have any effect on the rancidity of the extracted cocoa butter. Exposure of beans to radiation only or to radiation after heat treatment also did not affect the rate at which cocoa butter solidified. No significant differences in taste, colour and flavour were detected in cocoa drink prepared from cocoa powder produced from irradiated beans.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

There are several problems associated with fermented cocoa beans in storage. However, mouldiness of beans is a very serious problem, as it imparts a musty taste to chocolate. Some of these fungi causing mouldiness can break down fat, leading to rancidity (*Penicillium roquesforti*) (Hansen, 1975). Others (eg *Aspergillus flavus*) produce mycotoxins which are toxic to humans.

Fungi may grow on the surface of the beans and appear white or coloured and powdery. Some of the fungi can also grow and penetrate the beans internally causing internal mouldiness (Plate 1). Some of the beans appear wholesome externally but are mouldy internally. Cocoa beans become contaminated with fungal spores during fermentation, drying and storage (Rohan, 1957).

Conditions that influence fungal growth are; inadequate drying, unfavourable temperature (30 ^{0}C and above) and humidity in storage (75 – 98 %) and wetting of beans by rain. A number of species of fungi have been found to be associated with mouldiness of cocoa beans. However, the number and types of species contaminating cocoa beans under the varying storage conditions is not fairly well understood. There is therefore the need to characterise the fungi associated with mouldiness at the various stages of processing.

Traditionally, mouldiness is prevented by drying the beans properly by exposure to the sun on mats, with frequent heaping and turning of the beans. This is very laborious and not very efficient where large quantities of beans are involved. Sometimes, unfavourable weather conditions also prevent proper drying. Therefore some beans become mouldy. It is therefore necessary to handpick mouldy beans since consignment with as low as 4 % of mouldy beans makes the consignment

Plate 1. Mouldy Beans

Pictures showing (upper left) external (lower left) internal mouldiness

subgrade and therefore rejected. Handpicking is a very tedious, time - consuming exercise which is not very efficient. It also results in loss of beans even though the amount lost is not well known.

Other methods of preventing mouldiness in storage such as the use of chemical disinfectants, have not been effective in controlling mouldiness and have been found to leave residues on the beans. Use of ionising radiation is one relatively novel method of preventing spoilage in food by insects and micro-organisms. Rays from a radiation source have a high penetrating ability and they pass through the food whether packaged or not, killing insects and other spoilage inducing organisms without adversely affecting the quality of the food (WHO, 1981). Ionising radiation has been used successfully in controlling storage insects on cocoa (Amoako-Atta, 1979). The usefulness of this technology in preventing mouldiness in cocoa beans must be explored.

The quality of cocoa butter affects the quality of chocolate prepared from it, and it is important to ensure that treatment of the beans with ionising radiation does not adversely affect the quality of cocoa butter.

The objectives of the study were:

1. To establish the sources of contamination and assess the extent of mouldiness in cocoa beans at (a) the farmers' level i.e. on-farm on drying mats (b) buyers' level i.e.-depots (c) the store at the Tema port.
2. To isolate and characterise the range of fungi associated with the beans.
3. To characterise aflatoxin from mouldy beans.
4. To determine the potential of using irradiation only or in combination with heat to control mouldiness and assess the effect of the application of radiation or other technologies on the quality of fermented cocoa beans.

CHAPTER TWO

2.0 LITERATURE REVIEW

Cocoa, *Theobroma cacao,* is the only commercial specie among twenty species of *Theobroma. Theobroma cacao* is divided into two main groups viz.'Criollo' and 'Forestero'. There is a third group, the 'Trinitario', which is a cross between the two main groups. 'Forestero' is the most important commercial type (Minifie, 1989).

Cocoa is a tropical crop which is native of the Amazon. It was introduced in Ghana In 1879 and is cultivated in the forest belt of Western, Central, Eastern, Ashanti, Volta and Brong Ahafo Regions of Ghana where annual rainfall ranges between 1,100 and 1,800 mm. The tree takes three to five years to fruit.

2.1 The Cocoa industry

Cocoa has since 1910 been a major industry in Ghana. Until recently, the industry contributed more than 60 % of foreign exchange earnings, thus providing the bulk of Government revenue for development of the country (Birminham *et al.*, 1966; ISSER, 1993). The Government, therefore, attaches much importance to the industry and provides much support for research and extension services.

2.1.1 Field problems (Pests and Diseases)

In the field, cocoa is attacked by two major diseases namely the swollen shoot The virus disease (SSVD) transmitted by the mealy bug (*Pseudococcoides njalensis*) and the Black pod disease caused by two fungi, *Phytophthora palmivora* and *P. megakarya.* (Dand, 1993). Swollen shoot is currently controlled by cutting down diseased trees and Black pod by copper fungicides. Other diseases include, *Lasiodiplodia* pod rot and *Ceratostomella* wilt. *Monilia* pod rot does not occur in Ghana but only in the Americas (Minifie, 1989). The most damaging and widespread insect group on cocoa are called capsids, the major ones being *Sahlbergella singularis*

and *Distantiella theobroma*. Insecticides being recommended currently for the control of capsids are Unden 200 EC and Gammalin 20 (GCB, 2000). These pests and diseases are of economic importance as they cause heavy losses in yield if not controlled.

2.1.2 The cocoa pod

The mature tree produces pods each containing between twenty and forty beans (Minifie, 1989). The bean is surrounded by a mucilagenous pulp when the pod is ripe. The ripe pods are harvested, cut open and the beans removed. The pod has several uses. The husk is used for fertilizer, animal feed and soap. The sweatings for the production of assorted alcoholic drinks, jelly and pectin. The most important of the pod is the bean which is normally referred to as 'cocoa'. This is processed into cocoa butter and powder. Cocoa butter is used for chocolate, soaps, pharmaceuticals, cosmetics and pastries (GCB, 2000).

2.2 Preparation of cocoa for the market

2.2.1 Fermentation

The beans are fermented in heaps on banana or plantain leaves or in boxes. Fermentation takes five days in the Criollo variety and six days in the Forestero variety. The fermentation process involves lactic acid bacteria, acetic acid bacteria, aerophillic yeast eg. *Saccharomyces cerevisae* (Roelofsen, 1958) and bacilli eg. *Bacillus cereus* (Carr *et al.,* 1980). Aerophillic yeasts ferment sugar present in the thick pulp which surrounds the beans to ethanol. This is then converted to acetic acid through bacterial degradation. The breakdown of the high sugars cause a collapse of pulp cells and allows aerophillic flora to penetrate (D'Aoust, 1977). As the adhering pulp becomes liquid and drains off, the temperature rises to 45 °C and 50 ^{0}C till fermentation is completed. The bean must be killed before drying is done. If not slatiness may occur

and this results in loss of typical cocoa flavour. The two most important factors causing the death of the bean are temperature and acidity, and these depend to a large extent on aeration of the fermenting mass (Rohan, 1963). The living bean is killed during the first 30-40 hr by the combined action of temperature and increase in acidity arising from fermentation. Investigations by Chick *et al.,* (1981) indicated that acetic acid contributes to the death of the beans, prevents the colonisation by putrefactive micro-organisms, and creates an environment conducive to the formation of flavour and aroma precursors within the bean cotyledons.

The cotyledons gain moisture and the texture changes from a cheesy coherent mass to a fissured structure. When dried, they are friable and readily break into pieces called 'nibs' in industry. There is also a significant colour change. With the Forestero types, the unfermented bean is slaty grey, passing through purple, purple-brown to rich dark brown. With Criollo types, similar changes occur but the final colour is a light brown due to the fact that their cotyledons contain no purple anthocyanins (Minifie, 1989). It has been thought that the dried cocoa bean should be fully brown in colour. It is difficult to effect complete destruction of the pigments in Ghanaian cocoa. Attempts to do so by increasing fermentation time may result in over-fermentation which, is attended by loss of cocoa flavour and the development of off-flavours (Wadsworth, 1951). Knapp (1937) showed that Forastero cocoa are characterised by cells of an intense violet colour dispersed among the other cells.

Passos *et al.* (1 984), working on the characterisation of lactic acid bacteria, found in traditional fermentation of cocoa beans revealed the presence of members of families *Lactobacillaceae* and *Streptococcaceae*. The species found were *Lactobacillus plantarum, L casei, L. delorueckii, L acidophilus, L. lactis, Pediococcus cerevisiae, P. acidilactici, Streptococcus lactis and S. homolactis.* Other heterolactic species were *Leuconostoc mesenteroides* and *Lactobacillus brevis*. Cascante *et al.* (1991) identified

thirty eight yeast strains while working on Criollo and Forestero varieties in Costa Rica. Three of the yeasts identified at the alcoholic stage were *Saccharomyces cerevisiae, S chevalieri and S. microellipsoides*. Filamentous fungi mostly of the genera *Penicillium Exosporiella, Geotrichum, Fusarium, Rhizoctonia and Rhizopus microflora.* developed at the end of the alcoholic phase.

There is a lower limit for heap size below which it is not practicable to ferment because of increased heat losses due to an increase in surface area per unit volume. The safe minimum weight for normal heap fermentation is 100 kg (BCCCA, 1996). However, experimental samples of about 50 kg can be fermented provided care is taken to ensure a low surface to volume ratio to minimise heat loss. Heaps of between 250 and 500 kg are typical in West Africa while in south east Asia and Brazil, where box fermentation is practised, bed depth of between 40 and 100 cm are normal and can hold quantities 500 - 2000 kg of wet beans. For practical reasons, about 2000 kg wet beans is considered to be the upper limit for a single fermentation (BCCCA, 1996).

Aeration is also important in fermentation. Reducing the amount of beans during fermentation increases the degree of aeration. Occasionally, the beans are stirred to aerate and to ensure that the beans are all equally exposed to the temperature in the interior of the heap. Beans fermented in boxes are turned at two days interval. Boxes are sometimes arranged in tiers and the turning is replaced by transferring from one box to the other (Minifie,1989).

2.2.2. Drying

The beans are dried after fermentation. Marshall (1970) outlined two processes governing drying. These are (a) transfer of heat into the bean to provide the energy necessary for evaporation and (b) movement of the vaporised moisture from some point within the bean to the surrounding drying air. The rate of drying is determined

by whichever of these two processes is the slower step. Sun-drying is the usual method of drying but in regions of heavy rains where sun drying is problematic, artificial methods of rapidly drying the beans at high temperature have been developed. The rate of water loss from the shells at such elevated temperatures is faster than the rate of migration of acids from the beans to the shells. Consequently, water is evaporated and lost in preference to the acids which are concentrated in the cotyledons or nibs where they do not only give rise to an excessively acidic taste but also, inhibit the cocoa flavour during subsequent roasting (BCCCA, 1996). Sun-drying ideally takes 6 - 10 days. A much longer period may allow fungi to develop inside the beans, leading to off-flavours. In Ghana, sun-drying is usually done on a platform on raffia mats. Prolonged storage of drying cocoa on rolled mats often causes a reduction in quality (Rohan, 1963). During sun-drying, the radiant heat of the sun warms the surface of the exposed cocoa bean. The air in contact with the bean is then itself warmed by convection, its vapour pressure is increased and the drying mechanism initiated. The removal of the moisture - laden air is carried out by wind with a contribution from the natural convectional currents set up above the cocoa as a result of the convective heating of the adjacent air (Mc Donald *et al.*, 1981). The system of mobile trays is in use at Cocoa Research Institute (CRIG) which permits 6 trays to be stored under a roof. The beans are dried to 6 – 7 % moisture. The mat system is quite efficient but during sudden rains, it is not easy to cover a number of them quickly enough to avoid some getting wet. Covering is effected by rolling up the mats and covering with palm fronds. At CRIG, a system of mobile trays is in use which permits 6 trays to be stored under a roof. The trays roll on metal ranks and are relatively easily removed.

2.2.3 Storage

In order to obtain good beans, fermentation, drying as well as storage and transportation are very important. In West Africa, cocoa is stored in jute sacks. In

humid areas e.g. West Cameroun, polythene lining of jute sacks is recommended (Rohan, 1963). Seradio *et al.* (1982), working on the storage of cocoa in Brazil, reported that cocoa stored in 0.1 mm clear polythene bags for 6 months presented no problems with respect to fungal development and insect infestation, and the product remained in good condition. They therefore recommended this packaging for cocoa storage on the farm provided the beans are bagged in proper phytosanitary conditions with moisture content of not more than 7.5 %. In Cameroun, Powell and Wood (1959) noted that, the inside of buildings used for temporary storage had an average relative humidity of about 82 %. They recommended the use of polythene liners to the jute sacks and these delayed moisture gain, and permitted transport and local storage under high humidity. In many cocoa growing areas, the growers aim at getting the cocoa to the point of purchase as soon as possible.

Cocoa is bagged, loaded into trucks or trains and transported to the seaports for export. There are two major storage problems. These are insect infestation and mouldiness. The former is controlled with appropriate fumigants (Minifie, 1989) .

2.2.4 Marketing

2.2.4.1 Terminologies used in assessing the quality of fermented cocoa.

Beans with internal and external fungal infection are described as **mouldy**. When over ripe pods are harvested the beans may have already germinated or do so during the beginning of fermentation. Later, the embryo falls out leaving a round hole in the shell. **Germinated beans** are not objectionable but are more likely than normal beans to develop serious defects such as internal mould or insect infestation during storage.

Slaty beans can be recognised by their slaty colour and "cheesy" texture. Chocolate made from them is dark grey, extremely bitter, astringent and lacks the typical

chocolate flavour. Slaty beans generally result from inadequate mixing during fermentation, allowing the surface beans to dry out before fermentation is completed.

Over-fermented beans do not give a full chocolate flavour. Some over- fermented beans are dark brown and others tend to be pale yellowish-brown.

Smoky beans are beans contaminated by smoke during drying. They have objectionable flavour which is impossible to remove from chocolate. The off- smell caused by one or two smoky beans in a lot destroys the quality of the entire lot for processing (Dand, 1993). **Flat or shriveled beans** are imperfectly developed beans containing very little of the useful cotyledons. Their presence reduce yield of nib and they are removed by sieving and grading machine. **Purple beans** are objectionable because they produce bitter and astringent flavour. They exhibit a bright purple colour associated with inadequate fermentation. Normal fermentation will however give a proportion of beans described as partly brown and partly purple. Such beans are not defective; in fact it is desirable that the samples should contain at least 20 % of beans in this category. As the proportion increases, the bitter and astringent flavour of inadequately fermented cocoa will tend to predominate and 50 % should be regarded as the upper limit for this class of beans.

2.2.4.2 Grading

The Biscuit, Cocoa, Chocolate Confectionery Alliance (BCCCA, 1996) has prepared a booklet on the manufacturing requirements of raw cocoa. A summary of these is as follows: Beans should be clean, plump, well fermented and when cut, the nib should range from purple to brown. The average weight of a bean determined on 100 beans should not be less than 1 gram. The cut test provides an assessment of the beans from which analysts may infer certain characters. It is simple with regards to the equipment, and training, and is widely used in the cocoa industry.

2.2.4.3 The Cut Test

A composite sample is drawn from a batch (30 bags at buying centres and 300 bags at port). The sample is thoroughly mixed and then 'quartered' down to leave a heap slightly more than 300 beans. The first 300 beans are counted off regardless of size, shape or condition. These are weighed and cut length wise to expose most of the internal surface. The cut surfaces are examined. The number of defective beans are recorded as percentage of each defect as mouldy, slaty, germinated, insect damaged, germinated, flat or shriveled beans. The cut test is done on every batch of cocoa (Dand, 1993; BCCCA, 1996).

2.2.4.4 Specific Requirements:-

In the grading of cocoa, the specific requirements stated below have to be met (BCCCA, 1996).

- The average weight of the beans, determined on samples of not less than 100 beans should not be less than 1gram. The beans should be uniform in size and not more than 12 % should be outside the range of 1/3 of the average weight above or below the mean.
- The shell should be loose but unbroken and should be less than 12 % of the bean weight.
- The fat content of the nib calculated on dry matter basis should not be less than 55 %.
- The beans should be free from foreign matter.
- The beans must be free from foreign flavours e.g. smoke, insecticides.
- The beans should be free from mould especially internally and should be dried below an absolute maximum of 8 % and preferably to 6 % moisture.
- Unfermented or slightly fermented beans with slaty or full purple coloured nibs should be absent.

- Insect damaged, germinated or flat, shrivelled or immature beans should constitute a very low proportion of the sample.

Marketable cocoa in Ghana is graded according to the following; (BCCCA, 1996; Dand, 1993).

Grade I: Thoroughly dry, free from foreign matter, smoky beans and any evidence of adulteration, batch contains not more than 3 % of mouldy beans, and not more than 3 % of slaty ones and not more than 3 % beans with other defects.

Grade II: Thoroughly dried and free from smoky beans and foreign matter. Batch should not contain more than 4 % mouldy beans, 8 % slaty beans and 6 % of beans with other defects.

Sub-Standard: This is all cocoa below Grade II. The bags are marked 'ss' and are marked only under special contract.

Grading is done before purchasing and also at the port before shipment.

2.2.5 Purchase of Cocoa (CMB, 1987; GCB, 2000)

The Cocoa Board was established as the Gold Coast Marketing Board in 1947.

The various divisions of the Cocoa Board control different activities in cocoa industry. Until recently, the local purchase was solely the responsibility of the Produce Buying Agency of the Board. The Quality Control Division is responsible for inspecting storage sheds and issuing Certificate of Registration for premises used as scheduled grading centres or depots. The staff of the division stationed in the 84 Operational Districts within the 6 growing regions are responsible for inspection, sampling, grading and sealing of cocoa before evacuation for shipment or delivery at the factories. A certificate is issued for any consignment graded. Currently, the division operates 1890 purchasing centres. The farmers bring their produce to the purchasing centres or sheds where they are graded. They are then brought to collection depots from where they are carted to the port for export. The quality of

cocoa is greatly enhanced by good storage facilities built by the Board to store cocoa awaiting evacuation. The staffs work in the sheds to ensure good preservation.

Prior to 1961, local purchases were done by individuals and firms licensed by the Cocoa Marketing Board and they were known as Licensed Buying Agents. From 1961 Mid-Crop season, the United Ghana Cocoa Farmers Co-operative Council was in charge of local purchases till 1966 when the purchase went to the Produce Buying Agency under the Cocoa Board. Cash requirement was based on projections of purchases which were submitted to the Bank of Ghana. The latter then arranged for disbursement of promissory bills which were discounted with the Commercial Banks. (CMB, 1987).

The bulk of money being carried and distributed in over 4000 buying centres at the time presented problems to the Provisional National Defence Council Government. They therefore abolished the system of paying cocoa farmers with fiscal cash and introduced the Akuafo cheque system. This system sought to eliminate the misappropriation of cocoa funds by some Produce Clerks and District Purchasing Officers. The cheques received by farmers as payment for their cocoa was presented at the Commercial Bank for cash.

Late 1993, the Government again introduced the purchase of cocoa by private companies with the Cocoa Board still in charge of produce inspection and grading. Currently, nineteen private local companies purchase cocoa alongside the Produce Buying Company. These are: Cashew and Spices Products Ltd, Kuapa Cocoa Ltd, Universal Crop Protection (Gh) Ltd , Ghana Cooperative Marketing Association, Adwumapa Buyers Ltd, Geomco Services Ltd, Goldcrest Commodities Ltd, Premus Trading Company, Aboafo Buying CO. Ltd, Agrotrade Ltd, Federated Commodities, Kiku Marketing Ltd, Seel Ghana Ltd, Alhaji Sulemana Industries Ltd, Akuafo Adamfo

Marketing Company, Transroyal (Gh) Ltd, Cocoa Merchants (Gh) Ltd, United Farmers Marketing Co Ltd and Bowohoso Ltd (GCB, 2000).

2.2.6 Sale of Cocoa

The sale of the beans and semi–finished products is handled solely by the Cocoa Marketing Company of the Cocoa Board. About 20 % of the annual production is processed locally by three factories to cocoa powder, cocoa butter and chocolate. The Company has branch office in London which is organised to receive bids from buyers for transmission to Accra for decision. Overseas sales are made by private treaty on the basis of world market price which is controlled by the Biscuit, Cocoa, Chocolate and Confectionery Alliance. The principal importers are the United States, Germany, the Netherlands, United Kingdom, Russia and France (C.M.B. , 1987; Dand, 1993).

2 .2.7 Quality of cocoa

2 .2.7. 1 Cocoa Flavour

Practically, all the cocoa in the world today are of Forastero, Amelonado or Amazon type and their crosses referred to as ' bulk cocoa. The cocoa produced in West Africa is an example of bulk cocoa. This type of cocoa has a good chocolate flavour. There are however appreciable differences from country to country, depending upon the method of preparation for the market. It is a requirement with beans from a particular country that, there is uniformity of flavour not only from shipment, but also from crop to crop. (Minifie, 1989). The fine grade cocoa that come from various growing areas are used essentially for their flavour value in blending with bulk cocoa, particularly to make high quality chocolate.

All cocoa flavour is affected by the genetics of the plant and the cultural practices of the farmer, especially, fermentation and drying. It is important that thorough fermentation and drying are carried out. Not only are fermentation and drying necessary for a good chocolate flavour, but also they avoid the occurrence of

off-flavour in the subsequent chocolate (Powell, 1982). According to BCCCA (1996), the major causes of off-flavour are:

- Mouldiness as a result of prolonged fermentation, slow or inadequate drying and storage under very humid conditions. Germinated or damaged beans are prone to becoming mouldy.
- Smoky beans from contamination by smoke.
- Excessively acid taste from deep box fermentation, multiple turning or too rapid drying.
- Excessive bitterness or astringency as a result of the variety or lack of fermentation.

2.2.7.2 Cocoa Butter

The composition of the whole bean is assumed to be nib 88% and shell 12%. The fat content of roasted bean, nib and shell are approximately as follows; nib, 55% and shell, 3%. Cocoa butter which is obtained by pressing cocoa liquor is a light yellow fat, solid at room temperature, exhibiting a distinct brittle fracture below 20 ^{o}C, a fairly sharp complete melting about 35 ^{o}C with an incipient fusion and softening around 30– 32 ^{0}C (Dand, 1993). The complete liquid fat has marked tendency to supercool, a fact which must be taken into account in the processes of chocolate manufacture (Minifie, 1989). The other important characteristic of cocoa butter is contraction which allows the moulding of chocolate products and helps give a good finishing (Dand, 1993).

Although the chocolate flavour is of a non-liquid origin primarily, cocoa fat, is of great importance because of its unique crystallisation behaviour in confectionery product. Cocoa butter has a very complicated cooling curve as the fat displays four separate polymorphic forms α, β, β^{1} and γ, each having its own properties (Dand, 1993). The γ form is unstable and has a melting point of 17 ^{0}C. This shows how the temperature could drop without the fat solidifying. The stable fat β form melts at 34 –

35 ^{0}C (Dand, 1993). All cocoa butters are not the same; hence their properties are given in ranges. They comprise a mixture of different compounds. Their characters are largely dependent on the variety and the environment affecting the development of the pods. According to Kattenberg (1981), butter from West African beans, has marginally higher melting points than butters from Brazil. Most manufacturers contend that Brazilian butter is softer than butter from West African beans, and that, Ghanaian beans provide the most consistent type of butter (Kattenberg, 1981).

Chemically, cocoa butter consists of predominantly one group of fats, the triglycerides. They vary greatly in their composition but have a similar underlying structure as below:

Triglyceride structure

Glycerol molecule with three carbon atoms	O- fatty acid R [O- fatty acid R_1 I O- fatty acid R^2

The fatty acid composition of cocoa fat is palmitate 26 %, stearate 34 %, oleate 35 %, linoleate 3 %, arachidic 1 % and trace amounts of several acids (Erickson *et al.*, 1973). According to Minifie (1989) the composition of fatty acid is as below (Values in brackets indicate the number of carbon atoms). Myristic (C14) 0.1 %, Palmitic (C16) 25.8 %, Palmitoleic (C16:1) 0.3 %, Stearic (C18) 34.5 %, Oleic (C18:1) 35.3 %, Linoleic (C18:2) 2 .9 % and Arachidic (C20) 1.1 %, and trace amounts of other acids (Bracco *et al.,* (1970). Phospholipids content of cocoa butter vary between 0.1 and 0.9 %, depending on the recovery (Parsons *et al.,* 1970). Free fatty acid content is usually less than 1 %. The unsaponifiable fraction is approximately 0.3 % and is mainly sterols (Bracco *et al.,* 1970).

Prawoto (1989) reported that studies carried out on fatty acid composition of cocoa butter showed that it contains fatty acids of the following number of carbon atoms; C14, C16, C16.1, C17, C18, C1:1, C18:2, C18:3 and C20. Of these C16, C18 and C18:1 are the main fatty acids, their contribution being more than 96 %. Fine flavoured cocoa butter has a percentage of unsaturated fatty acid which increases with a combination of increasing humidity and average daily temperature. The percentage of unsaturated fatty acids of beans harvested in the early season (May - June) is higher than that of those harvested in the late season (November - December). However, the influence of harvesting season on the degree of fatty acid saturation is small. Indonesian bulk cocoa has a higher percentage of saturated fatty acids than cocoa butter from Ghana. However, the Indonesian fine flavoured butter has almost the same percentage of saturated fatty acids as Malaysian cocoa butter.

2.3 Mouldiness

Fungi grow rapidly on the cocoa bean during drying and sometimes during fermentation; these are externally mouldy beans. When the beans are well dried, penetration of the shell may not occur. If drying is too slow or insufficient, the fungal hyphae penetrate the shell and cotyledons and cause internal mouldiness of the beans. Development of fungi inside the bean is a very serious defect because even a small percentage of mouldy beans will cause the chocolate to taste musty. Some of the fungi eg *Penicillium roqueforti* can cause the breakdown of fat, leading to rancidity. Some of the fungi e.g. *Aspergillus flavus,* are known to produce mycotoxins which are detrimental to health. The average moisture content of the beans should be maintained between 6 and 7 % to avoid mouldiness.

2.4. Micro-organisms associated with dried cocoa beans in storage

Work done on cocoa beans by Dade and Bunting in the Gold Coast date back to 1928. They investigated the fungi responsible for internal mouldiness and identified six fungi namely *Aspergillus flavus, A. glaucus, A. ochraceus, A. tamarii, A. niger* and *Penicillium* sp. Hughes (1952), listed the following from cocoa; *Aspergillus niger, Beltrania africana, Eutypella theobromicola, Graphium rhadiopheum, Menisparopsis theobromae, Nectria flavo-lunata, Nectria haematococca, Nectria inventa, Nectria jumpneri, Nummularia anthracodes var. gliricidiae, Pseudocamptoum fasciculatum, Stachybotrys theobromae, Stachylidium bicolor, Tryblidiella rufula, Zygosporium oscheoides.*

Hughes (1953) further listed 8 more fungi on cocoa. These included *Botrydiplodia theobromae,Fracchiaea bromeaina, Gliocladium roseun, Hermatomyces sphaericum, Hymeniela socia, Hypoxylon rubiginosum, Phaeobotrycephaeria plicatula, Schizophyllum commune.* He however did not indicate which part of the cocoa the fungi were found.

Piening (1962) attempted to catalogue all fungi in Ghana up to 1958. Most of the fungi he recorded on cocoa have also been listed by Dade and Hughes. In his check list, Piening listed the fungi, the host, the place and the year each fungus was identified but failed to give the part of the plant it was isolated from. He listed 78 fungi on cocoa. However some of the genera listed eg. *Aspergillus* are known to occur on stored products. These included: *Absidia capillata, Absidia cristata, Abisidia glauca, Absidia regnieri, Absidia truchisii, Acrostalagmus cinnabarinus, Armillaria mellea, Aspergillus candidus, Aspergillus carbonarius, Aspergillus chevalieri, Aspergillus fischeri, Aspergillus flavipes, Aspergillus flavus, Aspergillus fumigatus, Aspergillus gracilis, Aspergillus japonicus, Aspergillus niger, Aspergillus ochraceus, Aspergillus sydowii, Aspergillus tamarii, Aspergillus tamarii var. castus,*

Aspergillus ustus, Aspergillus wentii, Botryodiplodia theobromae, Calonectria rigidiuscula, Cephaliophora tropica, Ceratocystis paradoxa Chaetomium funicola, Choanephora cucurbitarum, Corticium solani, Curvularia geniculata, Discella cacaoicola, Eutypella theobromicola, Fomes lamaoensis, Fomes lamaoensis, Fomes lignosus, Fomes noxius, Fracchiaea broomeiana, Gliocladium roseum, Glomerella cingulata, Hermatomyces sphaericus, Hypoxylon rubiginosum, Marasmius byssicola, Marasmius equicrinis, Marasmius scandens, Menisporopsis theobromae, Nectria dealbata, Nectria flavolanata, Nectria haematococca, Nectria inventa, Nectria jungneri, Nectria pityrodes var. saccharina, Nenrospora sitophila, Nigrospora sphaerrica, Nummularia anthracodes var. gliricidiae, Pestalotiopsis owensii, Pestalotiopsis owensii var. major, Pestalotiopsis papposa, Pestalotiopsis theae var. minor, Pestalotiopsis versicolor, Phaeobotryosphaeria plicatula, Phaeoisaria cornui, Phytophthora palmivora, Poria hypobrunnea, Pseudocamptoum fasciculatum, Roselinia arcuata, Schizophyllum commune, Sporidesmium tropicale, Stachybotrys theobromae, Stachylidium bicolor, Syncephalastrum cinereum, Thielavia setosa, Thyronectria pseudotrichia, Trachysphaera fructigena, Trametes corrugata, Tryblidiella rufula, Ustulina deusta, Zygosporium oscheoides.

Maravalhas (1966), studied mouldiness during fermentation, drying and storage in Bahia -Brazil, and observed that the species of fungi varied with both the crop and the environment (temperature and moisture).

- During fermentation: The most common specie encountered was *A. fumigatus*.
- During drying: *Penicillium* sp., *A. fumigatus* and *Geotrichum candidum*.
- During storage: The most conspicuous fungi encountered were *A. glaucus*, *Penicillium* sp. and *G. candidum*.

Ostovar and Keeney (1973), reported large populations of fungi (not specified) yeast and bacteria on fermented and dried cocoa beans in Bulgaria.

In West Africa, some work has been reported in Nigeria. Oyeniran and Adejini (1974), identified thirty - one species of fungi causing internal mouldiness in dried cocoa beans, including twelve in *Aspergillus* and four *Penicillium* spp. The major fungi were *Aspergillus chivalieri, A. flavus, A. fumigatus, A. niger, A. penicilloides, A. ruber* and *A. tamarii; Botrydiplodia theobromae, Mucor pusillus, Paecilomyces varioti; Penicillium citrinum, Rhizopus nigricans; Syncephalastrum racemosum.* The minor fungi included *Absidia corymbifera, Aspergillus aculens, A. nidulans, A. ochraceus, A. pseudoglaucus, A. restrictus, Curvularia lunata, Cylindrocarpon tonkinense, Fusarium oxysporum, F. solani, Geotrichum candidum, Macrophoma* sp., *Penicillium decumbens, Penicillium steckii, Penicillium variable.*

Oyeniran (1974 a) studied comparative infectivity of cocoa beans by moulds. He also investigated the effect of temperature on the growth and development of storage fungi of cocoa; and the storage environment on the quality of commercial cocoa with special reference to mouldiness of the beans (Oyeniran, 1974 b). Oyeniran (1979), stored cocoa at different humidities and isolated eleven fungal species including xerophytic fungi such as *A. chevalieri, A. ruber* and *A. penicilloides, A. niger* and *Syncephalastrum racemosum.* The xerophytic fungi have also been found to be the primary agents of deterioration which pave the way for other types of fungi by producing more moisture on drying crops.

Hansen (1975) examined samples of surface sterilised cocoa beans from various cocoa producing areas using seven different culture media, and identified bacteria and fungi from the *Aspergillus glaucus* group and *Mucor pusillus* (constituting over 75 % of the contaminated beans). Stantschew (1976), studying contamination of

cocoa beans in Trinidad identified eight species of fungi including *A. flavus* and *A. fumigatus.*

Cocoa plays a vital role in the economy of Ghana but comparatively, very little work has been done on mouldiness including the preliminary work by Appiah, (1978) and a study on the degradation of the cocoa testa by *Aspergillus* isolated from mouldy cocoa beans samples in Tema by Abitey (1982). Abitey isolated 21 fungi listed below *; A. flavus, A. restrictus, A. ruber, A. fumigatus, A. nidulans, A. niger, A. phoenicis, A. sydowi, A. tamarii, A. ustus, A. wentii, Botrydiplodia theobromae, Fusarium solani, Mucor pusillus, Neurospora crassa, Penicillium citrinum, Penicillium* sp. *1, Penicillium* sp. *2, Rhizopus nigricans, R. oryzae and Mycellia sterilia.*

2.5. Mycotoxin production in storage

Mycotoxins are secondary metabolites of fungal origin which when ingested, inhaled or absorbed through the skin can cause sickness or death in humans or animals. Currently, over 200 mycotoxins have been reported (FAO, 1986, 1990). Mycotoxins are low molecular weight compounds which do not produce immediate symptoms as bacteria toxin. Mycotoxin contamination of foods and feeds highly depends on environmental conditions that lead to fungal growth and toxin production. It varies from species to species and variation within a species depends on the type and strain. The tropical climate with an all year round high temperature and relative humidity provides optimal conditions for the growth of toxigenic fungi. Fungal toxins cause acute or chronic intoxications depending on the animal, sex or breed and dose (Coker, 1979). According to the World Development Report (1993), diseases caused by mycotoxins lead to reduced life expectancy in the developing countries (Miller, 1996).

Some of the fungi and their mycotoxins are the following. Aflatoxins are produced by *Aspergillus flavus, A. parasiticus* and *A. nominus* (Lillehoj *et al..*, 1976; Stultz and Krumperman, 1976; Jemmali and Guibot, 1970; Bradburn *et al.*, 1993). *A. ochraceus* produces ochratoxins (Huff *et al.,* 1979; FAO, 1986). Rugulosin is produced by dark *Penicillium* (Austwick, 1975) and citrinin by *P. citrinum* and *P. viridicatum* (Scott and Kennedy, 1973), Patulin is produced by *P. patulum*, and *P. claviform* (Reiss, 1975). Other mycotoxins include Penicillic Acid produced by *P. cyclopium*, P. *roqueforte, A. ochraceus* and *A. sulphureus*; Sterigmatocystin by *A. versicolor, A. nidulans;* Rubratoxin by *P. rubrum*, and *P purpurogenum*; T-2 Toxin by *Fusarium tricintum*, *F. roseum*, *F. solani* and *F. lateritium*; and Zearalenone produced by *F. roseum* and *F. moniliforme* among others (FAO, 1990). Some fungi isolated from cocoa beans have been found to produce mycotoxins Out of the mycotoxins, those of agricultural importance are aflatoxins, fumonisins, Ochratoxin *A.,* zearalenone and deoxynivanelo*l* (IARC, 1993).

According to Miller (1996), 40 % productivity is lost to diseases in developing countries due to diseases caused by aflatoxins. Yet many people in the region are not aware of the effect of mouldy products. The four major aflatoxins are B_1, B_2, G_1 and G_2. Aflatoxins M_1 and M_2 are hydroxylated metabolites of G_1 and G_2 in animals. Aflatoxin is a very powerful hepatocarcinogen and naturally occurring mixtures of aflatoxins have been classified as a class 1 human carcinogen (IARC, 1993). Aflatoxins have also been reported in cocoa (Lenovich and Hurst, 1979). Lenovich (1981), on studying 13 cocoa bean types, concluded that caffeine is an effective inhibitor of aflatoxin production and that is why aflatoxin does not accumulate in cocoa beans. Buchanan *et al.,* (1978), studied the growth and aflatoxin production in *Aspergillus parasiticus and* concluded that the anti-aflatoxigenic activity of cocoa

bean is not due to theobromine. Adachi *et al.*, (1992) detected aflatoxin B_1 in imported cocoa beans.

There are 9 known ochratoxins. Ochratoxin A is the most frequently found in food and animal feeds and which also possesses the highest level of toxicity. Other forms Ochratoxin B and C have also been isolated from food but are rare (Rates and Matisstek, 1999). Ochratoxin A was first isolated from *Aspergillus ochraceus* (*A. alutaceaus*) but later other fungi in *Aspergillus* and *Penicillium* species e.g. *P. verrucosum* (Van der Merwe e*t al.*, 1965). Ochratoxin A is found as natural contaminants in foodstuffs including cereals, dried fruits, wine and poultry eggs and milk. Kpodo (1996) detected Ochratoxin A in 5 out of every 20 fermented corn doughs. Ochratoxin A is immuno suppressive, teratogenic, genotoxic and mutagenic and International Agricultural Research Council has classified it in group 2b as possibly carcinogenic to human (IARC, 1993). Sedmikova *et al.* (2001), found that ochratoxin A can increase the mutagenic ability of aflatoxin B_1 in case of the two simultaneously occurring in the same crop.

Ochratoxin A has recently caused much concern because it has been detected in cocoa beans and products. Initial research on ochratoxin A in cocoa reported that it was present at a detectable level in 67 % of cocoa samples tested. There was no correlation between the ochratoxin A and the visible mould on the beans, and that all origins of beans were affected. The issue is still being investigated (Raters & Matissek, 2003).

The European Union has produced draft regulatiion stating that ochratoxin levels in cocoa beans and powder imports must not exceed two parts per billion (ppb) while finished cocoa products e.g. chocolate, must not contain more than 1ppb. The final recommendation on the regulation was expected to be implemented by December 2003 (ICCO, 2003). Mycotoxins already formed are resistant to radiation (Miyaki, 1967). Van Dyck *et al.* (1982), irradiated an aqueous solution of

aflatoxin B_1 to 20 kGy and reported near complete degradation. However, aflatoxins in food are much more resistant to degradation (Chiou, 1996). Fenell (1966), working on groundnuts, observed no reduction in toxicity of aflatoxin $B_1$when irradiated .Temcharoen and Thilly (1982) reported that an aflatoxin – contaminated groundnut meal required a high dose of 50 or 100 kGy to eliminate the effect of aflatoxin on a bacterial test system. Doses as high as 180 kGy have been reported to degrade only 10 % of aflatoxin in a dry environment (Aibara & Miyaki, 1970). Patel (1989), reported that simultaneous treatment with hydrogen peroxide and gamma radiation resulted in a synergistic inactivation of aflatoxin B_1in contaminated groundnuts . Pure ochratoxin dissolved in methyl alchohol was found to be stable up to a dose of 75 kGy (Paster *et al.*, 1985).

2.6 Detection methods

Until 1996, the majority of mycotoxins were determined by Thin Layer Chromatography. Methods that have been developed to detect mycotoxins in cocoa include the use of high-performance liquid chromatography (HPLC) with flourescence to determine patulin, penicillic acid, zearalone and sterigmatocystine (Hurst *et al.*, 1987), and liquid chromatography to determine aflatoxin (Hurst *et al.*, 1982). HPLC methods are currently being used more frequently because the separating capacity is higher and the analysis time shorter (Raters & Matissek, 2003). Enzyme-linked Immunosorbent assay (ELIZA) is also used to detect aflatoxin (Adachi *et al.*, 1992).

2.7 Factors influencing mouldiness and mycotoxin production

Fungi may contaminate agricultural produce during growth in the field and during fermentation, sun-drying, handling, transportation and storage. In maize and

groundnut for instance, fungi such as *A. flavus* may invade mechanically-damaged and insect–damaged wet seeds in the field and in the wet produce. Here, the factors influencing mouldiness are type of substrate (crop), variety and vigour, insect infestation and the extent of fungal contamination of produce. *A. flavus* may be able to survive through drying and cause mouldiness in storage. The contamination of food by toxigenic fungi may lead to the production of mycotoxins, though conditions governing the growth of fungi may be different from those required for toxin production (Northolt *et al.*, 1977). Factors influencing the production of mycotoxins include; fungal species and strain, the substrate, the temperature and Water activity (a_w i.e. water available for growth of the fungus).

Fungi appearing in fermentation heaps of cocoa may be killed by chemical changes occurring during the process or during drying. Those appearing during drying may be killed if drying is complete. Incomplete drying causes mouldiness. Wetting during storage may lead to fungal growth. The critical factors governing mouldiness are moisture content of the bean, the storage environment (relative humidity, temperature and mycoflora present), and the duration of storage (Bunting, 1928; Maravalhas, 1966). Mouldiness is enhanced when storage and transportation facilities are inadequate. For example poor roads, and lack of vehicles to cart the beans to the port and improper protection from rains during transportation contribute to the beans becoming mouldy.

The beans tend to pick up moisture in areas of high humidity. Work done by Powell and Wood (1959) in Cameroun showed that the average relative humidity during temporary storage was 82 %. Under these conditions, the beans were in equilibrium at a moisture content of over 8 %. They observed that when cocoa beans are placed in an atmosphere with relative humidity above 80 %, they attain equilibrium moisture content in a matter of days, while cocoa in a free-standing bag may take

several days. Oyeniran (1979), working on the influence of storage environment on the quality of commercial cocoa, found that beans with 6.9 % moisture content atttained moisture contents of 6.5, 7.1, 7.4, 8.5, 9.2, 11.7 and 17.98 % when stored, respectively, at relative humidity of 65, 71, 75, 81, 84, 90 and 98 % for 3 months. He observed that internal mouldiness occurred at R.H. between 75 and 98 %. In Nigeria, it was found that in large stacks, temperature increased several degrees above ambient leading to a fall in the moisture content of the inner bags. The outer bags may however gain moisture slightly (Rohan, 1963). Results of studies on the moisture sorption isotherms of cocoa under 55-95 % R.H. indicated that the moisture content of beans increased from an initial level of 6.4 % to 7.7 % and 8.9 % at 55, 65 and 75 % R.H. respectively after 6-8 days (Amoako- Atta *et al*., 198). Beans stored under 85 and 95 % RH continued to absorb moisture from their respective environments indefinitely during the 64 days storage period.

2.8 Preservation of cocoa

The current method of preventing mouldiness in storage is by handpicking mouldy beans before bagging. Although handpicking is effective when small quantities are involved, it is tedious and time consuming. Besides, some beans with only internal infection or with slight external fungal growth may be considered to be good. Incubation of such beans often shows that some of the externally occurring mycelia and spores are viable and under favourable conditions can cause both external and internal mouldiness in storage. Therefore if such mycelia and spores are killed, mouldiness in storage would be prevented.

Cocoa with the moisture content between 6 and 7 % is stored in jute sacks and stacked in ware houses with good phytosanitary conditions to ensure that the moisture content of the beans do not exceed 7.5 %. In Brazil, jute sack lined

with polythene is recommended for storage on the farm to slow down moisture absorption by the beans (Serodio and Prado 1982). Other methods of preventing spoilage such as the use of chemical disinfectants have not been effective and leave residues on the beans. There does not seem to be any effective chemical for controlling internal mouldiness.

In Ghana, the Quality Control Division of Cocoa Board is responsible for pest control of stored cocoa. The Division has eighteen "Disinfestation Zones" within which trained pest control staff operate from strategic locations, including the ports, regional capitals and some district offices. The officers inspect storage sheds and carry out disinfestation of the produce by fumigating with Phostoxin to ensure that only insect–free cocoa is exported. In addition, rodent control operations are also carried out with rodenticides.

2.9 Food Irradiation

Food Irradiation is a relatively new physical means of preservation comparable to processes such as heat pasteurisation, controlled atmosphere processing, canning or freezing. It involves exposure of food either packaged or unpackaged to ionising radiation from a Cobalt-60 source, X-ray machine or electron accelerator. The energy from ionising radiation kills pests and micro-organisms in the food. The food does not become radioactive as a result of the exposure to the ionising radiation. This process rather reduces the use of chemical additives and renders the food wholesome for human consumption. Data from over forty years of research into the use of ionising radiation was evaluated in 1980 by the Joint FAO/IAEA/WHO Expert Committee on the wholesomeness of Irradiated food (JECFI). They concluded that irradiation of food commodity up to an overall dose of 10 kGy causes no toxicological, microbiological or nutritional hazard. Toxicological testing of food so treated therefore is no longer

required (WHO, 1981). After the evaluation of available data on research on food treated with ionising radiation above 10 kGy, another JECFI meeting in 1997 came to a similar conclusion. These recommendations have in the past decade resulted in approval of one or more food items for irradiation and human consumption in 44 countries. Twenty-eight of these countries are using 50 commercial or demonstration irradiators for processing various food items for commercial purposes. Ghana gazetted standards for irradiated foods in 1997 (GS 210, 1997). The most common irradiated food products for commercial use are spices and dry vegetable seasonings. Some 200,000 tonnes are estimated to be irradiated in 1991 (Loaharanu, 1992).

Food Irradiation has been recommended for application by FAO, WHO and credible scientific bodies of many countries including the United States of America, the United Kingdom, Canada and the Commission of the European Union. The Codex Alimentarius Commission has published a Codex General Standard for Irradiated foods and Recommended International Code of Practice for the Operation of Radiation Facilities used for the treatment of food (CAC, 1984).

Food Irradiation can solve specific food storage problems. The benefits can be summarised as:

- reduction in post harvest losses
- production of safe food
- quality improvement
- promoting international trade

2.9.1 Reduction of post-harvest losses

Food Irradiation is able to contribute to reducing these losses through various means. It inhibits sprouting in potato, sweet potato, yams, onions, garlic, shallots, and ginger at low doses. Sprout inhibitors such as maleic hydrazide are effective but they leave residue which causes health hazards. Irradiation delays the ripening of fruits and

vegetables and extends shelf-life. A dose of 0.15 kGy delays ripening of banana by 9 days (IAEA, 1981) while a dose of 0.5 kGy delays senescence of egg plant for three weeks (Appiah *et al.*, 1990). Irradiation is less time consuming and can be applied even when food is packaged. With the banning of some of the chemicals used for fumigation irradiation is an effective alternative. Radiation disinfestation doses up to 1 kGy have been applied successfully to various foods grains, pulses, cocoa and coffee beans, dried and smoked fish, and dried nuts (Appiah, 1989; Ahmed *et al.*, 1989; Matin *et al.*, 1992). Irradiation is the only method which kills the mango seed weevil within the seed (Ahmed, 1993). At sublethal doses, irradiation causes reduction in feeding and slows down development. Unlike fumigation, irradiation leaves no residue on food. The process causes no danger to the operator as he is nowhere near the radiation source.

2.9.2 Effect of ionising radiation on micro-organisms in food

The use of ionising radiation has been shown to be extremely effective in controlling food borne pathogens (Table 1). Ionising radiation is capable of causing chemical changes in the cells of the micro organism especially the DNA, which may result in damage or death of cells. Factors influencing radiation resistance include the nature and amount of direct damage produced the number, nature and lifetime of radiation–induced reactive chemical entities and the inherent ability of the cell to either repair or tolerate radiation damage, and the influence of intracellular and extra cellular environments on the above factors (Grecz *et al.,* 1983). Factors influencing the survival of such cells are; temperature, phase of growth, water activity, pH, the nature of gaseous environment and the chemical composition of the food (Grecz *et al.,* 1983; Davies, 1976).

The lethality of radiation to micro organisms is expressed in D_{10} values (The dose that reduces the population to 10%). Since it is difficult to determine cell numbers

from mass of hyphae in hyphae-producing fungi, their radiation sensitivity is usually not expressed in the form of a D_{10} value except for spores whose numbers can be determined (WHO, 1999).

2.9.3 Irradiation and its effects on food quality

Food irradiated in accordance with established practice is microbiologically, toxicologically and nutritionally safe and wholesome for consumption (WHO, 1981). Sometimes, irradiation offers additional benefits. Exposure of dried fruits, pulses and vegetables to ionising radiation especially above 4 kGy can reduce the toughness, rehydration time and cooking time. Flatulence causing oligosaccharides in certain legumes including soybeans, red and green gram, and navy beans can be effectively eliminated by irradiating the germinated legume seeds with doses 2.5-10 kGy (Hasegawa and Moy, 1977; Rao *et al.*, 1983). Irradiation increases the yield of juice from grapes without affecting the wine-making quality (Kiss *et al.*, 1974). It also increases the rate of drying of some fruits such as prunes (Kiss *et al.*, 1974).

Meat is tenderised to some extent by exposure to substerilising doses for shelf-life extension mainly because the activity of the enzymes is not inhibited (CAST, 1989). Very low doses applied to air-dried barley and other grain, retards root growth so that high-quality malt can be achieved (Satin, 1993). A dose of 18 kGy raised diastatic power, beta-amylase and alpha-amylase in Nigerian sorghum (Uwaifo, 1983). Frozen shovel-nosed lobsters often exhibit a garlic-like off odour which is removed by irradiation with a dose of 25 kGy (Freeman *et al.*, 1985). Sweet potato brandy is irradiated to improve the sensory quality (Diehl, 1991).

2.10 Irradiation of Cocoa

Ionising radiation has been applied successfully to disinfest stored cocoa against insects. Results of the application of gamma radiation to four insects *Cadra cautella, Lasioderma serricorne, Araecerus fasciculatus* and *Tribolium castaneum* isolated

Table 1: List of some organisms that have been controlled with radiation

Organism	Product	Reference
Bacillus cereus	Spices	Powers *et al*, 1976
Clostridium perfringens	Minced bacon	Strong *et al*, 1963
Campylobacter jejuni	Ground pork, ground beef	Collins *et al*, 1996;
Escherichia coli	Ground beef	Maxcy & Tiwari, 1973
E. coli 0157.H7	Mech. deboned chicken	Thayer & Boyd, 1992
Aspergillus flavus	Ground spices	Flannigan & Hui, 1976
Aspergillus flavus	Maize	Nout & Saint- Hilaire, 1983
Salmonella niloese	Poultry	Farkas 1988; Giddings & Marcotte1991
Salmonella spp	Fish, shellfish	Nickerson *et al.*, 1983
Salmonella spp.	Shrimp	Nerkar &Bandekar, 1990
	Clams, herring	Carver *et al.*, 1969
Microbial load	Protein serum	Tsuji, 1983
Trichinella spiralis	Pork	Brake *et al.*, 1985
Byssochlamys fulva	Apple juice	van der Riet & van der Walt, 1985

from Ghanaian cocoa showed that, a dose of 0.8 kGy completely attenuated insect activity in room cultures as well as on cocoa beans (Amoako-Atta, 1979). In a similar study in Malaysia, the relationship between effective doses including mortality and sterility in several storage beetles were investigated. To induce 100 % mortality, doses ranging between 0.05 - 0.1 kGy require weeks; 0.2 - 1.0 need 1.5 - 3 weeks and 2 kGy require 1 week for *Tribolium casteneum, Oryzaephilus surinamensis* and *Lasioderma serricorne*. (Rahim-bin-Muda *et al.*, 1991).

Amoako-Atta *et al.,* (1981), observed that pre-irradiation storage humidities are important in the determination of the effectiveness of gamma radiation dose in preventing fungal growth. Barata and Mansur (1995), reported that a dose of 3.99 kGy drastically reduced the number of mesophilic non-spore forming aerobic bacilli in fermented beans while a dose of 1.05 kGy eliminated coliforms from Brazilian cocoa beans.

Chemical analysis of cocoa after irradiation at doses of 0.1, 0.2, 0.5, 2.0 and 5.0 kGy showed no significant difference in reducing sugar, total amino acids, total fats, free fatty acid value, saponification value, refractive index, slip point and specific gravity, alkaloids and protein contents (Takyi and Amuh, 1979).

In a separate study, Takyi and Offori-Mensa (1979), incorporated up to 35 % (v/v) roasted and ground cocoa beans irradiated at 5 kGy into the normal diet of rats for 18 weeks. There was no significant difference in the general behaviour, haematology, gross pathology and weight of vital organs between those fed on ordinary feed and those fed on irradiated cocoa.

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CHAPTER THREE

3.0 ESTIMATION OF THE EXTENT OF MOULDINESS IN COCOA BEANS IN GHANA

3.1 INTRODUCTION

In Ghana, cocoa is grown by peasant farmers in small holdings. The mature cocoa pods are harvested and cut open to remove the beans which are fermented, dried and bagged in jute sacks for marketing. Cocoa is graded during purchasing. In order to meet the set standards, farmers remove all defective beans during drying and bagging. The farmers are grouped into societies and purchasing of cocoa is done at the societal level. At each society the cocoa purchased is bulked together and dried again to ensure proper drying. During this time any defective bean is removed and the cocoa is rebagged, inspected by the Produce Inspection Division and sealed. The cocoa is carted to the collection centres from where they are conveyed to the ports either by rail or road. At the port, grading is done again before storage for shipment.

The defective beans that are removed by the farmer are discarded or may be sold at a very low price. Some buyers also sort out and discarded defective beans. There is no available report on any research carried out in Ghana to determine how much is lost by the farmer before beans are sold or how much is discarded at the buyers level. It is of interest to quantify this loss especially that due to mouldiness. Although data on the cut test is routinely collected at the port as part of the quality assessment, analysis of beans for mouldiness is not done.

The objectives of the present investigation are:

1. To determine the quantity of beans lost especially mouldy beans at the farmers' level.

2. To determine the quantity of mouldy beans discarded at the buyers' level (before rebagging and sealing).
3. To determine the quantity of mouldy beans at the port level before shipment.

3.2 MATERIALS AND METHODS

3.2.1. Determination of loss of beans due to mouldiness and other defects at the farmers' level

3.2.1.1 The questionnaire approach

Five hundred cocoa farmers representing about 10 % of the number of cocoa farmers in the Tafo District were targeted for the survey. Farmers were selected from each of the 19 societies (Appendices 1 and 2). The questionnaire (Appendix 3) was designed to provide information on the farm size, yield (average) of marketable beans, quantity lost and the cause of loss. With the assistance of the Head of the Cocoa Services Division at Tafo, the questionnaire was explained and was then distributed to the farmers. The literate ones answered the questions themselves while the illiterate ones were assisted. The questionnaire was collected and the data compiled and analysed. In answering the questionnaire, the farmers estimated the volumes of discarded beans using volumes of containers, such as "American tin" which were later converted into weights.

3.2.1.2 The discarded beans collection approach.

Twenty-four farmers were selected from the societies with the assistance of Extension Staff during the 1996 major (October - March) and minor (June - September) seasons for data collection. Each farmer was asked to keep aside discarded beans from harvesting to drying. The discarded beans were grouped into batches according to whether they were mouldy, insect infested, germinated, slaty or flat. The volume of the

discarded beans was measured using the following containers and were converted to weights as shown below:

Bag	=	64.00 kg
Load	=	30.00 kg
American tin	=	1.50 kg
Margarine tin	=	0.25 kg
Milk tin	=	0.10 kg

The farmers were also asked to provide information on the farm size and the yield of cocoa beans for the season (Appendix 4). As visits were paid to the farmers, their cocoa was found to be at various stages of drying (Plate 2). Some of the farmers had two or three mats with cocoa at different stages of drying. On the first day that they spread the freshly fermented beans to dry, they removed mainly pieces of debris and some rope like pieces. Then as the days went by, they removed the defective beans such as mouldy and germinated beans and dried them separately (Plate 3). At the end of the season they had a collection of defective beans (Plate 4) which was sorted out and weight determined (Plate 5).

The total weight of beans for each farmer, the total discarded beans and percentage of the mouldy beans over the yield were calculated. The data was analysed statistically using "Minitab" software and the defects for the two seasons compared.

3.2.2. Determination of the quantity of mouldy beans at the buyers' level.

Twelve buying centres were visited during the 1996 major cocoa season to find out how they handled the cocoa beans before transportation to the collection centres and finally to the port. The buyers were asked to set aside discarded beans which were used for this study. The discarded beans obtained were grouped according to the

Plate 2. Farmer spreading fermented cocoa beans on a mat to dry.

Plate 3. Cocoa at various stages of drying.

Note: (a) Woman picking defective beans and placing them in her left hand with a daughter looking on.

(b) Defective beans being (arrowed) dried separately from the marketable beans.

Plate 4. A basket containing discarded beans removed from a farmer's cocoa during drying.

Plate 5. Discarded beans grouped according to various defects.

Black pod: Beans extracted from pod infected with the fungus causing the disease.

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various defects and weighed. The quantity of beans from which the discarded beans were taken was also recorded. It was observed that the buyers bulked all their purchases together. They then redried them on tarpaulin (Plate 6) to ensure that the beans were well dried. To compensate for weight loss during redrying the weight of the bags purchased was 64 kg instead of 62.5 kg. After redrying they were collected in heaps and then rebagged using new bags supplied by Cocoa Board (Plate 7). The Produce Inspectors (Quality Control) assessed the quality of the cocoa using the cut test before the bags were sealed and labelled for transportation to depots. The tags bearing the name of the centre was also put on the bags. The bags were then ready to be transported to the depots or collecting centres. They were not opened at the depots unless there was a problem. From there, they were transported to the port for export. The buyers were asked about removing and discarding defective beans during redrying. Nine of the buyers said they do not sort out any defective beans during redrying. A heap of defective beans obtained from a buying centre is shown in Plate 8.

3.2.3 Determination of mouldy beans and other defects at the port

Each consignment of cocoa which arrived at the port during 1992/93 major and minor seasons was off-loaded into the sheds packed in groups of 300 bags on pallets (Plate 9). After the necessary records were taken 300g samples of beans taken from each of 30 baags using a mental horn assessement and bulked together (Plate 10). A quarter of the composite sample was taken and 300 beans taken out for the cut test. This consists of cutting lengthwise the beans so as to expose maximum cut surface cotyledon area for inspection. The cut test was done by trained personnel of the Quality Control Division (Plate 11) and the cut pieces grouped according to defects (Plate 12). The number of beans showing each of the following defects was recorded

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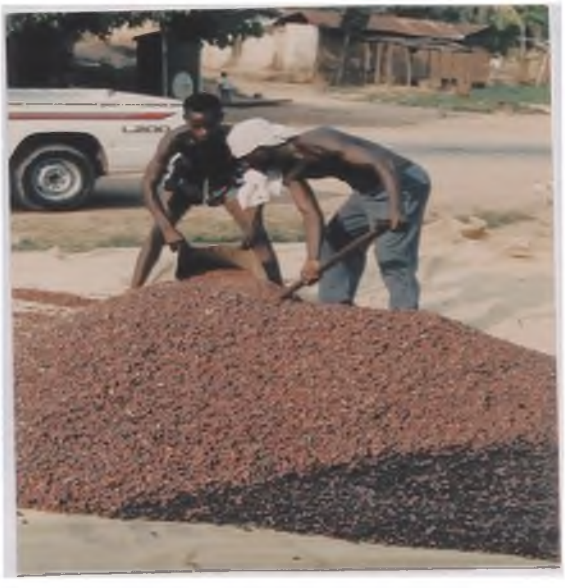

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Plate 6. Cococa being dried on tarpauline in a buying centre.

Plate 7. Rebagging after drying at a buying centre.

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Plate 8. A small heap of discarded beans (right) separated from the rest.

Plate 9. Stacking of cocoa in the shed at the Tema Port.

and expressed as percentage: mouldy slaty insect infested germinated, flat or shrivelled for each sample. Thus, by records, the average percentage defects for the period 1992/93 could be calculated. The total weight of beans exhibiting the various defects could be calculated using the formula derived below:

The percentage defective beans (a) = $\frac{\text{No. of defective beans (y) x 100}}{300}$

$$a = \frac{y}{3}$$

The weight of defective beans per bag (62.5 kg.) = a x 0.625 kg

Wt. (kg) of defective beans in S number of bags = a x 0.625 x S kg.

Where S = number of bags

The bags were not opened again before shipment. However, if bean moisture content in some of the bags was high, such bags were opened and redried (Plate 13). The cocoa was reconditioned by picking out all mouldy and infested beans before rebagging (Plate 14). For shipment, the bags of cocoa were packed into containers lined with polythene to prevent wetting during shipment (Plate 15 & 16). A total of 97 shipments were done during the major season and nine during the minor season (Appendices 7 & 8). In order to know the average mouldiness of cocoa beans at the port over a period, data of percentage mouldy beans from 1988 to 1992 were collected from the Quality Control Officers and analysed as described above.

3.3 RESULTS

3.3.1 Determination of mouldy beans at the farmers' level

Out of 500 farmers who received the questionnaire, 407 were returned.

The analysis of the data collected by questionnaire have been presented in Table 2

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Plate 10. A composite sample taken for the cut test.

Plate 11. Quality control staff carrying out the cut test.

(a) Working table demarcated to give working space to each other.

(b) Box container sample for the test.

(c) Cut beans lined up for examination (internal).

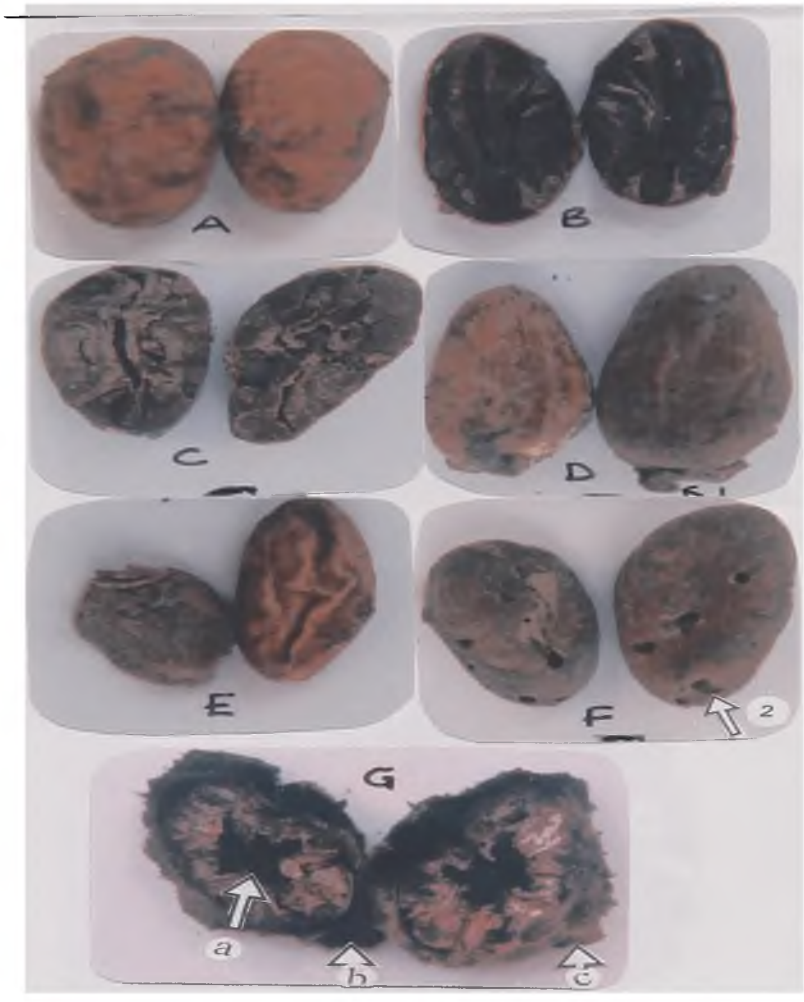

Plate !2. Grouping of cocoa beans based on cut test.

A&B - Good beans .
Note - The cut surface is purplish brown (dark brown in picture).
C - Slaty beans (Surface is light brown).
D - Germinated beans (1=Radicle protruding from beans).
E - Flat beans (size smaller than normalbeans).
F - Weevil infested beans (2=holes made by weevils).
G - Mouldy beans.

a Internal fungus (*A niger*) .
b&c External fungi (*A. flavus* and *A. niger* growing as external fungi on surface of bean).

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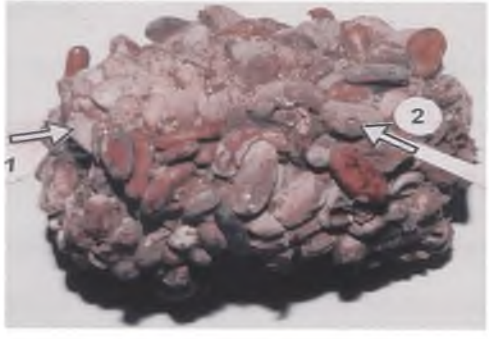

Plate 13 (a). Wet Cocoa being re-dried and rebagged at the Port.

Plated 14 (b). Caked beans (resulting from wetting) obtained from the Tema Port showing both (1) and insect infested beans (2).

and Fig.1-3. The farm size of the 407 farmers ranged between less than 1 hectare (ha.) to 50 ha. The estimated total marketable cocoa beans produced per year by the 407 farmers was 355,605 kg (355.61 T) and the estimated total quantity of beans lost due to various defects was 5,669.83 kg which was 1.73 % of the marketable beans. Of this, mouldy beans were estimated to be 551.20 kg which was 0.16 % of the marketable cocoa (Table 2) and 10 % of the total quantity lost (Fig. 1). The estimated weight of beans discarded by the farmers was between less than 1 kg and 128 kg. The majority (42 %) discarded 1.1 –5 kg; 17 % lost 5.1- 10 kg of beans per year while some farmers (13 %) indicated they did not incur any loss through the defects year (Fig 2). The farmers attributed the loss of beans to a number of factors including over-drying, germination, blackpod disease, flat beans, rodent attack, insect infestation, mouldiness and mechanical damage (Fig. 3). Most farmers gave more than one cause of loss.

The results of the survey conducted using the bean collection approach has been presented in Tables 3, 4 & 5 and Fig. 4. In the 1996 - 1997 major seasons, the total weight of marketable beans for the 24 farmers was 9,568.33 kg from 45.9 ha of farms. The total loss was 74.2 kg (Table 3) which was 0.78 % of the marketable beans. During the minor season of the same year, the total marketable cocoa beans produced by the 24 farmers weighed 3,733.6 kg from 48.2 ha of cocoa farm (Tab le 4). The loss weighed 146.37 kg which was 3.92 % of the marketable yield (Table 5). The total marketable yield for the year was 13,301.80 kg. Total loss for the year was 220.70 kg which was 1.66 % of the marketable beans. The loss due to mouldiness for the year was 17.3 kg which was 0.13 % (Table 5). Individual farmers lost between less than 1 and 33.3 kg in the minor season and between less than 1 and 9.2 kg of beans. The incidence of mouldy beans in the two seasons differed. During the minor season, 20

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Plate 15. Container lined with polythene for shipment of cocoa.

Plate 16. Loading bags of cocoa into a container for shipment.

Table 2: Estimated weight (kg) of cocoa discarded by farmers due to various defects in the questionnaire approach expressed as % of marketable beans.

Description	Wt. of beans	% of marketable beans
Estimated marketable Yield	355,605	-
Total defective beans	5,669.83	1.73
Overdrying	64.73	0.02
Over ripening	6.25	0.002
Flat beans	556.74	0.16
Blackpod	650.14	0.18
Rodent damaged	313.83	0.09
Insect-infested	302.77	0.09
Germinated	2015.12	0.57
Mouldy	551.20	0.16
Mechanical damage	721.9 3	0.20
Under developed	487.12	0.14

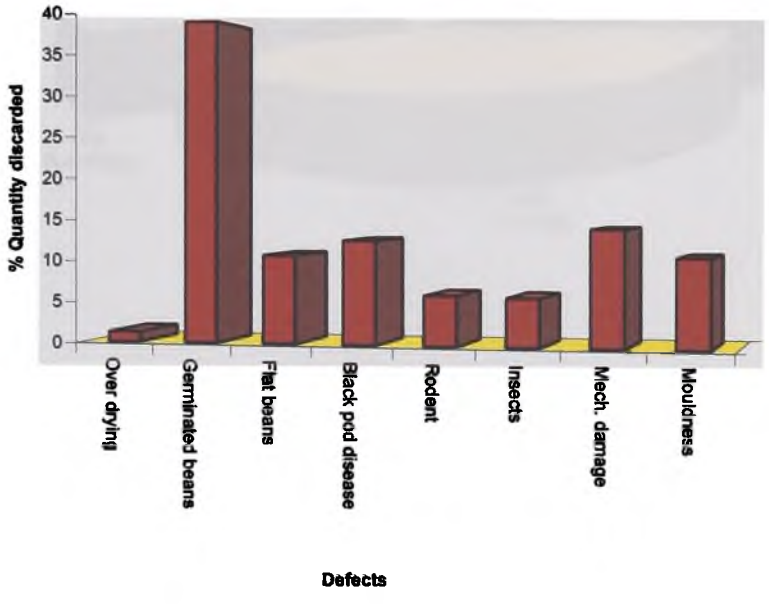

Figure 1. The estimated quantity of beans discarded by farmers expressed as % of total loss.

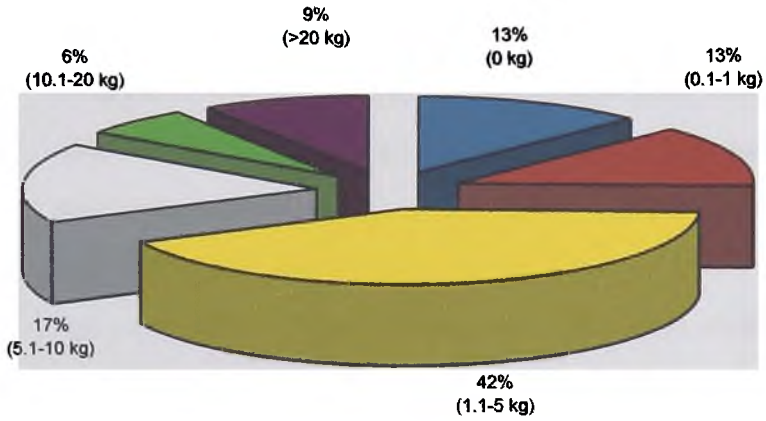

Figure 2. Percentage of farmers discarding different quantities (kg) of cocoa beans per year.

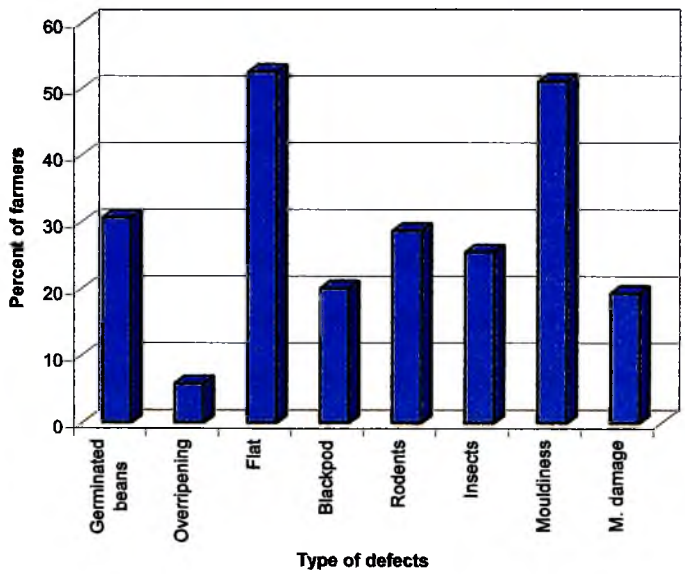

Figure 3. Percentage of farmers reporting various defects in the survey M. damage = Mechanical damage.

out of 24 farmers discarded 16 kg of mouldy beans which was 0.42 % as against 9 farmers discarding 1.3 kg of mouldy beans which was 0.01 % in the major season. Mouldy beans formed 10.93 % of the defective beans in the minor season (Table 4) as against 1.8 % in the major season (Table 3). In the minor season mouldy beans ranked 4th after blackpod disease, rodent attack and insect infested beans. In the major season, however, mouldy beans ranked last (8th) in the defects (Fig. 4). When each of the defects was analysed separately for the two seasons the defects with highly significant differences ($p<0.01$) were mouldy beans, blackpod disease and insect infested beans (Appendix 6).

3.3.2 Determining mouldy beans at the buyers (societal) level

The result of the survey at the buyers' level is presented in Table 6. Nine out of twelve buyers said they did not sort out any defective beans during drying. Three of the buyers picked out defective beans during redrying. All the three said they normally did not have any mouldy beans during the major season. It was during the minor season (June-July in the rainy season) that mouldy beans might be found.

The total marketable beans was 2,230 bags (126,875 kg). The total defective beans weighed 8 kg 200.83 g which was 0.0006 % of the marketable beans. The mouldy beans weighed 25.4 g which was 0.00002 % of the marketable beans.

3.3.3 Determination of mouldy beans at the port

The results of the survey at the Tema port have been presented in Tables 7, 8 & 9 and Fig. 5. The weight of cocoa carried by various ships from Tema port ranged between 50 and 5,473 T. The total weight of cocoa shipped during the minor season from Tema port was 14,938.8 T (239,020.8 bags) and for the major season 93,135.8 T (1,490,173 bags) (Table 7). The percent average mouldy beans was 0.80% in the minor season and lower in the major season at 0.52 %. The total of cocoa exported

Table 3: Data collected from farmers on discarded cocoa beans during the major season of 1996-97

No.	Farm Size	Yield (kg)	Yield (/ha)	Insect infes-	Ro- dent	Mouldy	Black Pod	Under Dev.	Flat	Germi- nated	Slaty	Total discarded
1	0.8	128	160	0.3g	5.25	-	-	0.38	0.2	0.5	0.75	7.38
2	1.4	133	95	-	0.35	-	-	-	1.5	0.75	0.75	3.35
3	1.2	300	250	1.5	5.0		-	0.5	1.0	1.0	0.25	9.25
4	3.2	650	203.1	-	0.15	-	-	-	0.25	-	0.13	0.73
5	2.2	750	340.5	0.2	3.0		0.1	-	-	1.5	0.25	5.05
6	2.0	625	284.1	-	0.13	0.1	-	-	0.1	0.23	0.05	0.76
7	1.0	195	195.0	0.15	0.3	-	-	-	0.2	0.25	0.25	1.2
8	1.1	184.0	161.4	0.2	0.75	0.1	-	1.0	0.2	0.25	0.10	2.6
9	2.0	281.2	140.6	0.2	0.2	0.1	-	-	0.1	0.13	0.15	0.98
10	1.6	250	156.3	0.3	1.5	0.2	-	-	0.3	0.5	0.25	3
11	1.6	312.5	195.3	0.25	0.1	0.1	-	-	0.25	0.13	0.15	0.73
12	1.0	240	240	-	0.75	-	-	-	0.75	-	-	3
13	2.2	250.5	113.9	-	4.5	-	1.5	-	-	0.75	-	6.25
14	2.2	625	284	-	1.23	0.2	1.0	-	0.15	-	-	1.58
15	0.8	128.0	160	-	0.74	-	-	-	0.20	-	0.3	1.49
16	2.1	687.5	326.3	0.25	4.5	0.3	-	0.27	0.5	0.2	0.2	6.47
17	1.1	250	227.3	0.5	0.5	-	-	0.54	0.2	0.1	-	1.64
18	1.2	312.5	250	0.3	1.58	-	-	0.38	0.1	-	0.1	2.16
19	1.8	1.25	69	-	1.23	0.1	-	1.15	1.0	0.2	0.3	3.98
20	2.0	615.0	307. 5	-	1.4	-	-	-	1.0	0.1	0.3	2.8
21	2.2	563	255.9	-	1.5	-	-	-	-	0.25	-	1.75
22	2.8	750	261.9	-	2.5	-	-	-	0.2	1.5	-	4.2
23	3.2	842.5	263.3	-	0.75	-	-	-	-	0.3	-	1.05
24	4.2	920.25	219.2	-	2.5	-	-	-	-	0.3	-	2.8
Total	44.7	9877.97		9.45	40.41	1.3	3.0	4.72	9.70	6.94	3.98	74.2
% of marketable bean s				0.10	0.42	0.01	0.03	0.05	0.10	0.07	0.04	0.78
% of Total loss				5.59	44.33	1.8	3.2	63.6	10.51	1 8.14	4.58	

Table 4: Data collected from farmers on discarded cocoa beans during the minor season of 1996-97

No.	Farm Size	Yield (kg)	Yield (/ha)	Insect infes-	Ro-dent	Mouldy	Black Pod	Under Dev.	Flat	Germi-nated	Slaty	Total discarded
1	1.2	49	40.8	-	-	1.3	-	-	-	0.5	0.2	2.00
2	1.4	149	106.4	-	2.52	-	1.5	1.0	-	0.8	0.1	5.90
3	1.3	98	75.4	0.80	-	0.5	2.1	-	-	-	0.8	4.27
4	1.4	90	64.3	1.5	2.1	-	28.8	-	0.3	0.3	0.8	33.80
5	1.2	115	95.8	0.7	1.8	0.3	-	-	0.4	0.3	0.3	3.80
6	1.5	25	16.7	2.5	0.4	2.3	9.7	1.0	0.4	0.3	-	16.60
7	1.0	15	15.0	1.19	3.2	1.1	1.5	-	-	0.3	-	7.29
8	2.0	93	46.5	-	0.4	-	-	-	-	0.1	0.1	0.60
9	1.8	312	173.3	-	-	0.5	0.5	0.4	0.5	0.5	0.2	2.60
10	0.8	16	20	3.0	1.2	3	-	-	-	0.1	0.1	7.40
11	1.2	59	49.2	0.4	1.6	1.2	0.91	0.1	3.1	0.8	-	8.11
12	2.0	240	120	0.8	2.6	0.4	-	0.2	-	-	-	4.00
13	1.8	340	188.8	0.8	0.5	0.8	9.8	-	0.4	-	0.1	12.40
14	1.4	63	45	-	0.3	-	-	-	0.1	-	0.2	0.60
15	1.2	250	208.3	0.8	-	0.4	1.8	-	-	-	-	3.00
16	2.0	96	48.0	-	2.1	0.1	-	0.1	0.1	0.1	0.3	3.60
17	0.8	26	32.5	1.2	3.2	2	0.4	0.2	0.2	-	0.5	5.90
18	2.2	120	54.5	0.7	0.4	0.2	-	1.0	-	0.2	-	2.50
19	3.2	152	47.5	0.5	0.5	0.2	1.5	-	0.1	-	0.1	2.90
20	2.8	120	42.9	2.0	1.6	0.5	-	-	0.2	-	0.1	4.40
21	2.0	110	55	-	1.2	0.6	2.5	0.1	0.4	0.1	0.1	5.00
22	1.0	64	64	-	-	0.1	-	-	0.1	-	0.3	0.50
23	0.8	711.5	889.4	0.8	0.3	1.1	3.5	-	-	-	-	5.50
24	3.2	90	28.1	0.8	0.2	1.2	0.4	-	-	0.2	0.1	2.90
	48.2	3733.6		18.4	25.9	16	64. 91	4.1	3.5	4.6	4.4	146.50
% of marketable beans				0.49	0.69	0.42	1.64	0.11	0.09	0.12	0. 1	3.95
% of Total loss				2.82	18.28	10.93	46.75	2.87	2.45	3.21	3.07	

Table 5: The total Wt (kg) of cocoa beans showing various defects during two seasons expressed as % of marketable cocoa in the bean collection approach.

Description	Major Season	Minor Season	Total /yr	%
Insect-infested	4.50	18.4 0	22.90	0.17
Rodent-damaged	40.41	25.90	66.31	0.49
Mouldy beans	1.30	16.00	17.30	0.13
Blackpod	3.00	64.91	67.91	0.51
Under-developed	4.72	4.10	8.82	0.07
Flat beans	9.70	3.50	13.30	0.10
Germinated	6.94	4.60	11.54	0.09
Slaty	3.98	4.40	8.38	0.06
Defective	74.20	146.50	220.70	1.66
Marketable	9,568.2 0	3,733.60	13,301.80	

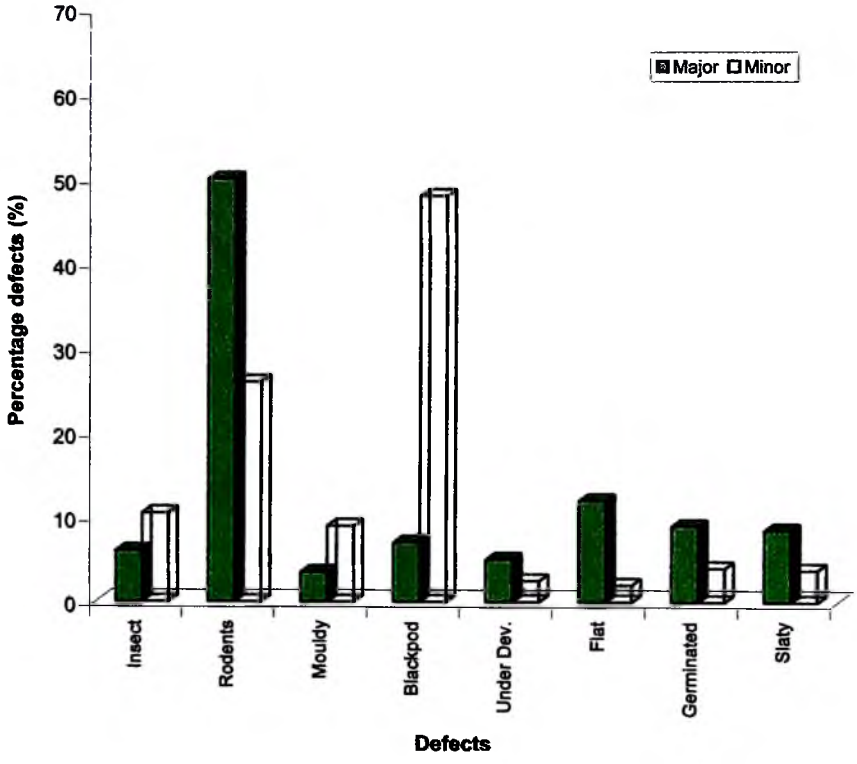

Figure 4. Type of damage observed on the discarded cocoa beans by farmer expressed as percent of the total discarded beans per season.

for the year through Tema port was 108,074.6 T and was estimated to contain 603.82 T mouldy beans which was 0.56 % of exported beans (Table 8). Out of 3,781.31 T of defective beans estimated in the major season mouldy, beans accounted for 484.31 T and ranked third compared to the other defects. In the minor season, out of 736.93 T defective beans, mouldy beans accounted for 119.51 T and ranked second after slaty beans. These weights expressed as percentages of total % defective beans showed the same trend. Apart from slaty beans, all the defects being compared have higher percentages in the minor season.

The average percentage mouldy beans in exported cocoa and the estimated weight of mouldy beans from 1988/89 to 1991/92 have been presented in Table 9. The quantity of cocoa exported for the five-year period ranged between 23,141.31 and 93,135.80 T with the average being 39,294.76 T/year. Out of this, mouldy beans were estimated to range between 129.9 T and 484.31 T for the period. The average percent mouldy beans for the five period was 0.69 %.

3.4 DISCUSSION

At the farmers' level, results obtained in the the bean collection apprroach complemented what was obtained in the questionnaire approach. Some of the observations made in the questionnaire approach were confirmed during the bean collection. Estimated annual % loss of cocoa beans determined by questionnaire and bean collection approach were 1.73 % and 1.66 % of the total yield respectively. The questionnaire approach showed that over 51 % of the farmers identified mouldiness as a problem. The estimated quantity of mouldy beans was 551.20 kg which was 0.16% of the estimated total yield of the farmers. In the bean collection approach, the total weight of mouldy beans lost by the farmers for the year was 17.3

Table 6. Weight (g) and composition of defective cocoa beans sorted out at three buying centres and expressed as % of marketable beans.

Name of Centre	No. of bags	Wt. of defective Beans	Slaty	Mouldy	Flat	Germinated	Under developed	Weevil infested
Mantukwa	160	135.6	0	25.4	46 2	0	64.0	0
Tetteh Nkwanta	1.770	8,000	8,000	0	0	0	0	0
Nankese	100	65.23	18.47	0	0	35.98	10.78	0
Total	2,030	8,200.83	8,018.47	25.4	46 29	35.98	74.78	0
Average		2733.62	2,672.8	8.35	15.30	11.99	24.92	0
% of marketable.								
Beans		0.006	0.006	0 .00002	0.0004	0.00003	0.00006	0

Table7: Total weight and mean % defects of exportable cocoa during minor and major of 1992/93.

Season	Total no. of bags	Total Wt.	Defect	mean % defect
Minor	239,020.8	14,938.8	mouldy	0.80 ± 0.16
			germinated	0.73 ± 0.12
			slaty	2.32 ± 0.22
			insect-infested	0.78 ± 0.40
			other defects	0.30 ± 0.12
Major	1,490,173	93,135.8	mouldy	0.52 ± 0.02
			slaty	0.06 ± 0.02
			germinated	0.60 ± 0.02
			slaty	2.61 ± 0.03
			insect-infested	0.24 ± 0.02
			other defects	0.09 ± 0.01

Table 8: Estimated weight (Metric tonnes) of defective cocoa beans in cocoa exported in the major and minor seasons of 1992/93

Defect	Major season	Minor season	total/year	% of exported
Wt. of defective beans	3,781.31	736.93	4,518.24	4,18
Mouldy	484.31	119.51	603.82	0.56
Germinated	558.81	109.05	667.86	0.62
Slaty	2,530.84	347.17	2,878.01	2.66
Insect infested	223.53	116.52	341.05	0.32
Other defects	83.82	44.82	128,64	0.12
Exported cocoa	93,135.8	14,938.8	108,064.6	

kg, representing 0.13% of the total marketable beans. This is very close to the earlier finding in the questionnaire approach that farmers in the Tafo District lose 0.16 % of their cocoa through mouldiness. The bean collection approach was better than the questionnaire approach since the former dealt with actual counts and the latter gave estimates. Knowing the actual quantities discarded as a result of various defects could help farmers to know the quality of their beans and to keep within the acceptable quality. At the buyers' level both the bean collection and the questionnaire approach were applied. By the bean collection approach the loss due to mouldy beans at the level in the Tafo District was found to be .000002 % of the marketable beans which was very low. This estimate is minimal because seventy-five percent (75 %) of the buyers interviewed did not do any sorting out as the removal of mouldy beans is done at the farmers' level. Under the Cocoa Buying Company, farmers are expected to remove all defective beans before purchase. Now that private buyers have entered the market there is competition. Therefore, some of the private buyers buy the cocoa and do the picking out themselves.

The quality of exportable cocoa determined by the cut test was found to be very high. The average % mouldy beans in 1992/93 were 0.56 % which represented 603.82 T. There were more incidence of mouldy beans in the minor season (0.8 %) than in the major season (0.52 %). The major cocoa season (October –March) occurs in the dry season when there is normally enough sunshine to dry the beans.The minor cocoa season (June – September), however, is within the rainy season when rainfall sometimes prevents proper dnying. The average mouldy beans at the Tema port for a 5-yr period based on major season exports was 0.69 %.

Table 9. Weight of mouldy beans in exported cocoa (tonnes)

Season	No. of bags	mean % mouldy	Weight of mouldy beans	Total weight exported
1988/89	370,450	0.97	224.46	23141.31
1989/90	448,376	0.75	210.18	28023.50
1990/91	450,124	0.67	188.50	28133.88
1991/92	403,894	0.54	129.81	24039.31
1992/93	1,490,173	0.52	484.31	93135.80
Average:	623,607	0.69	247.452	39294.76

The estimated quantity of mouldy beans lost by farmers, the buyers and the estimated weight of mouldy beans in exportable cocoa should give the quantity of mouldy beans in the country. The 0.13 % mouldy beans obtained for the Tafo district is reliable and representative for 1996 because the farmers were selected from all the societies in the district and actual weights were taken. Though the data was for only one year, it is close to 0.16 % and confirms the observation in the questionnaire approach that mouldiness of beans is less than 0.2 % of the marketable beans. However, this data cannot be extrapolated to cover the whole country. In order to have an estimated % mouldy beans for the whole country, studies have to be conducted in representative districts to estimate mouldy beans in the Eastern Region and subsequently in representative number of regions in the whole country over a period of time. Similarly, the % mouldy beans at the buyers' level is reliable for the Tafo District for one year. More surveys covering representative districts will have to be done to obtain the % mouldy beans in the country.

The % mouldiness of 0.56 % obtained at the port for 1992/93 is representative of % mouldy beans in exportable cocoa in the country for the period because it is based on data collection and also most of the country's cocoa are exported through the Tema port. The % mouldiness of 0.69 % is a reliable average in exportable cocoa in the country it is an average of five years data. In the grading system, the maximum permissible quantity of mouldy beans for Grade 1 cocoa is 3 % that cocoa exported from Ghana is of a high quality. Oyeniran and Adejini (1974) who conducted a similar study on cocoa at the reported an average % mouldy beans of the exported cocoa as 0.4 % using the cut test method.

From the investigations, mouldiness did not seem to be a major season problem to the cocoa farmer but is a problem in the minor season. It did not seem

to pose a problem at the buyers' level or at the port. However, due to the objectionable nature and the effects mouldy beans have on the manufacture of chocolate and the storage of cocoa butter, it is important that it is kept under control and hence the need for further research into the causes and possible control of mouldiness in dried cocoa beans.

CHAPTER FOUR

4.0 DETECTION, ISOLATION AND IDENTIFICATION OF FUNGI CAUSING MOULDINESS IN DRIED COCOA BEANS

4.1 INTRODUCTION

One of the problems of dried cocoa beans during storage is mouldiness. The critical moisture content of dried beans is 8 % corresponding to an equilibrium relative humidity of 80 % (Scott, 1928; Theimer, 1958).

Fungi causing internal mouldiness of cocoa in Ghana were first documented by Bunting (1928) and Dade (1928) who identified six species which were four species of *Aspergillus*, a *Penicillium* sp. and a *Mucor* sp. Hughes (1952, 1953) and Piening (1962), also listed several fungi occurring on cocoa but did not specify which ones cause mouldiness of the beans. The only available report on work done was by Abitey (1982) who identified twenty-one species including twelve species of *Aspergillus*. In order to find a solution to the problem of mouldiness in cocoa, it is necessary that this area is re-studied and the information on the species of fungi updated. The objective of the present study is to detect, isolate and identify fungi present on cocoa beans.

4.2. MATERIALS AND METHODS

4.2.1 Selection of surface sterilising method and culture medium for isolation

Before isolating the fungi a surface sterilising method and culture medium were selected. Different surface sterilising methods were compared in order to select one for the study. Thirty-five dried cocoa beans were divided into seven groups

of five. Each group was dipped in one of the following solutions before being placed on solidified agar: (a) 70 % ethyl alcohol for 5 min (b) 70 % ethyl alcohol for 1 min; (c) 5 % sodium hypochlorite for 2 min (d) 5 % sodium hypochlorite for 1 min; (e) 10 % sodium hypochlorite for 2 min. (f) 10% sodium hypochlorite for 1 min and (g) a batch without surface sterilisation placed directly on solidified agar as a control. The beans were incubated at 28 ^{0}C for seven days, during which the fungal colonies appearing were counted and later subcultured on potato dextrose agar (PDA) until pure cultures were obtained.

In order to select a culture medium, three culture media namely PDA, water agar (WA) and moistened filter paper were compared. The testa of 30 dried cocoa beans were removed and dipped in 70 % alcohol for 1 min. A batch of ten sterilised beans were then placed into either PDA, WA or moistened filter paper. The cultures were incubated for 10 days under room temperature during which they were observed for fungal growth. Percentage of beans with fungal growth, growth of the fungal colonies and identity of fungi were recorded.

4.2.2 Isolation and identification of fungi

Fungal isolations were made from different parts of the cocoa beans. Each batch of 10 beans were given one of the following treatments and cultured singly:

(a) testa were removed from beans and cultured (b) beans without testa (cotyledons) (c) cotyledons surface-sterilised with 70 % ethyl alcohol for 1 min (d) whole beans not sterilised but placed directly on solidified WA. Beans were incubated and observed for 10 days for fungal growth before isolation. To facilitate identification of fungi the following were observed; the pattern of

growth, the colour and texture of the cultures. In order to identify sexual stages of fungi belonging to *Aspergillus* and *Penicillium* genera, subculturing was done at 6 mm intervals on Czapek Dox Agar in McCartney tubes and incubated at 28 °C (CAB, 1968; Staples, 1973). Pure cultures were kept on PDA slants. Fungi were identified using standard reference materials (Raper and Fennel, 1965; Raper and Thom, 1949; Smith, 1960; Barnett and Hunter, 1972).

4.3. RESULTS

4.3.1 Selection of surface sterilising method and culture medium for isolation

When the beans were dipped in 70 % ethyl alcohol for 5 min none of them showed any fungal growth (Table 10). Those dipped for only 1 min had fungal growth after 4 days. There were seven distinct colonies which could be counted initially. All the concentrations of sodium hypochlorite at various times of dipping allowed the inner spores to grow, but growth was restricted. The medium surrounding the bean became brownish and fungal growth could not extend to the bean surface of the medium. Colonies were not as distinct as on the alcohol treated beans. Sodium hypochlorite at 5 % for 1 min gave seven colonies, and for 2 min gave five. At 10 % for 1 min seven colonies were counted and at 2 %, seventeen colonies were present. Out of the treatments, sterilising in 70 % ethyl alcohol for 1 min was found to give the best results and this was used in subsequent experiments.

When the unsterilised beans were cultured on the three different media, the beans on the PDA had 90 % infection on the 3rd day, and 100 % by the 4th day (Plate 17; Fig. 5). Those on Water agar showed 30 % infection on the 3rd day, 95

% by the 4th day and 100 % by the 5th day. On the 3rd day the beans on the moist filter paper did not show any growth, but on the 4th day the beans had 55 % infection and 80 % infection by the 6th day. 100 % infection was recorded on the moist filter paper after 10 days. Fungal

growth on the beans sterilised with 70 % alcohol started later than on the unsterilised beans. On the 4^{th} day, beans on PDA had 50% infection. Beans on Water Agar had 35% infection and those on filter paper showed no infection. By the 6th day, beans on PDA had 100% infection. Those on Water Agar had 90% and beans on filter paper had 5% infection. By the 12th day, the beans on filter paper had 30% infection. In general, the beans cultured on PDA showed larger fungal colonies which grew faster than the beans on the other media and soon covered the beans making it difficult to see the slower fungi.

4.3.3 Isolation and identification of fungi

Fungi encountered on different parts of the beans consisted of both external and internal ones The unsterilised beans had the highest number of fungi (Table 11). Surface-sterilised beans had 48 species (Table 11), all being internal ones. The testa yielded only fungi on the surface of the beans. Ten fungal species appeared as external fungi only (Table 13). A total of fifty eight (58) species of fungi were isolated; twenty-nine (29) being recorded for the first time (Table 12). Twenty six (26) belong to *Aspergillus,* five to *Penicillium,* eight to *Fusarium*, in addition to nineteen (19) other species (Tables 12 & 13). One of the isolates could not be fully identified. The descriptions of the different fungal species which were used as criteria for identification are as follows.

Table 10: Detection of fungi from cocoa beans surface-sterilised by different methods

	Number of colonies							
	Unsterilised	70% Alcohol		$NaOCl_3$				
Fungal spp		**1 min**	**5 min**	**5% for 1 min**	**5% for 2 min**	**10% for 1 min**	**10% for 2 min**	**Total colonies**
A. flavus	2	3	0	2	1	1	4	13
A. tamarii	2	1	0	2	1	1	4	11
A. glaucus	2	1	0	1	1	0	1	6
A. niger	1	0	0	0	1	0	3	5
A. ochraceus	0	1	0	1	0	0	1	3
P. phoeniceum	3	0	0	0	0	0	1	4
P. cyclopium	0	0	0	0	0	1	1	2
Mucor	1	0	0	1	0	2	1	5
Rhizopus	1	0	0	0	0	1	0	2
Verticillium	1	0	0	0	0	0	0	1
C. lunata	1	0	0	0	0	0	0	1
Absidia	1	0	0	0	1	1	1	4
Cladosporium	1	0	0	0	0	0	0	1
Saccharomyces	1	0	0	0	0	0	0	1
Phoma	1	0	0	0	0	0	0	1
Fusarium	0	1	0	0	0	0	0	1
Total no. of colonies/ treatment	18	7	0	7	5	7	17	61

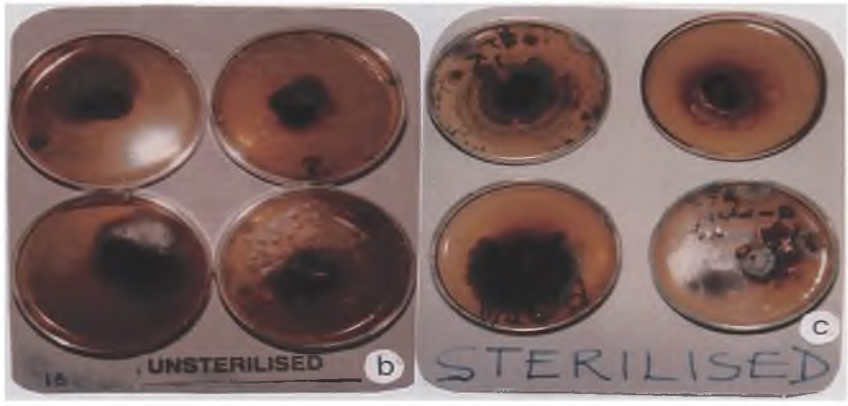

Plate 17. Fungus growth on the cocoa beans incubated on different media for 4 days.

A - Filter paper - fungal growth just starting on beans.

B - Water Agar - fungal growth cover some of the beans.

C - Potato Dextrose Agar - fungal growth cover all beans.

Plate 18. Fungal growth on cocoa beans 8 days after incubation on Water agar.

b) Unsterilised beans showing growth from external and internal fungi.
c) Surface-sterilised beans showing growth generating from internal fungi.

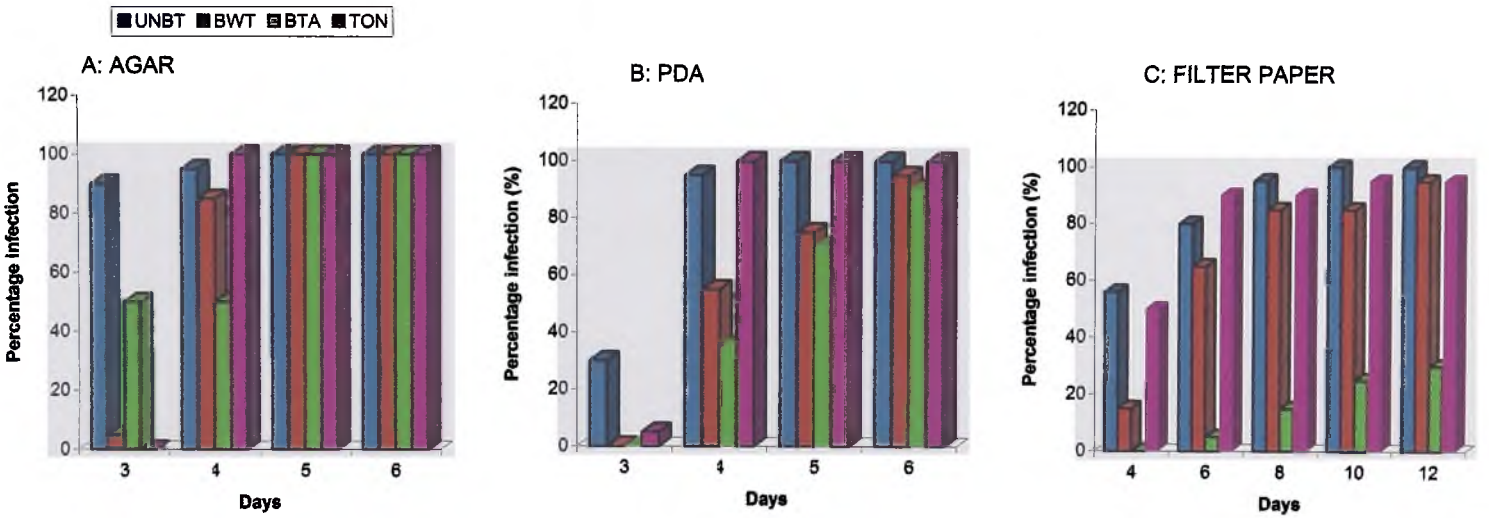

Figure 5. Percentage infection of cocoa beans plated on different media after different treatments.
UNBT : unsterilised beans with testa; BWT: Beans with testa removed;
BTA : Beans with testa treated with alcohol; TON: testa only

Table 11. Fungi Occurring on Dried Cocoa Beans after Different Treatments

Fungi	Unsterilised bean	Alcohol sterilised	Testa Removed (Unsterilised)	Testa (Unsterilised)
A. penicilloides	+	+	+	+
A. amstelodami	+	+	+	+
A. chivalieri	+	+	+	+
A. itaconicus	+	+	+	+
A. nidulans	+	+	+	+
A. flavus	+	+	+	+
A. oryzae	+	+	+	+
A. fumigatus	+	+	+	+
A. wentii	+	+	+	+
A. tamarii	+	+	+	+
A. versicolor	+	+	+	+
A. fischeri	+	+	+	+
A. ruber	+	+	+	+
A. echinulatus	+	+	+	+
A. pseudoglaucus	+	+	+	+
A. phoenicis	+	+	+	+
A. niger	+	+	+	+
A. glaucus	+	+	+	+
A. terreus	+	+	+	+
Aspergillus sp.	+	+	+	+
Penicillium variable	+	+	+	+
P. cyclopium	+	+	+	0
P. chrysogenum	+	0	0	+
P. phoeniceum	+	+	+	+
Fusarium nivale	+	0	0	+
F. monilforme	+	0	0	+
F. oxysporum	+	0	+	+
Fusarium lateritium	+	0	+	+
F. rigidiuscula	+	0	0	+
F. solani	+	+	+	+
Fusarium sp.	+	0	0	0
Fusarium roseum	+	+	0	+
Absidia corymbifera	+	+	+	+
Rhizoctonia sp.	+	0	+	+
Sporendonema sp.	+	0	+	0
Paecilomyces varioti	+	+	+	+
Geotrichum candidum	+	+	+	+
Pullularia pullulans	+	0	0	+
Rhizopus nigricans	+	+	+	+
Nigrospora sp..	O	0	+	+
Syncephalastrum sp	+	0	0	0

Table 11. (contd.): Fungi occurring on Dried Cocoa Beans after Different Treatments

fungi	unsterilised bean	alcohol sterilised	testa removed unsterilised	testa
A. parasiticus	+	+	0	+
A. sydowi	+	+	0	+
A. lutescens	+	+	+	0
A. sulphureus	+	0	+	0
A. japonicus	+	0	+	+
Scopulariopsis	+	0	0	0
P. citrinum	+	+	0	+
C. lunata	+	0	+	+
Chaetomium sp.	+	+	0	+
C. globulosum.	+	+	0	+
Byssochlamys fulva	+	+	+	0
Mucor sp.	+	0	0	+
Mucor haemalis	+	0	0	+
Monascus purpureus	+	0	0	+

\+ *means present*
0 means absent

Table 12: Internal fungi identified on dried cocoa beans.

**Absidia corymbifera.*
**A. amstelodami.*
A. chivalieri.
**A. echinulatus*
A. flavus.
**A. fischeri*
A. fumigatus
**A. itaconicus.*
**A. lutescens.*
**A. japonicus*
A. nidulans.
A. niger.
Aspergillus sp.
**A. oryzae*
**A. parasiticus*
A. penicilloidess
**A. phoenicis.*
**A. proliferans*
**A. pseudo-glaucus*
A. ruber
**A. sulphureus*
A. sydowi
A. tamari.
A. terreus
A. ustus.
**A. versicolor*
A. wentii

**Byssochlamys fulva*
**Chaetomium globulosum*
**Curvularia lunata.*
**F. lateritium*
**F. moniliforme.*
**F. oxysporum*
**F. rigidiuscula.*
**F. roseum*
F. solani.
Geotrichum candidum.
**M haemalis*

Mucor sp.
**Paecilomyces varioti*
Penicillium citrinum.

** P. cyclopium.*
**P. phoeniceum.*

**P. variable*
**Phoma*
Rhizopus nigricans.
**Syncephalastrum racemosum*

** Fungi being recorded first time on cocoa in Ghana*

Table 13 : Fungi occurring on the surface of the beans

Chaetomium sp.	*Fusarium* sp,
Fusarium nivale	*Monascus purpureus*
Nigrospora sp.	*P. chrysogenum*
Pullularia pullulans	*Rhizoctonia* sp.
Sporendonema sp.	*Scopulariopsis* sp.

Absidia corymbifera (Cohn) Sacc

The culture grows rapidly appearing white, then grey. The sporangia are small and rise from stolons. The columella of the sporangia is funnel-shaped. Conidiophores are often in whorls. The spores are smooth and hyaline. (Plate 19).

Aspergillus(Eurotium) amstelodami (Margin) Thom & Church

Culture is deep green changes to deep yellow from the middle. It produces deep yellow perithecia. The reverse of the culture is colourless. Ascospores are rough with prominent furrows. Conidiophores have single phialides. (Plate 20).

Aspergillus candidus Link

Culture is white and grainy. The mycelia are white. The heads are small. The conidiophores are smooth walled with small vesicle and metulae covering about 1/2 or 2/3 of the surface with a second row of phialides. Some of them have single phialides. The conidia are round. (Plate 21).

Aspergillus chivalieri (Mangin) Thom & Church

Growth is bluish green changing to cream. Culture may be wrinkled or slightly fluffy in the centre. Reverse is orange red. Large yellow perithecia are produced. Ascospores are smooth-walled with prominent crest appearing like a pulley. Conidiophores have single phialides. Conidia are rough. (Plate 22).

Aspergillus echinulatus

Cultures growing slowly with a mixture of bluish-green conidial heads and orange-red perithecia with reverse pale to deep red. Perithecia yellow embedded in red hyphae growing close to the surface of the medium. Ascospores have broad furrow with irregular edges. Conidiophores are long broadening upward and have phialides. Conidia are elliptical and very rough. (Plate 23).

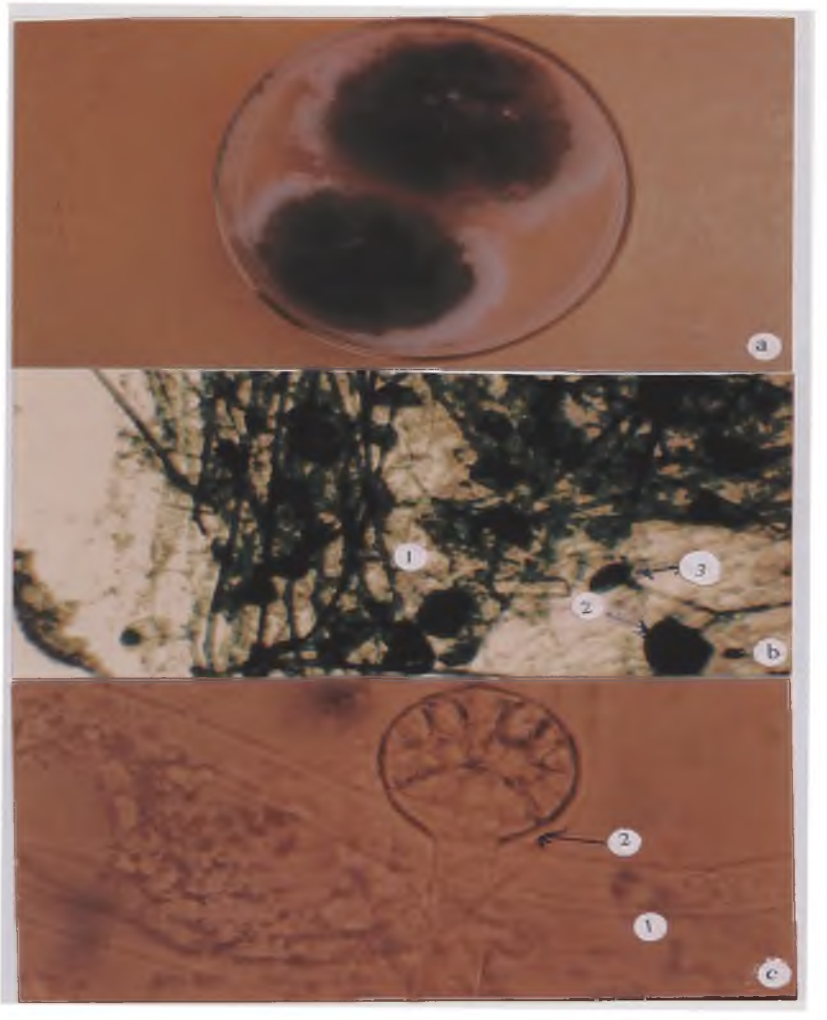

Plate 19. *Absidia corymbifera* (Cohn) Sacc.

a) Eight day old grey culture on PDA at room temperature.

b) Portion of culture showing stolons (1) and sporangia (2) and funnel-shaped (3) based x 400.

c) A single sporangium showing a characteristic shape and funnel-shaped columella of sporangium (2) and hyaline spores (1) x 800.

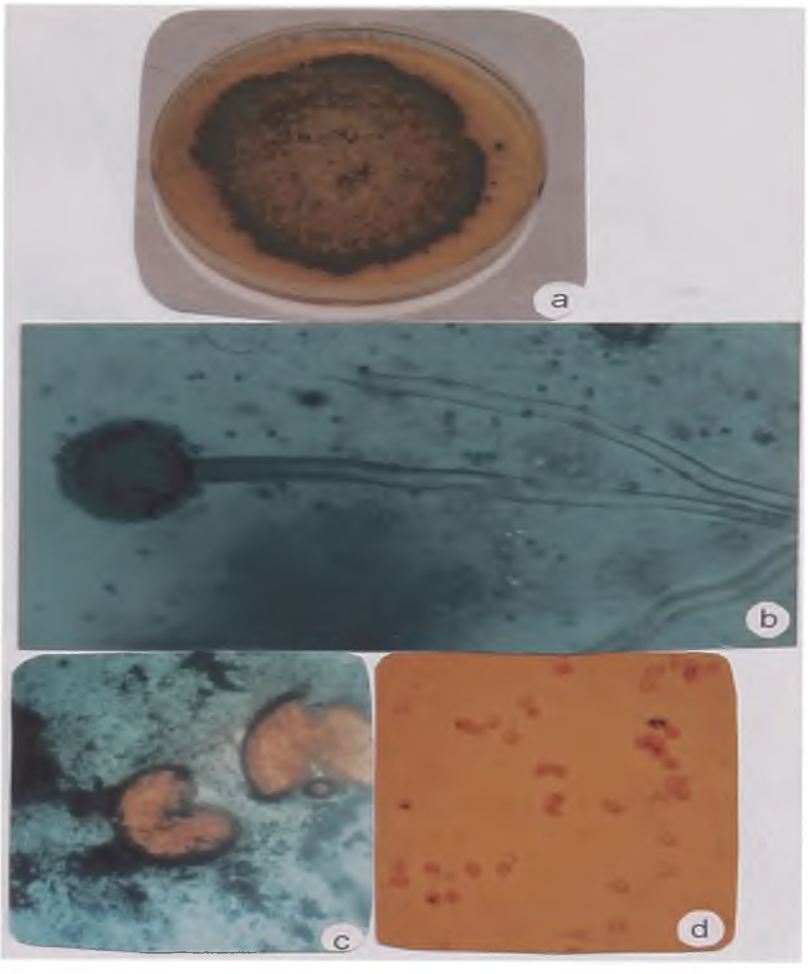

Plate 20. *Aspergillus amstelodami* (Margin) Thom & Church.

a) Two weeks old deep olive-green and grainy colony on Czapek Dox Agar colour changes to bright yellow as perithecia develops.

b) Conidiophore ending in a vesicle covered with phialides and globose conidia x 400.

c) Bright yellow open perithecia x 200.

d) Ascospores x 200.

Plate 21. *A. candidus* Link

a) White grainy culture on Czapek Dox Agar

b) Smooth-walled conidiophore ending in double phialides and globose conidia

1 round conidia
2 double phialides
3 conidiophore

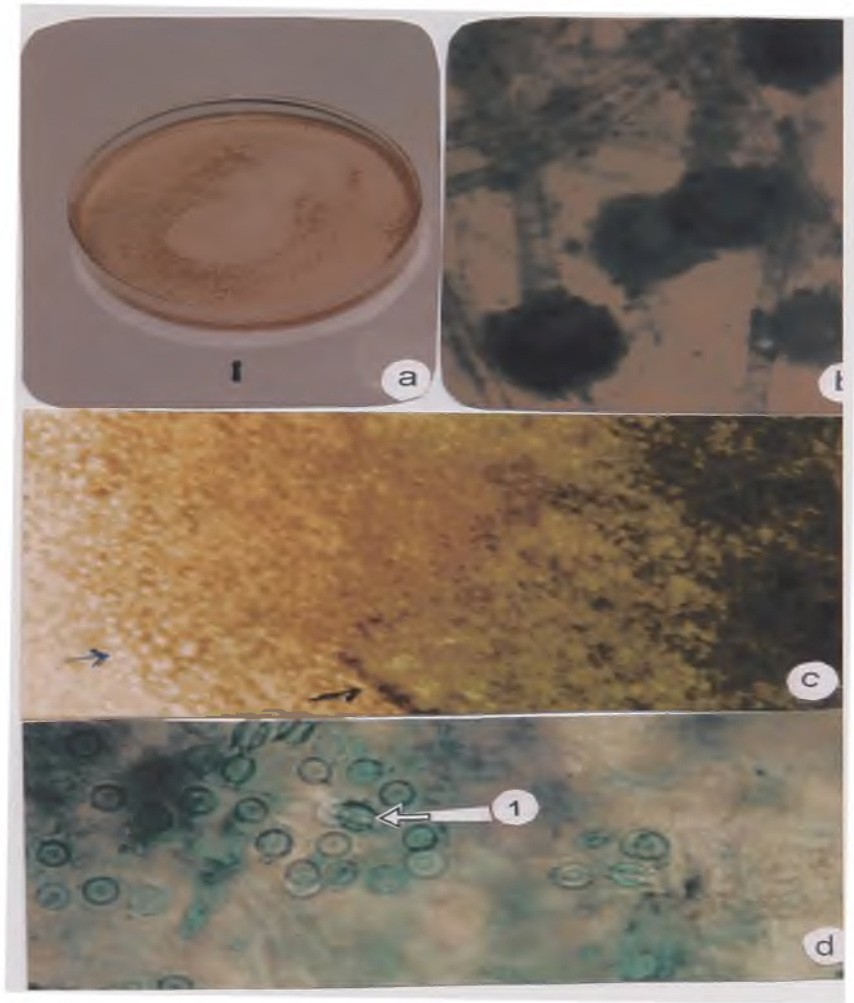

Plate 22. *Aspergillus chivalieri* (Mangin) Thom & Church.

a) Two weeks old cream colony (mostly perithecia) on Czapek Dox Agar.

b) Bluish-green conidial head. Conidiophore ending phialides

c) Yellow perithecia releasing yellow ascospores:

1 mass of ascopores
2 part of open perithecia

d) Ascospores showing deep pulley type of furrow.

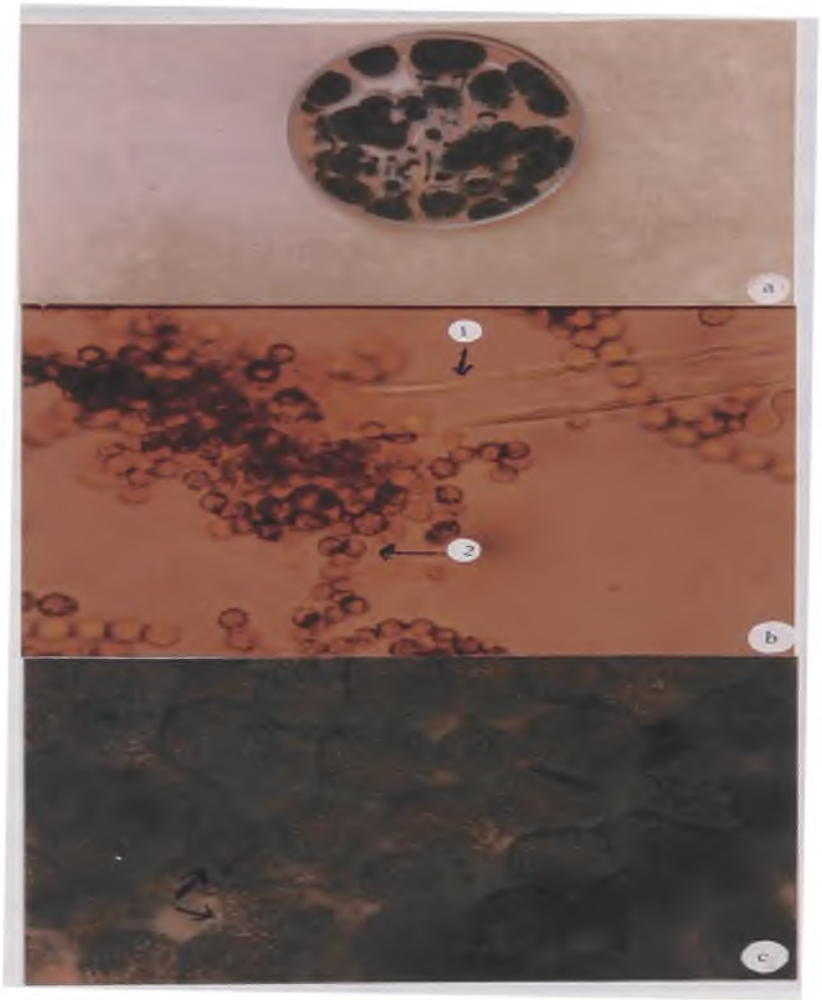

Plate 23. *Aspergillus echinulatus* Thom & Church

a) Eight days old culture in Czapek Dox Agar.

b) Conidiosphore (1) with phialides and chains of large spherical and conidia (2).

c) Perithecia (arrowed).

Aspergillus fischeri Wehmer

Colony is bluish – green; later it develops a grayish-pink colour in the medium. The vesicle is flask-shaped with phialides covering the upper portion . Culture is similar to *Aspergillus fumigatus* but produces light coloured perithecia with rough ascospores. (Plate 24).

Aspergillus flavus Link ex Fries

Culture starts as white and floccose, changing to green. Conidophore is rough and vesicle subglobose. Phialides are double. Conidia are smooth. Sclerotia are present. (Plate 25).

Aspergillus fumgatus Fresenius

Culture is bluish-green velvety with the reverse dark. The heads appear columnar with the conidia sticking together like beads. The vesicle is flask-shaped and covered with phialides on the upper surface. Conidia are rough and bluish. (Plate 26).

Aspergillus itaconicus Kinoshita

Culture is in bluish green shade and spreads. The vesicle is globose and fertile over the entire surface with single phialides. Conidiophores are long with rough conidia. No perithecia is found. (Plate 27).

Aspergillus japonicus Saito

The culture grows rapidly and changes from white to light brown and then to dark brown. The conidial heads are globose and the phialides cover the entire surface of the vesicle. The conidia are brown round and rough. Sclerotia are present. (Plate 28).

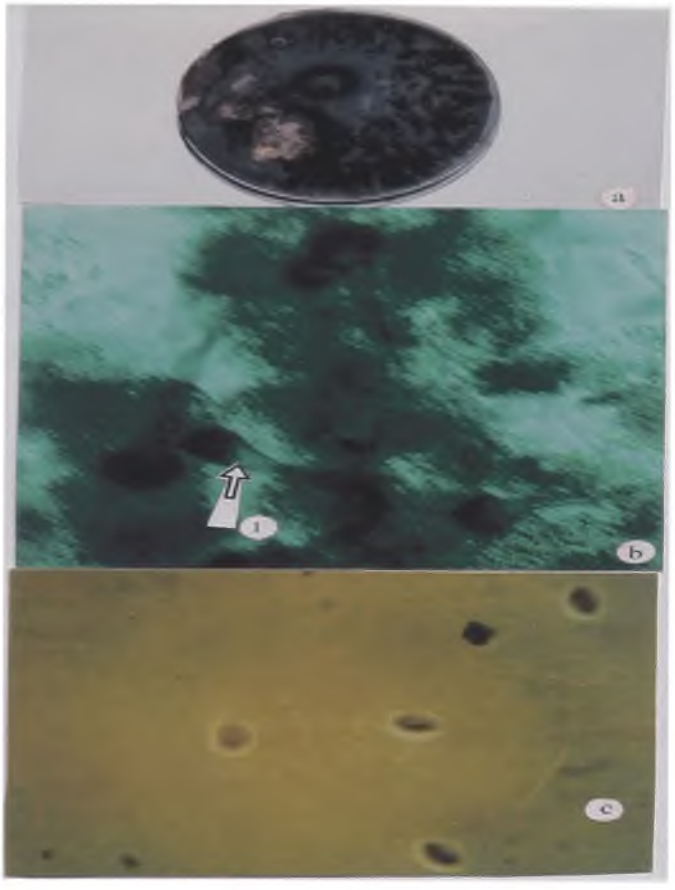

Plate 24. *Aspergillus fischeri* Wehmer

a) Two weeks colony showing grayish –pink patches as perithecia develop.

b) Columnar heads showing typical flask-shaped vesicle (1) bearing single phialides with masses of conidia (black part) x 400.

c) Ascospores.

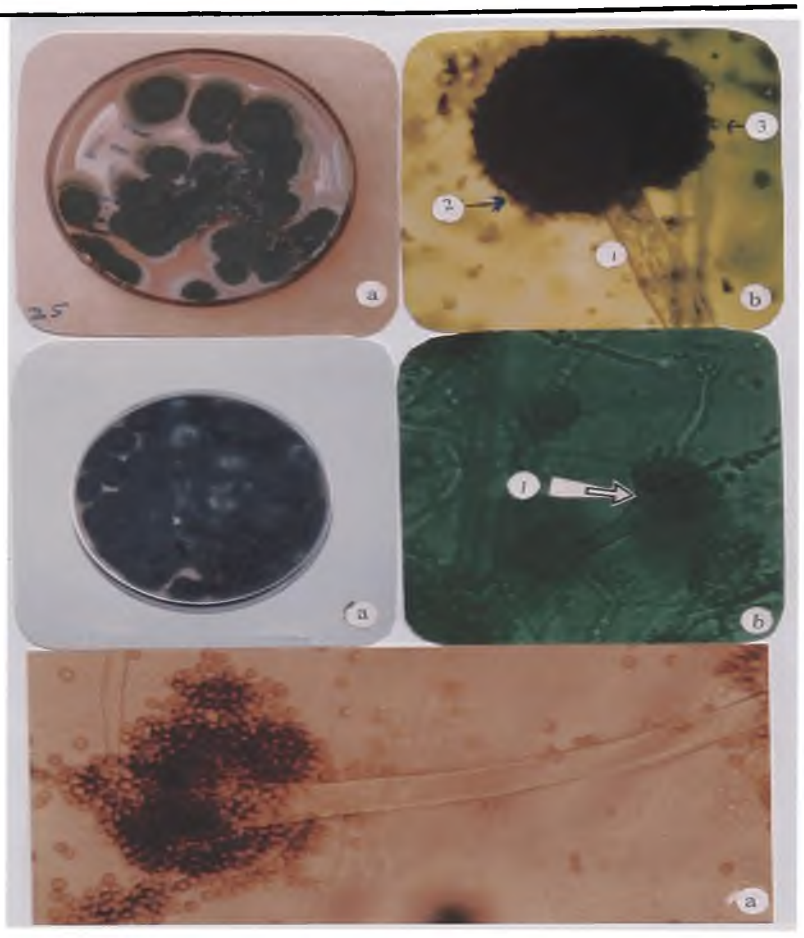

Plate 25. *Aspergillus flavus* Link

a) Eight days okd culture on Czapek Dox Agar. Culture is grainy.
b) Pitted conidiophore (1) ending in globose vesicle (2) with entire surface in covered by phialides bearing smooth conidia (3) x 300.

Plate 26. *Aspergillus fumigagus* Fresenius

a) Eight days bluish-green, velvet colony on Czapek Dox Agar
b) Conidiophores with typical club- shaped vesicle x 400.

Plate 27. *Aspergillus itaconicus* Konishia

a) Long smooth conidiophore with large, globose vesicle covered With chain of round rough conidia.

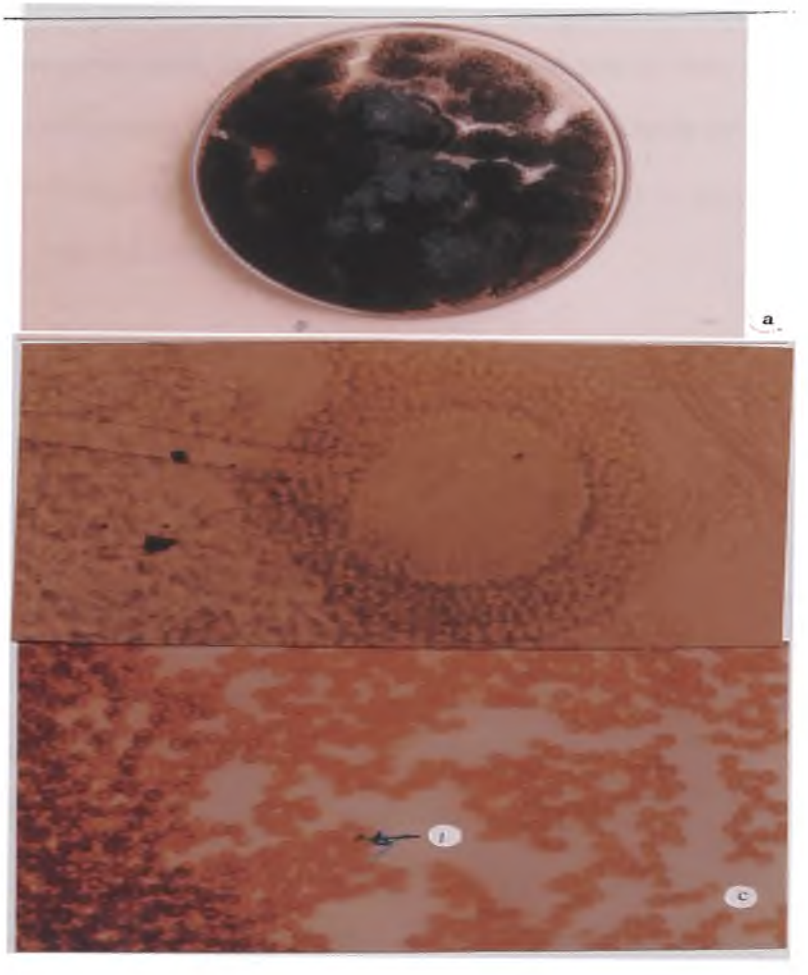

Plate 28. *Aspergillus japonicus* Saito.

a) Two week old brown and grainy culture on Czapek Dox Agar.

b) Long brown conidiophore (1) bearing a globose vesicle (2) covered with chains of conidia.

c) Masses of brown rough conidia of the fungus.

Aspergillus lutescens Brainier

Culture grows rapidly and appears grainy. The color starts as white, changing to cream and greenish, then later to light brown color. Conidial heads are radiate with distinct thick phialides separated from each other. The light brown spores are round, large, rough and string together like beads (Plate 29).

Aspergillus nidulans (Eidam) Winter

Culture is green which later develops cream patches and the reverse is red. The conidiophore is smooth-walled and slender. The vesicle is small and fertile over the upper half. Phialides are double with rough conidia. Perithecia are reddish surrounded by large round cells called hulle cells. Ascospores are red, smooth-walled with furrows (Plate 30).

Aspergillus niger van Tieghem

Culture appears white, yellowish and then black as heads develop. The heads are biseriate, large, with bige and globose vesicle fertile over the entire surface. The conidiophore is smooth and colourless and has large, globose and rough conidia (Plate 31).

Aspergillus sp.

Culture is orange, grainy, and spreads rapidly. Heads are big and conidiophores long. The conidiophore shows a shade of yellow and is pitted. The vesicles are globose and fertile over the whole surface. Philiades are double with the primary ones large and septate. Conidia are smooth and large. Sclerotia are present the fungus looks very much like *A.ochraceus* (Plate 32).

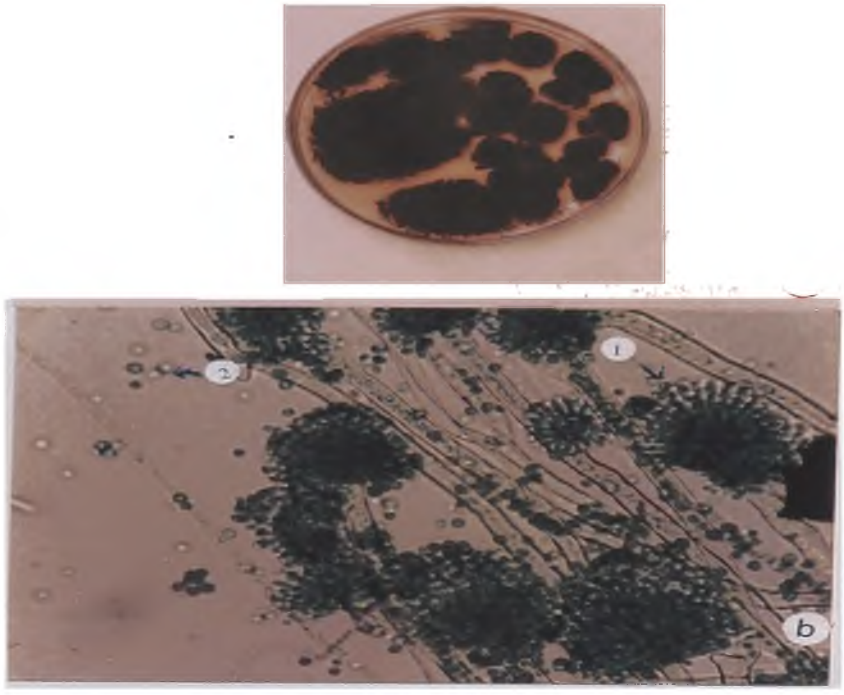

Plate 29. *Aspergillus lutescens* Bainier

a) Three week-old brown and grainy culture.

b) Radiate heads with distinct thick and distinct metulae and phialides (1) and globose, large conidia (2) x 400.

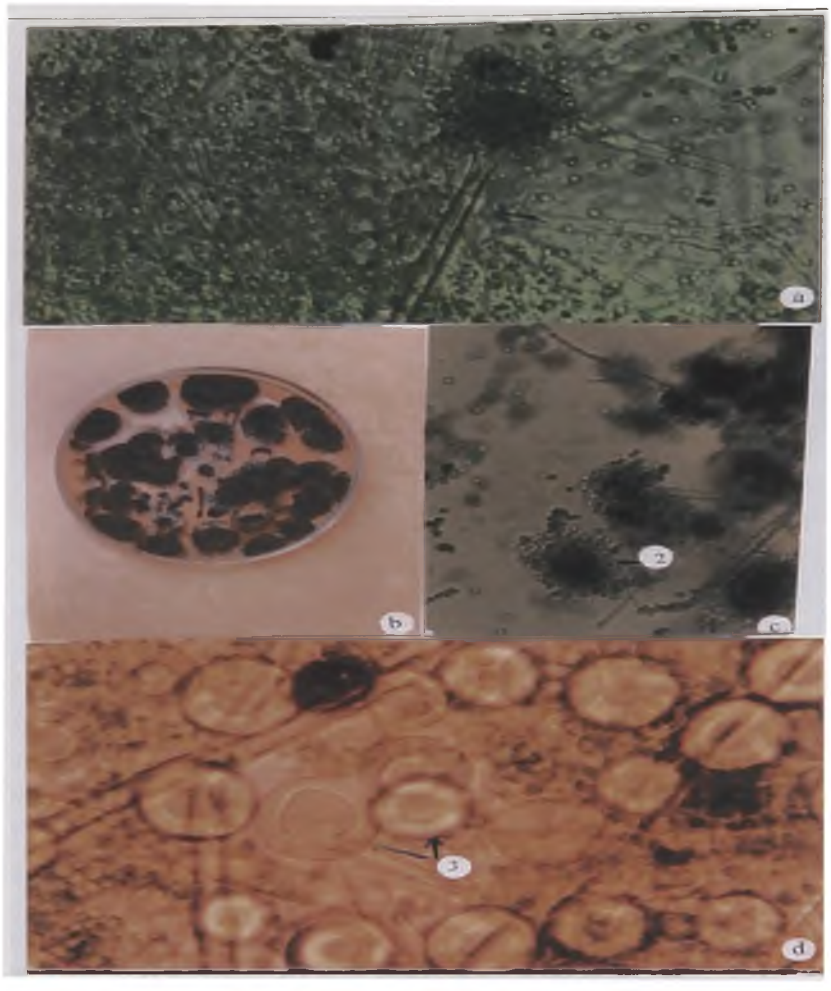

Plate 30. *Aspergillus nidulans* (Eidam) Winter

a) Smooth-walled conidiophore (arrowed) bearing vesicle with double phialides over the upper half x 400.

b) Two week old green and grainy culture on Czapek Dox Agar.

c) Perthecia surrounded by hulle cells (2) x 400.

d) View of slide under microscope showing globose hulle cells (3) x 300.

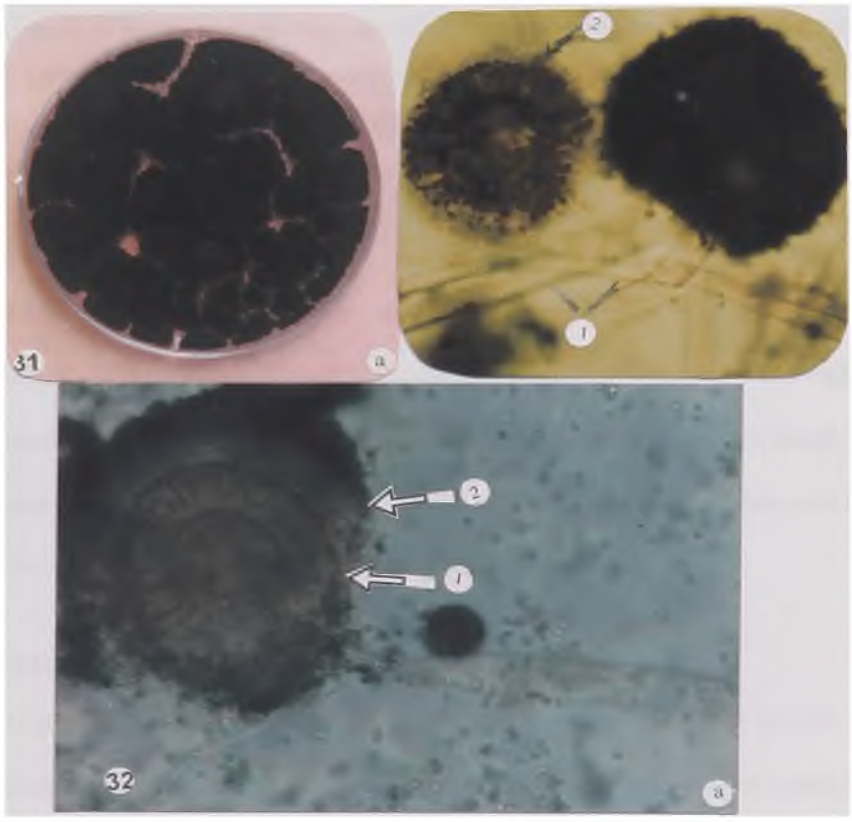

Plate 31. *Aspergillus niger* van Tieghem

a) Two week old black and grainy culture on Czepek Dox Agar.

b) Smooth-walled conidiophores (1) bearing black heads, and globose vesicle (2) with double phialides x 400.

Plate 32. *Aspergillus* sp.

Yellowish pitted conidiophore. Large orange head comprising a globose vesicle with long and septate metulae (1) and (2) phialides resembling *A. ochraceus*.

Aspergillus oryzae Cohn

Growth is rapid. Culture starts as white, changes to yellow, then to light greenish yellow and then to brown. The conidial heads are big, globose with double phialides conidiophore is long and rough (>800 μ). The conidia are rough. Sclerotia are dark and visible to the naked eye (Plate 33).

Aspergillus parasiticus Speare

Culture grows rapidly and is deep yellowish-green and appears grainy. The heads which are produced abundantly are radiate. The conidiophores are rough and about 400 μ long. Both single and double phialides are present in the heads. The conidia formed are green and rough. No perithecia or sclerotia were seen (Plate 34).

Aspergillus penicilloides Spejazzaric

Culture is bluish-green with wide margin with the centre becoming greyish. The surface is velvety and the growth is irregular. Conidial heads resemble a penicillium heads but with well defined and small vesicles. The conidiophores are long and slender and colourless. They have single phialides which are long with long chains of round, rough conidia. The foot cell is almost at right angle with the conidiophore (Plate 35).

Aspergillus phoenicis (Corda) Thom

Culture changes from white to black. It has numerous black spores and grows like a Mat on the surface of the medium. It is in the *A. niger* group. The pruning phialides are larger than secondary ones and have small conidia (Plate 36).

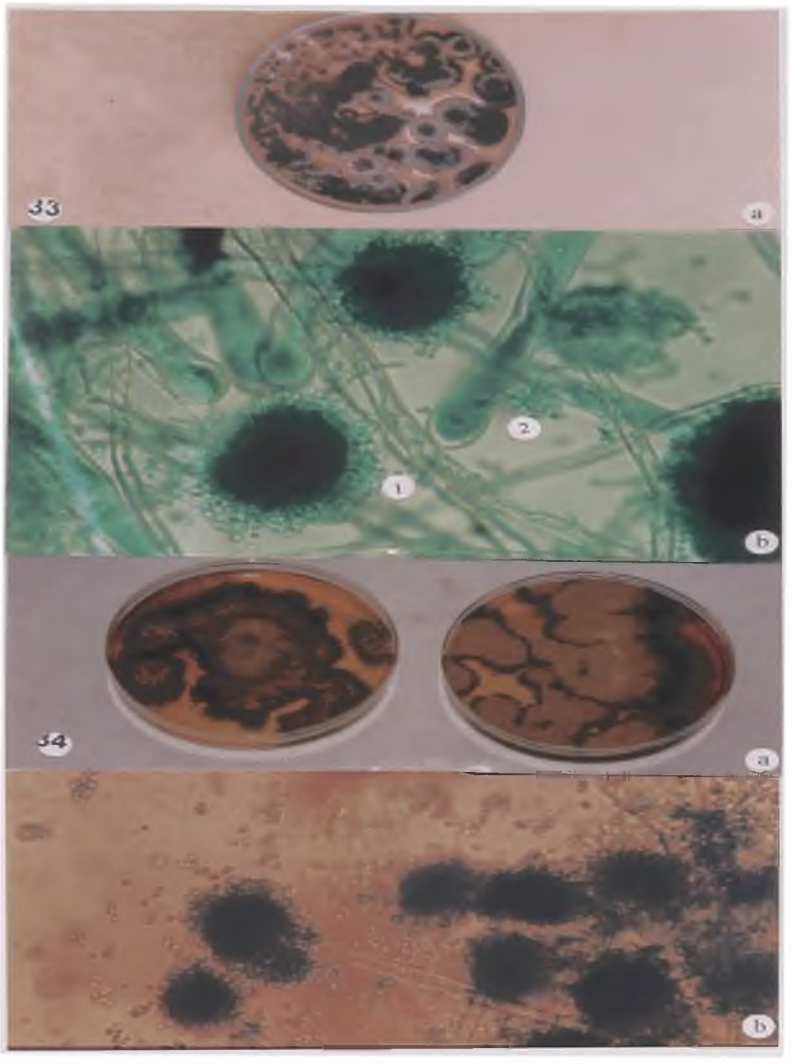

Plate 33. *Aspergillus oryzae* Cohn

a) Eight day greenish yellow with culture on Czapek Dox Agar.
b) Slide showing (2) developing conidiophore (1) globose heads x 400.

Plate 34. *Aspergillus parasiticus* Speare

a) Yellowish-green grainy culture on Czapek Dox Agar.
b) Rough conidiophore and globose heads.

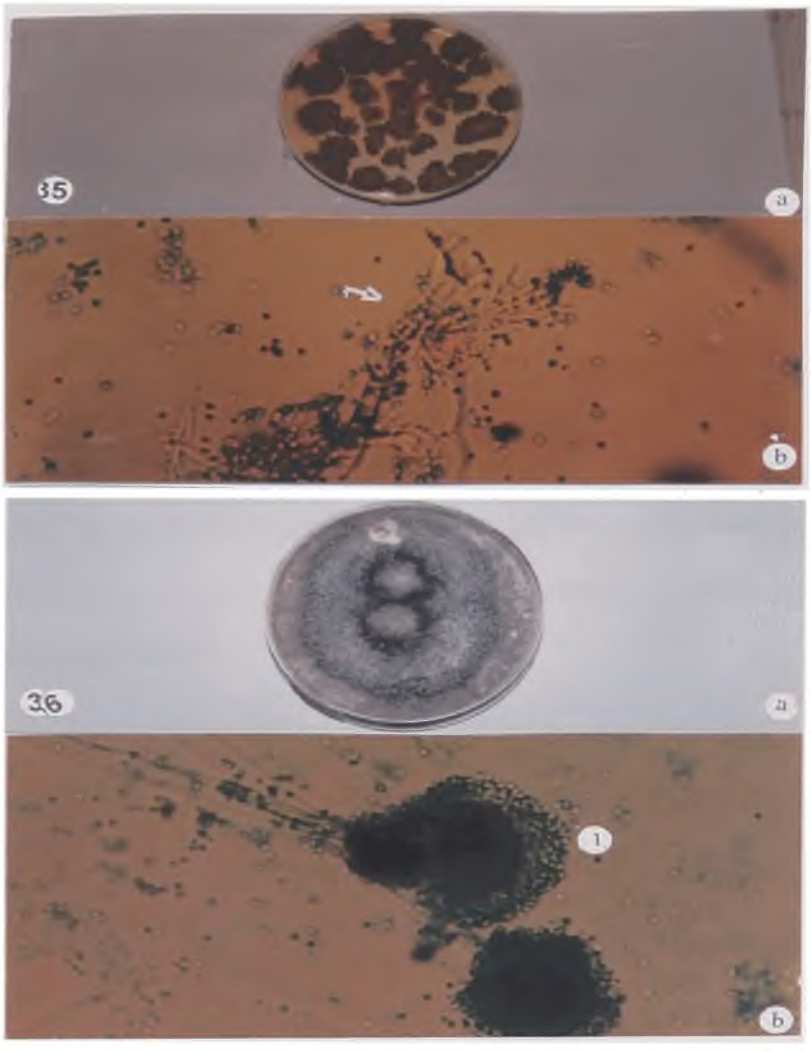

Plate 35 *Aspergillus penicilloides* Spejazzaaric

a) Two week old culture on Czapek Dox Agar.
b) Heads showing long "single phialide (arrowed).

Plate 36. *Aspergillus phoenicis* (Corda) Thom.

a) Eight day-old "mat-like" cultlure changing from white to black on Czapek Don Agar.
b) Black head on smooth-walled conidiophore. The metulae are longer than the phialides bearing small conidia x 400.

A*spergillus sulphureus* Fresenius

The culture grows rapidly changing from white to yellow then to yellowish orange. The conidial heads are globose with the entire surface covered with phialides which are double. the comidiophore appears yellow. It is in the *A. ochraceus* group (Plate 40).

Aspergillus sydowi Thom and Church

The culture consists of small round colonies resembling *Penicillium.* Cultures are velvety, bluish-green; reverse is reddish. The conidial heads are small. The conidiophore is slender and smooth, globose and has double phialides. Small vesicle may have a few of single or double phialides Conidia are globose and rough (Plate 41).

Aspergillus tamarii Kita

Culture changes from olive brown to brown and is grainy. The conidiophores are smooth and colourless. Vesicle is globose, fertile over the entire surface. The phialides are in one or two series. Conidia are thick walled, rough and elliptical (Plate 42).

Aspergillus terreus Thom

Colonies are small light brown with dark brown spore heads. The vesicle has double phialides on the upper half. Conidia are globose and large. The heads are small. The conidiophores are slender. There are colourless round cells (Hull cells) (Plate 43).

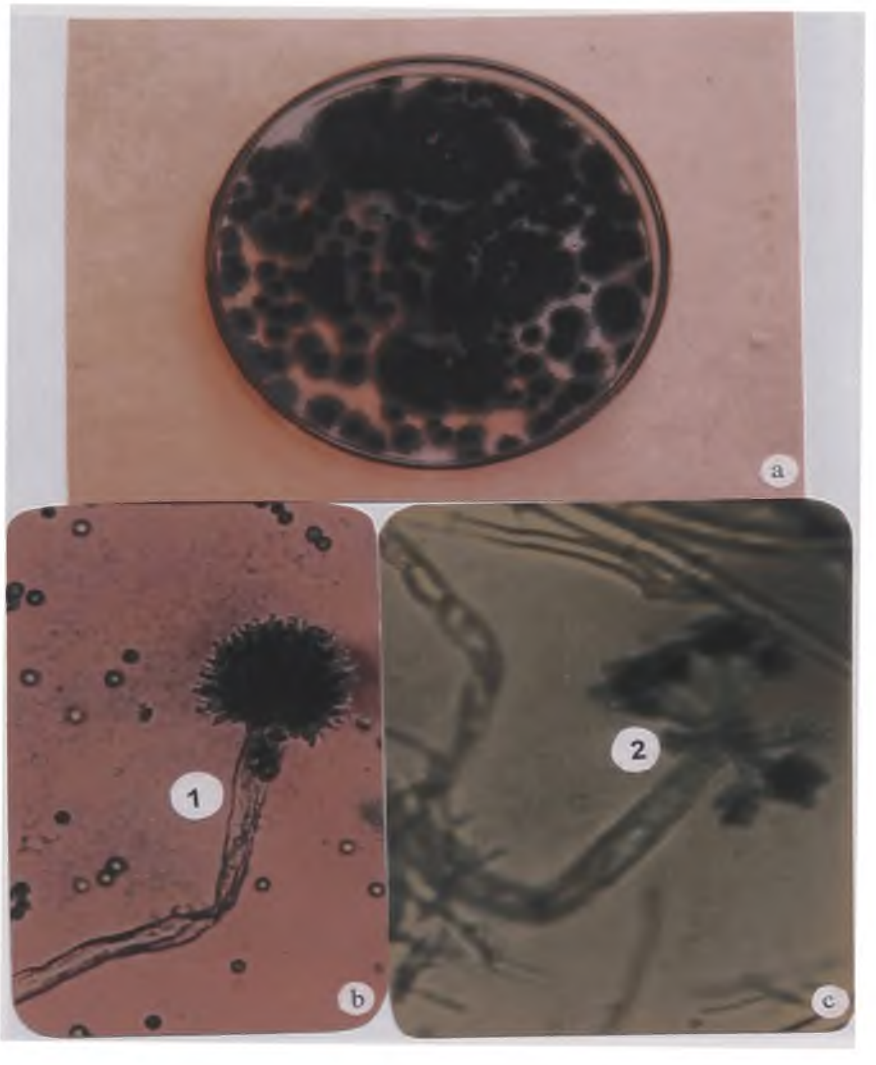

Plate 37. *Aspergillus proliferans* G. Smith.

a) Eight day old bluish green culture on Czapek Dox Agar.

b) Conidiophore with single head (1).

c) Conidiophore with secondary heads (2) x 400.

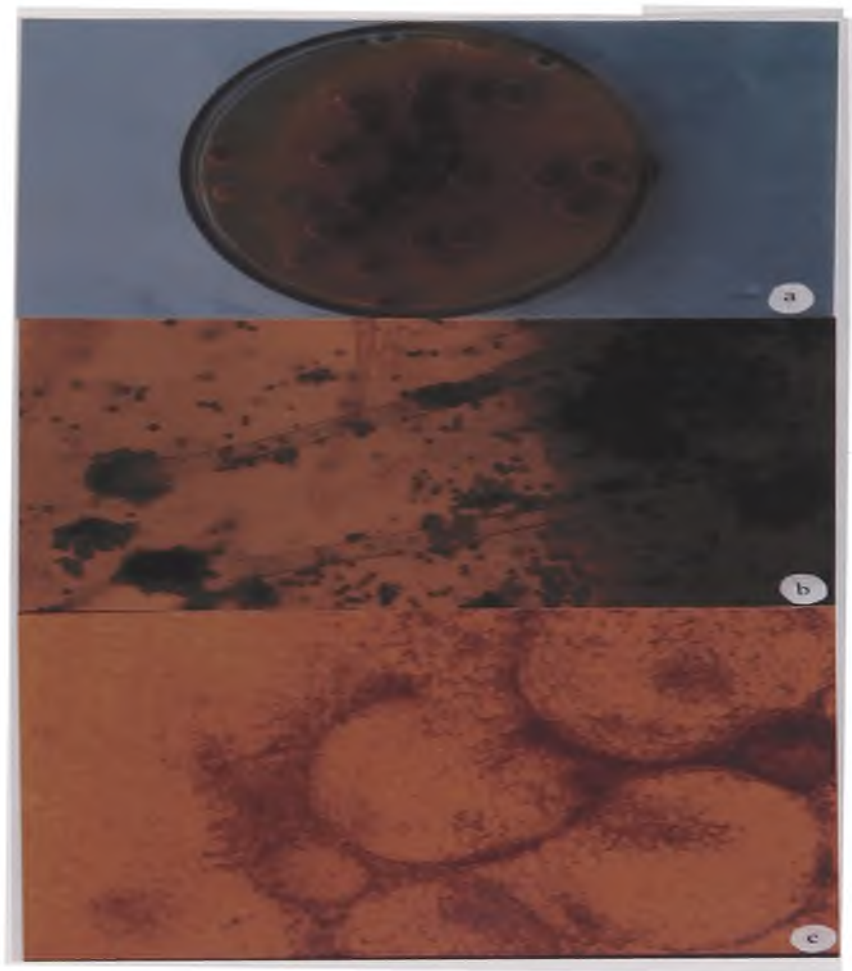

Plate 38. *Aspergillus pseudo-glaucus* Blotch.

a) Eight day old predominantly perithecial culture on Czapek Dox Agar showing small orange brown colonies.

b) Conidiophores ending in vesicle and single phialide and round conidia.

c) Orange spherical perithecia embedded in hyphae.

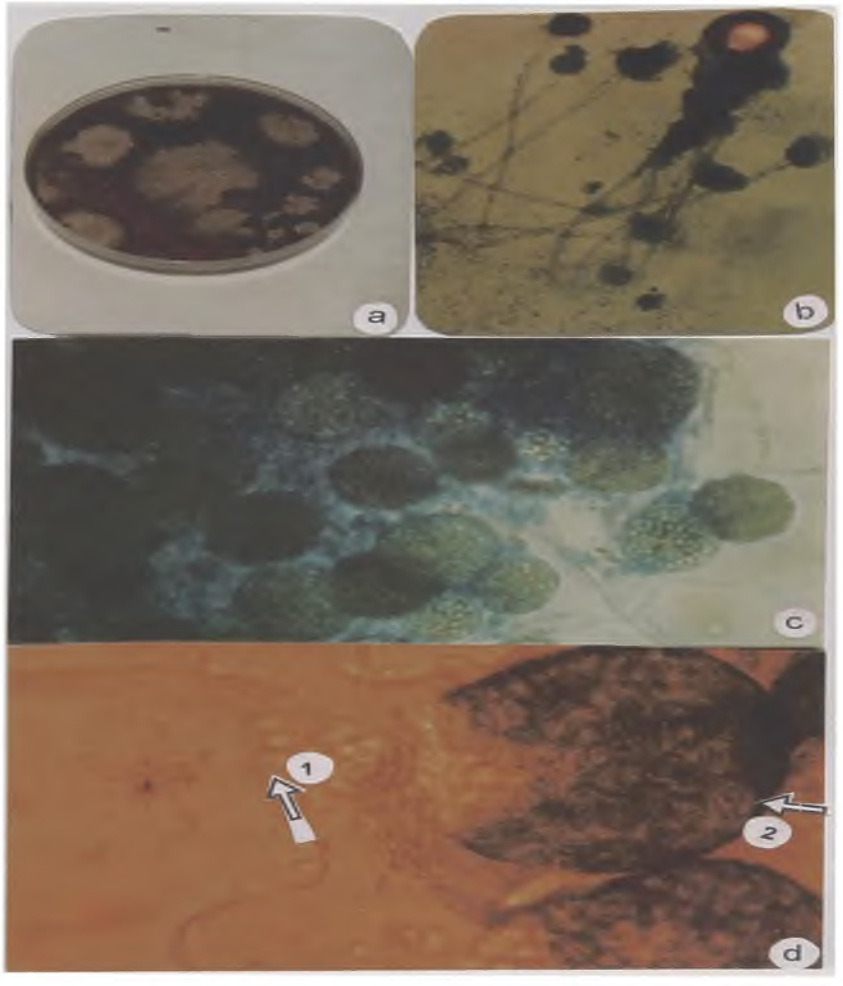

Plate 39. *Aspergillus rubber* Thom & Church.

a) Two week old reddish brown velvety culture on Czapek Dox Agar. Note red pigmentation of medium.

b) Conidiophores with radiate heads x 400.

c) Spherical yellow perithecia embedded in mycelia.

d) Broken perithecia (2) letting out asci containing smooth ascospores (1).

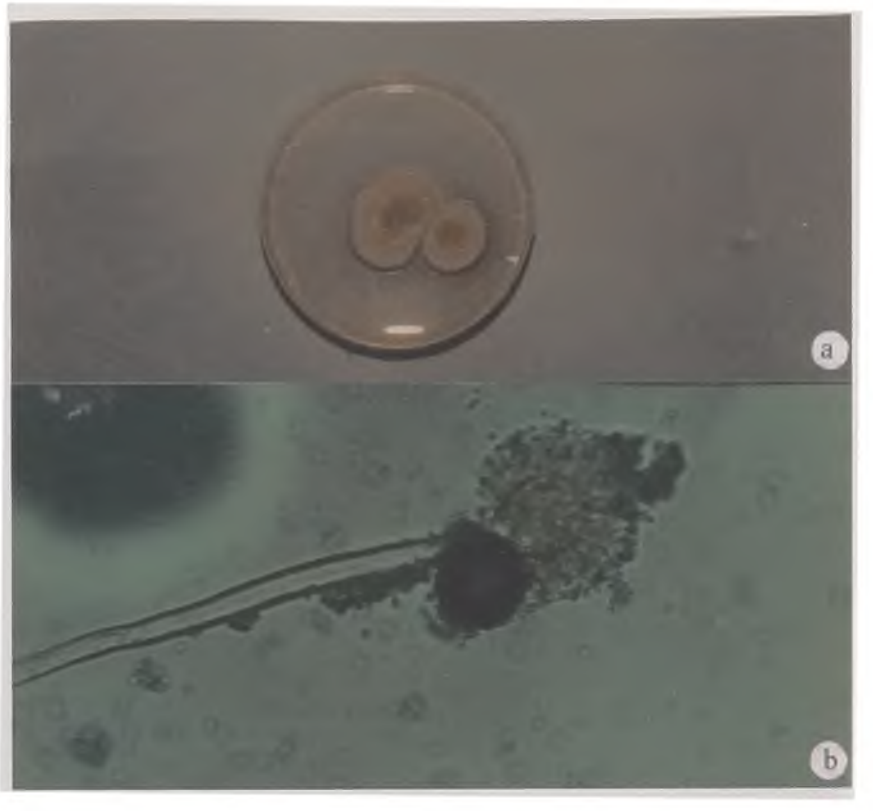

Plate 40. *Aspergillus sulphureus* Fresenius.

a) Eight day old yellow to orange culture on Czapek Dox Agar.

b) Conidiophore with smooth yellow wall carrying a yellowish head and double phialides.

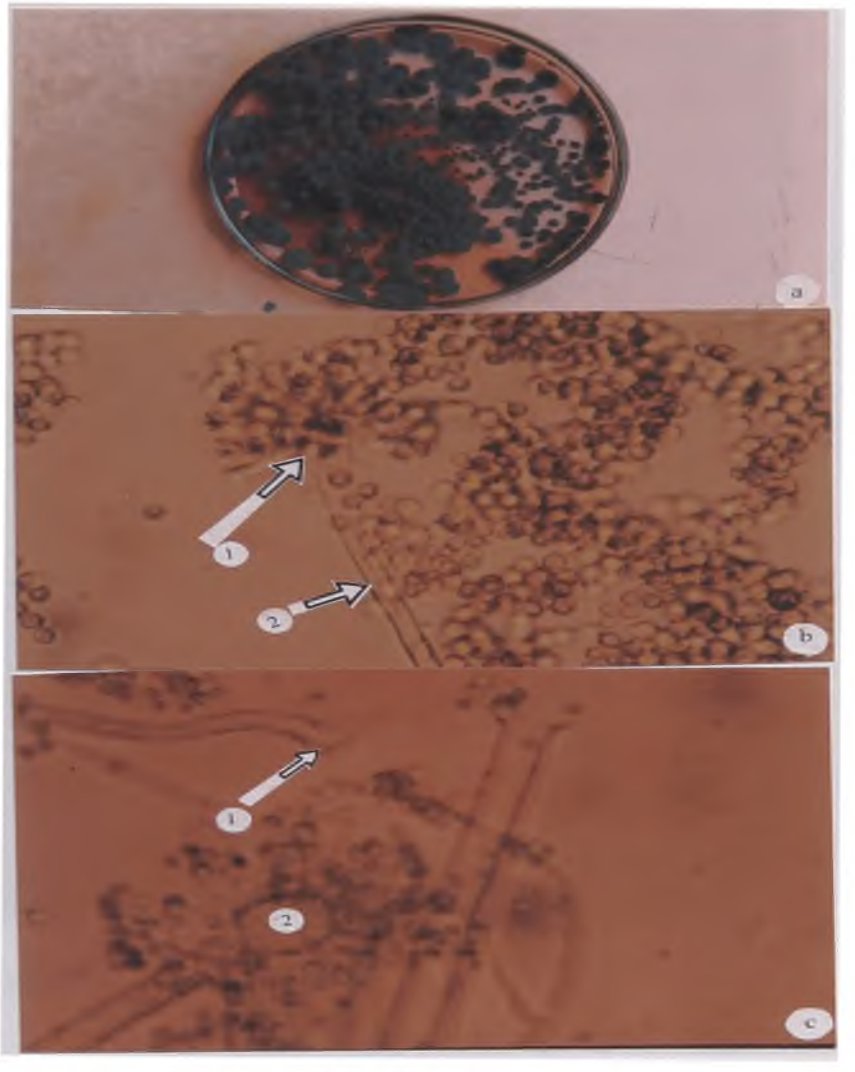

Plate 41. *Aspergillus sydowi* Thom & Church.

a) Two week old bluish green culture with small colonies resembling *Penicillium*. Note red pigmentation of medium.

b) Smooth slender conidiophore (2) carrying a radiate head and round rough conidia x 400.

c) Slide showing foot cell (1) and small globose vesicle (2) x 800.

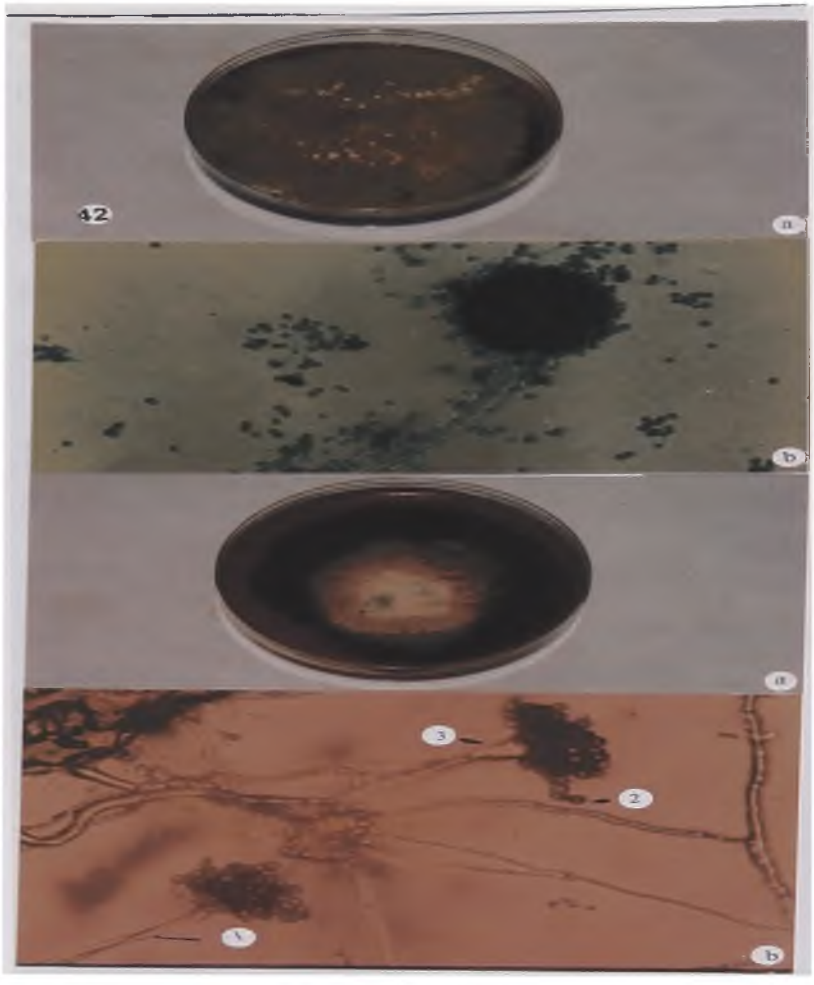

Plate 42. *Aspergillus tamari* Kita.

a) Three week old brown grainy culture on Czapek Dox Agar.

b) Smooth-walled conidiophore bearing a globose head with double phialides and globose conidia.

Plate 43. *Aspergillus terreus* Thom.

a) Two week old brown culture on Czapek Dox Agar.

b) Small brown head with colourless, smooth and slender conidiophore (1). Note the upper half of vesicle (3) covered with double phialides bearing chains of round conidia (2).

Aspergillus ustus (Bainier) Thom and Church

Colony is grainy. It starts as yellowish brown and becomes brown with age. Under the microscope, the conidiophores are short (500 μ), smooth and light brown. It has double phialides covering about 3/4 of vesicle and conidia are globose and rough. There is a foot cell which is almost horizontal to the conidiophore (Plate 44).

Aspergillus versicolor Toraboschi

Culture is grainy changing from white to yellow to pink with green patches. The reverse is pink to red. A second culture remained green. The heads are globose, fertile over the whole surface. The vesicle is small. Conidiophore is colourless and smooth (Plate 45).

Aspergillus wentii Wehmer

Culture is light yellowish brown becoming dark brown with age. The conidiophores are long and smooth. Conidial heads are large and globose. The vesicles are large and fertile over the entire surface. The double phialides are closely packed and bearing long chains of ovoid and round conidia (Plate 46).

Byssochlamys fulva Westling

Culture starts as white fluffy and later changes to brown. The conidiophores bear single oval conidia resembling *Paecilomyces*. The asci develop without any peridium, it is possible to see the 8 ascospores in it (Plate 47).

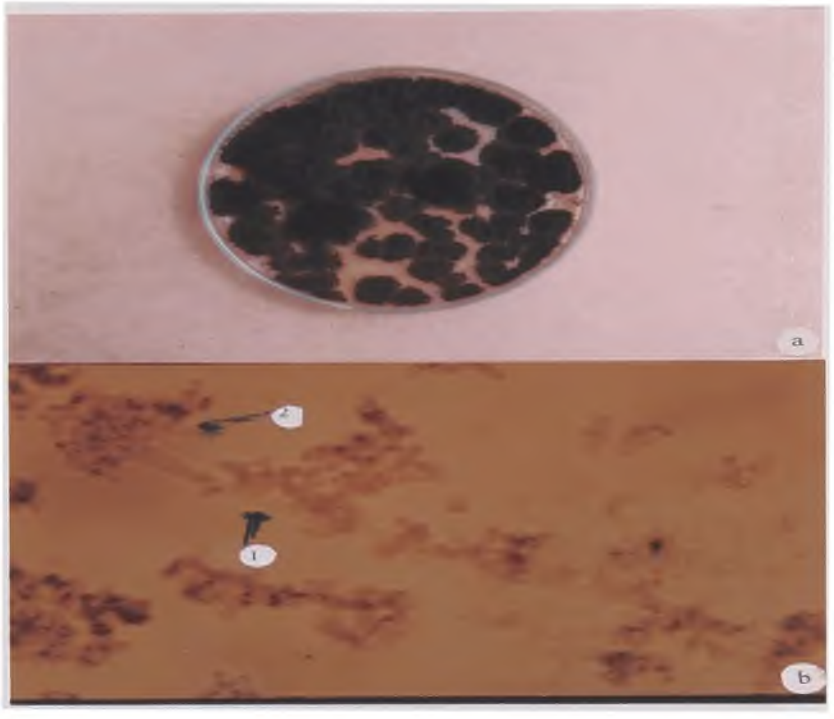

Plate 44. *Aspergillus ustus* Thom & Church.

a) Two week old yellowish brown grainy culture on Czapek Dox Agar.

b) Short, smooth, light brown conidiophore (1) bearing small vesicle with upper part by few radiate double phialides (2).

Plate 45. *Aspergillus versicolor* Toraboschi.

a) Eight day old green grainy culture on Czapek Dox Agar.

b) Long smooth conidiophore with "bushy" green head x 400.

c) Eight day old multicoloured culture. Note pink colouration of medium.

d) Multicoloured heads mounted in distilled water x 400.

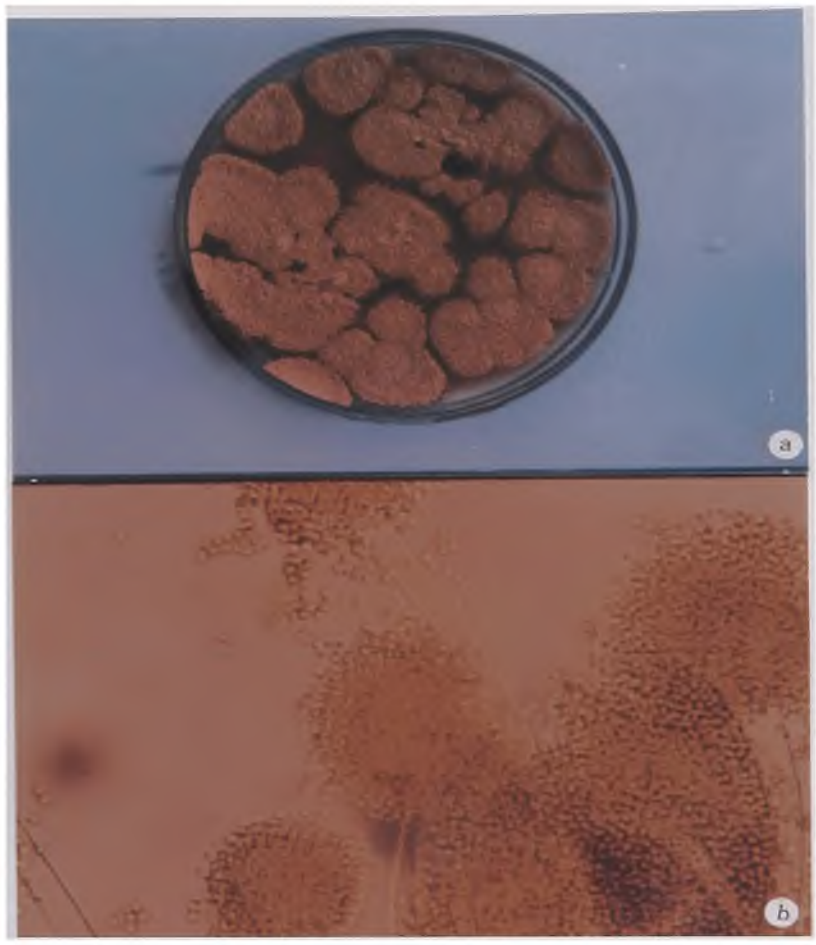

Plate 46. *Aspergillus wentii* Wehmer.

a) Eight day old orange, grainy culture on Potato Dextrose Agar.

b) Light orange large heads with long chains of round conidia on large globose vesicles.

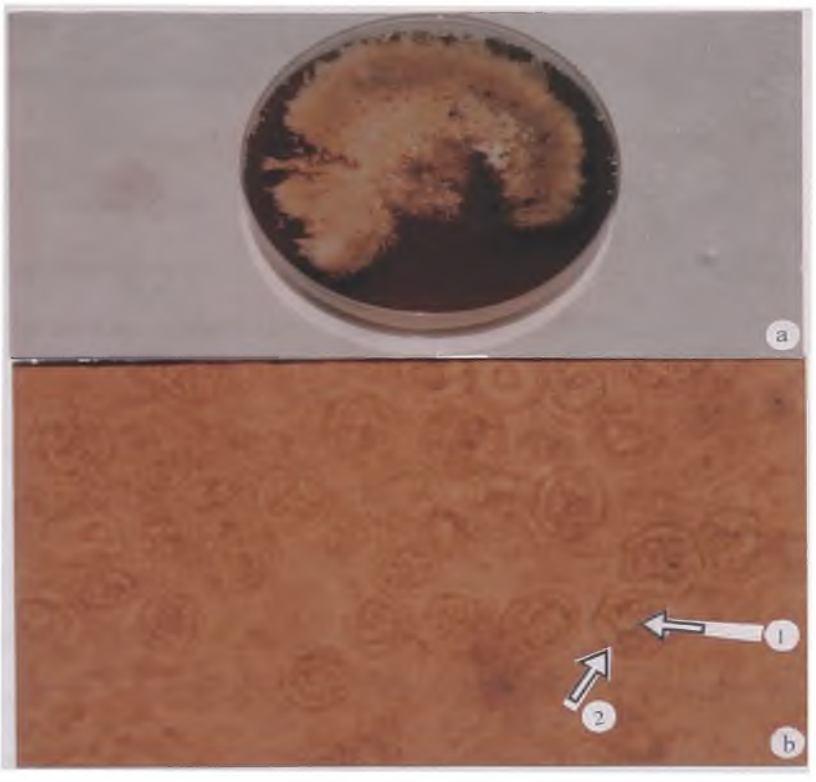

Plate 47. *Aspergillus fulva* Westling

a) Three week old brown fluffy culture on Potato Dextrose Agar.

b) Developing asci (1) without peridium. Note: Ascospores are visible in the asci x 400.

Chaetomium sp. Kunze ex Fries

White cottony growth surrounded by dark green submerged growth. Septate Hyphae are present. Black perithecia are present with thick numerous septate curly hyphae around them. The perithecia are irregular shaped and has a short neck. The ascospores are single celled brown and large (Plate 48).

Chaetomium globulosum Kunze ex Fries

Culture starts as white growing slowly. Later black spores visible to the eye on The medium. Under the microscope perithecia are globose (round), black and Covered with stiff hair-like structures. The conidia are large and brownish (Plate 49).

Curvularia lunata Boedjin

Culture starts as white and fluffy. Later, it shows bluish concentric lines about 3 cm in diameter on the medium with white reverse. In another culture concentric rings were absent but culture was wrinkled. The conidia are 3 - 5 celled and curved with the middle one enlarged (Plate 50).

Fusarium sp.

Culture is white to black and grows slowly. Conidia are small. They are rod shaped Or ellipsoid but not curved, 5.5 μ long and 0.9 μ wide (Plate 51).

Fusarium lateritium (Nees) Syder & Hansen

Culture is white cottony with patches of cream to light brown becoming dark in age. Growth looks a bit submerged. Reverse is brown with few portions dark brown patches. Macroconidia are long, thick-walled. 3-5 celled (size 63 μ long and 6 μ wide). Perithcia are formed. Chlamydospores present (Plate 52).

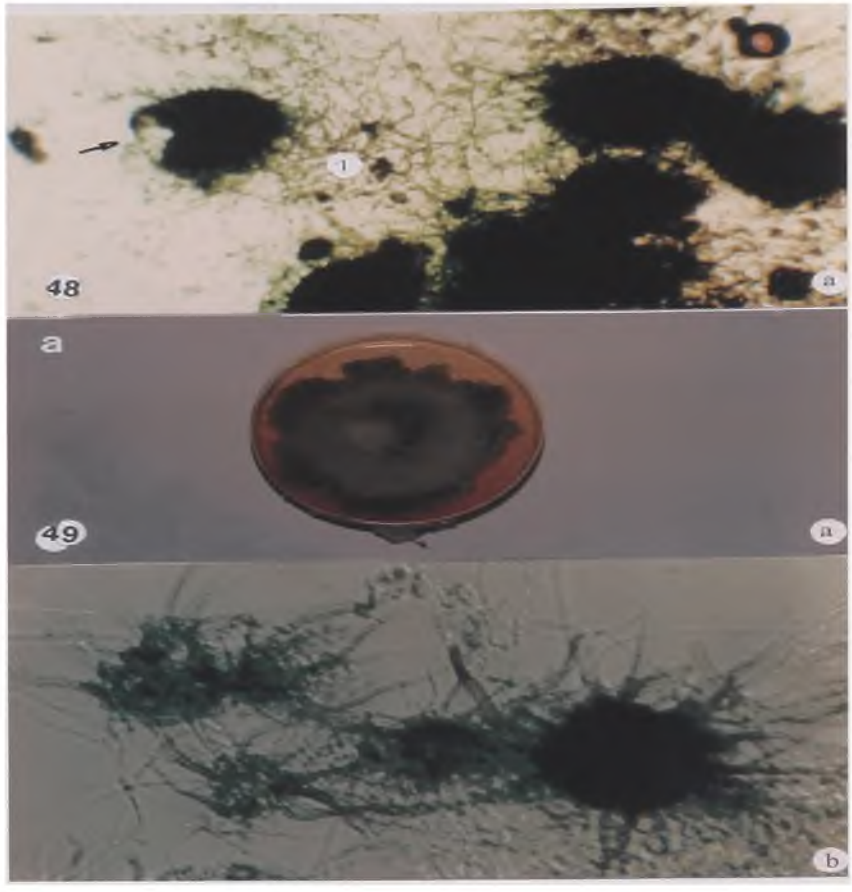

Plate 48. *Chaetomium* sp. Kunze ex Fries

Irregularly shaped black perithecia covered with stiff hairs (1) open perithecia (arrowed) showing conidia x 200.

Plate 49. *Chaetomium globulosum* Kunze ex Fries

a) Two week old grayish culture. Note black developing at the periphery of culture.
b) Spherical perithecia covered with stiff hairs x 200.

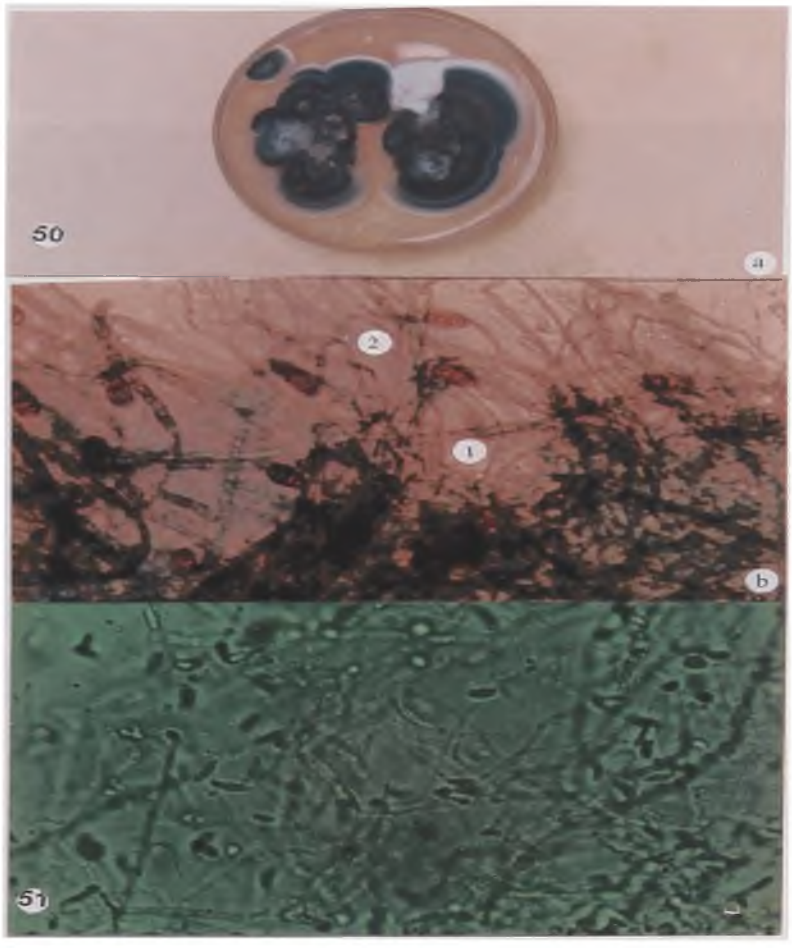

Plate 50. *Curvularia lunata* Boedjin

a) Three week old wrinkled culture on Potato Dextrosw Agar.

b) Mycelia with 3-5 celled curved conidia (1).

Plate 51. *Fusarium* sp.

Small rod shaped and curved macro-conidia x 400. Micro conidia (1- celled conidia are also present.

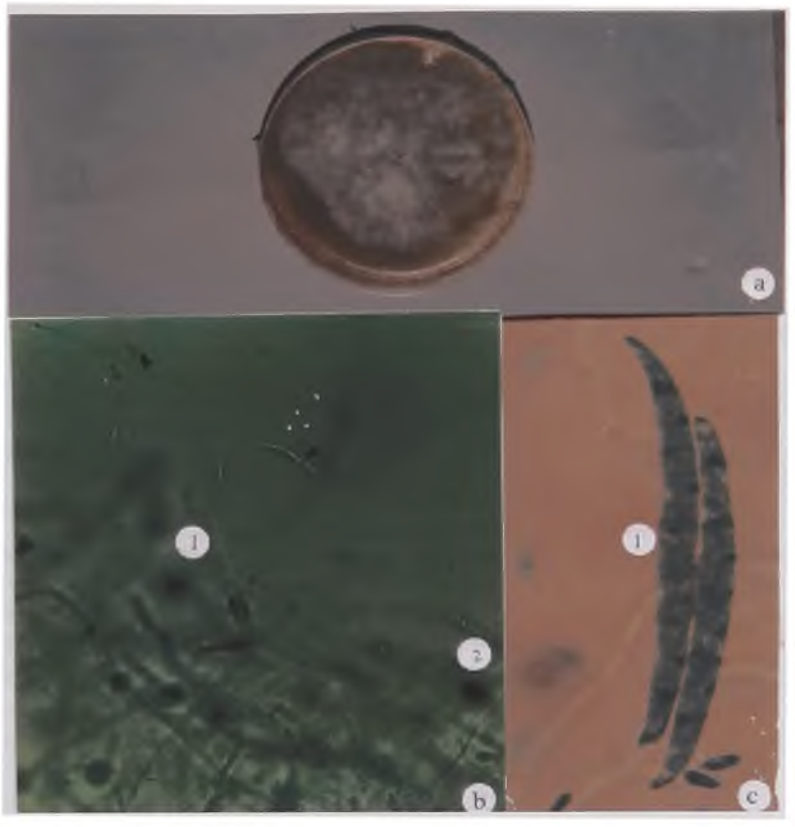

Plate 52. *Fusarium lateritium* Syder & Hansen.

a) Two week old grey culture on Potato Dextrose Agar.

b) Microconidia (1) and chlamydospores (2) x 400.

c) Large thick-walled macro-conidia (1).

d) Ascus (1) and conidiophores 2.

Fusarium moniliforme Sheldon

Culture is white, cottony developing brownish white to orange colour. Chlamydospores are absent. Microconidia are ovoid and borne on stalks or in chains in abundance. Macroconidia are narrow and thin-walled measuring 24 µ long and 4µ wide and 3-5 septations (Plate 53).

Fusarium nivale (Fr.) Ces. (syn. *Micronectriella nivalis* Booth)

Mycelium is white and fluffy and grows rapidly. Microconidia are absent. Macrospores are few. The microconidia are curved with a pointed apex and flat base. There are 1 3 septations. The 2.5 - 5 µ wide and 15 to 20 µ long (Plate 54).

Fusarium oxysporum Schlecht.

Growth is velvety in culture may be purple to pink with cottony growth covering the medium. Microconidia are oval and borne in clusters. *Fusarium* macroconidia of various sizes and shape are thin walled with blunt ends. Occasionally, chlamydospores are seen (Plate 55).

Fusarium rigidisciula.(syn. *F. decemcellulare)* Brick

Culture is white, cottony with grey patches. Later develops powdery appearances. Microconidia are oval and small. Occasionally may be one septation and may be borne in chains (like *Paecilomyces*). The macroconidia are thick-walled, large about 6.6 µ wide and 64 µ long with 5-10 septations. They can be about 130 µ long and 10 µ wide. One side is pointed and has a fool cell. The chlamydospores were observed (Plate 56).

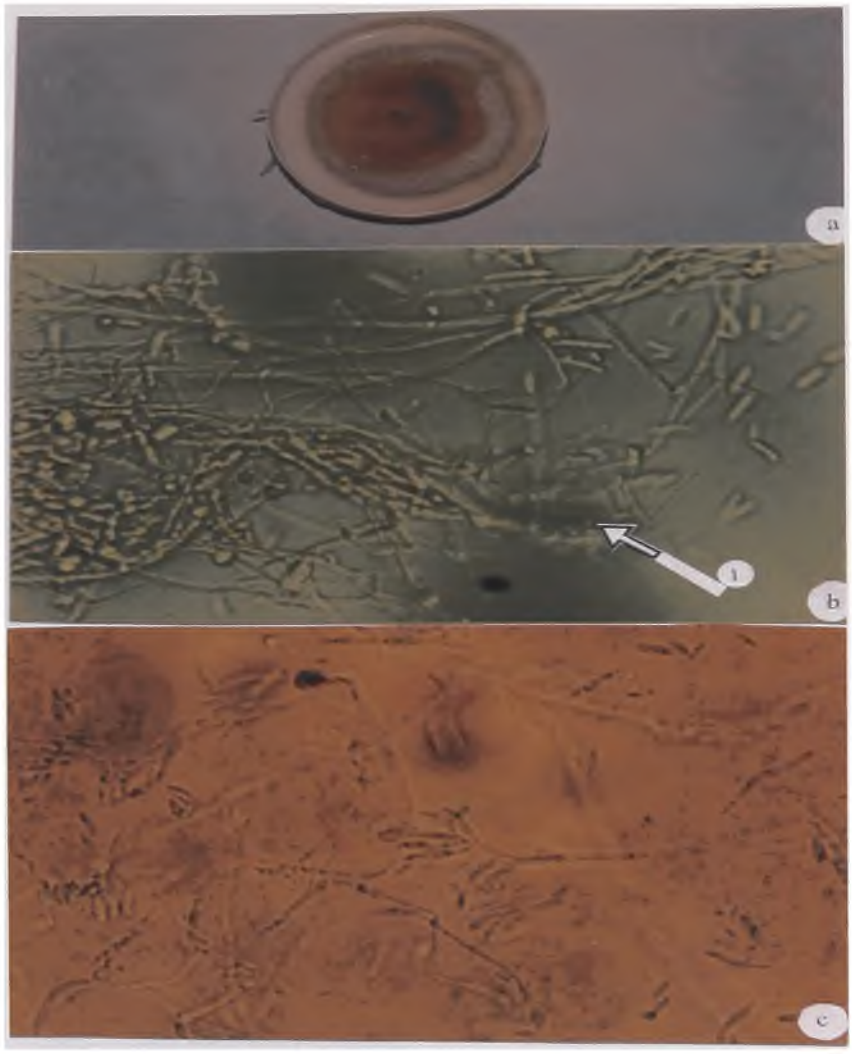

Plate 53. *Fusarium moniliforme* Sheldon.

a) Two week old culture on Potato Dextrose Agar.

b) Note deep yellow colouration with dark tinge developing from centre of centre.

c) Conidiophones (1) micro-conidia and macro-conidia x 800.

d) Clusters of macro-conidia on conidiophores.

Plate 54. *Fusarium nivale* (Fr.) Ces.

a) Two week old white fluffy culture on Potato Dextrose Agar.

b) Macroconidia are thick-walled and of various sizes. They have pointed apices and flat base x 400.

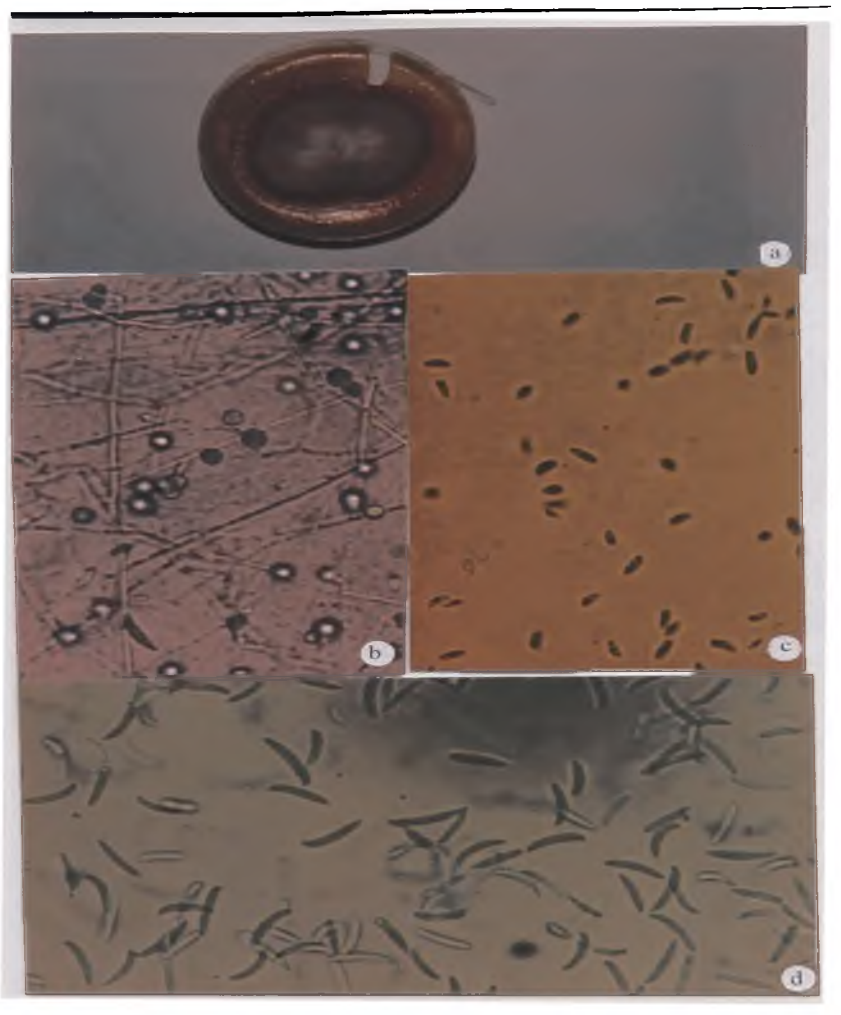

Plate 55. *Fusarium oxysporum* Schlecht.

a) Two week old mauve, velvety culture on Potato Dextrose Agar.

b) Single-celled chlamydospores.

c) Ovoid microconidia.

d) Thin-walled macroconidia

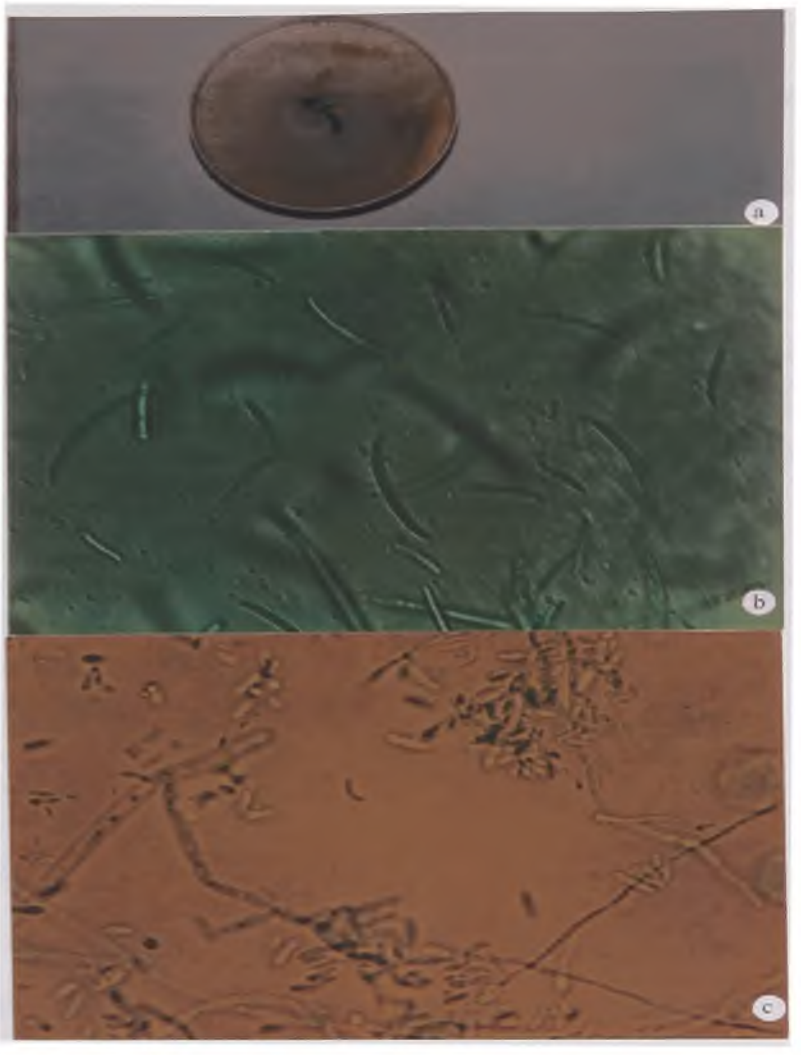

Plate 56. *Fusarium rigidiscula* Brick.

a) Two week old grayish cottony culture on Potato Dextrose Agar.

b) Large thick-walled macroconidia x 200.

c) Ovoid microconidia in clusters x 400.

Fusarium roseum Link

Culture is white to pink and sub-merged microconidia and macroconidia present. Macroconidia are 22.3 μ long and 4.2 μ wide and blunt at one end. Chlamydospores are rounded and 2-3 celled (Plate 57).

Fusarium solani (Wort) Daco

Growth is rapid. Culture starts as white changing to cream from the centre. Colony often has a bluish or violet tinge. It has microconidia. The macroconidia are 4.5-5.5 um in size, thick walled not ending in a fine point. They are 3-5 celled. Chlamydospores are present (Plate 58).

Geotrichum candidum Link

Culture is white changing to cream. Long mycelial strands are present in young cultures but they later break up at the terminal into short single cells (Plate 59).

Monascus purpureus Went

Culture is in small scattered pink colonies which changed to purple and become greyish with age. The reverse is dark purple. The mycelia is like *Paecilomyces* with round spores. Round structures like perithecia with stalks are present (Plate 60).

Mucor sp. Fresenius

The colony is grey, becoming greyish brown with age. The conidiophores are irregularly branched and unequal in length. The sporangia are globose and of different sizes. The collumella are ovate with collarette left after the spores are shed (Plate 61).

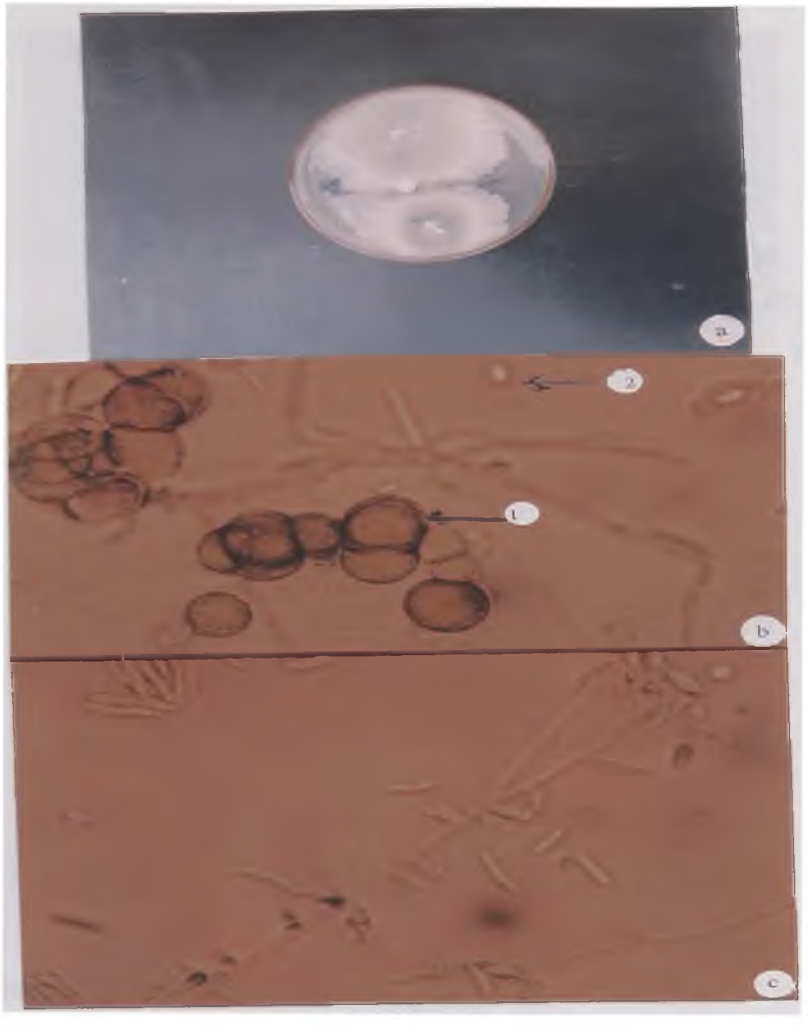

Plate 57. *Fusarium roseum* Link

a) Two week old grey culture on Potato Dextrose Agar.

b) Slide showing clusters of multi-celled chlamydospores (1) and microconidia (2).

c) Microconidia of various sizes x 400.

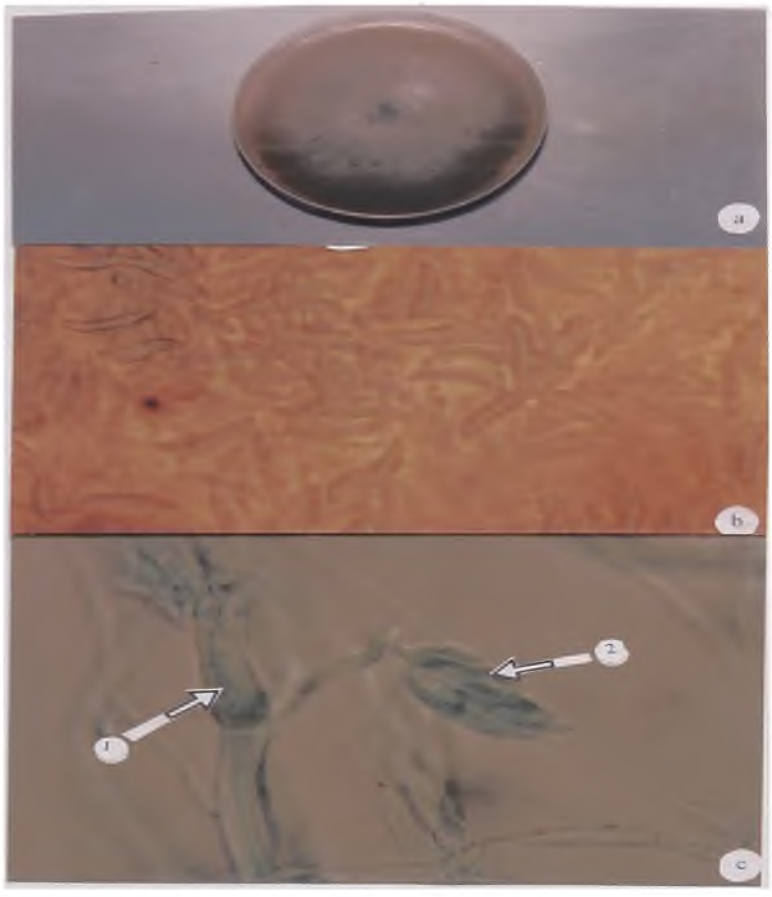

Plate 58. *Fusarium solani* (Wart) Daco.

a) Two week old white cottony showing dark bluish colouration at the periphery.

b) Think walled macroconidia x 400.

c) Part of conidiospore (1) bearing a cluster of macroconidia (2) x 800.

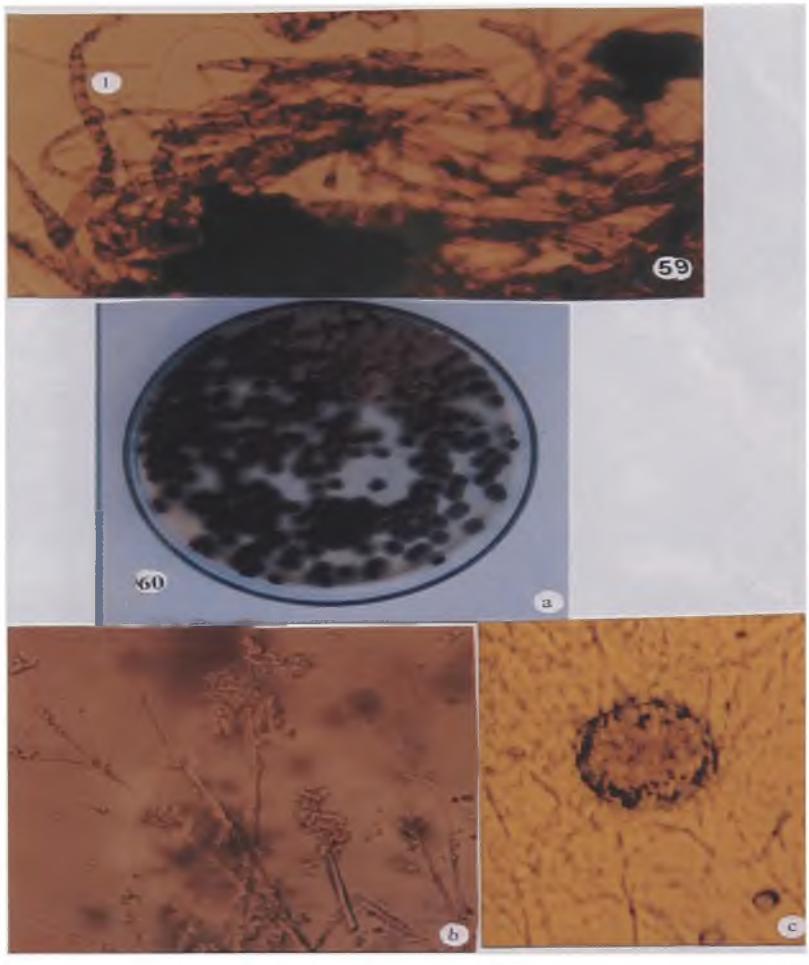

Plate 59. *Geotrichum candidum* Link.

Slide showing mycelia with some thick hyphae terminating in short septate cells (1) breaking off as conidia.

Plate 60. *Monascus purpureus* Went.

a) Culture on Potato Dextrose agar. Note: Small bluish colonies with pinkish areas resembling *Penicillium* grey with age.

b) Slide showing *Paecilomyces* type of mycelia. Note: The branched conidiophores carry globose conidia x 800.

c) Stalked spherical perithecia x 400.

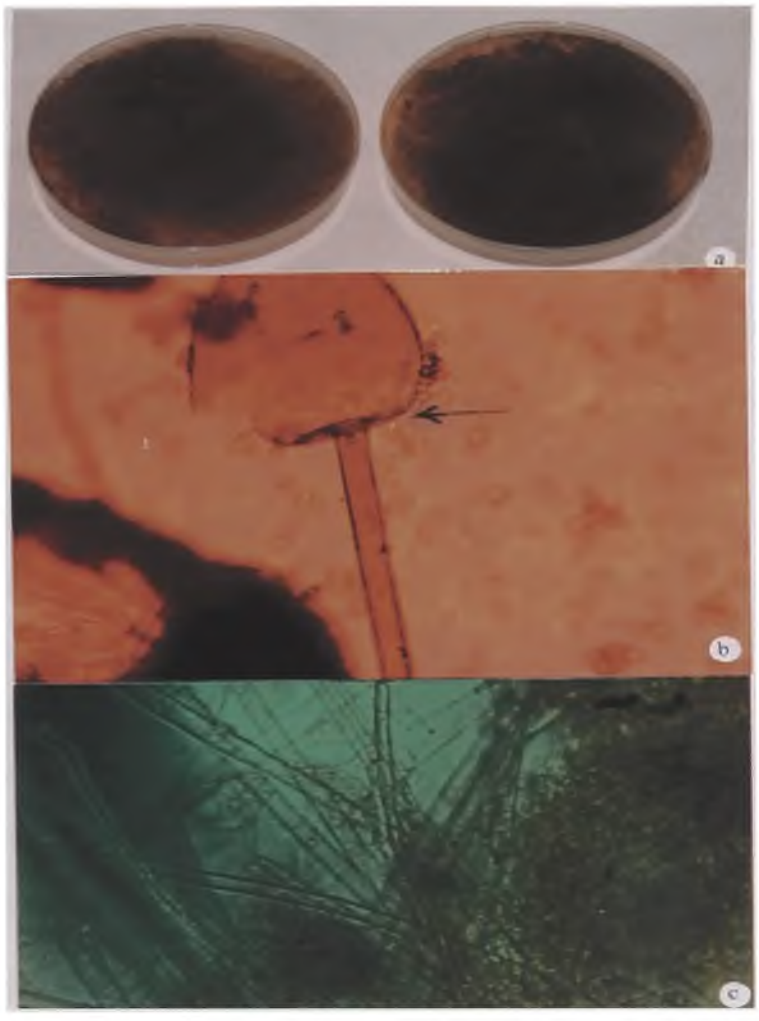

Plate 61. *Mucor* sp. Fresenius.

a) Greyish brown cottony culture on Potato Dextrose Agar.

b) Sporangiophore showing spherical columella with collarete (arrowed).

c) Part of stolons an sporangiophores.

Mucor haemalis Wehmer

Culture starts as white and fluffy changing to grey, sporangiophore are branched alternately but rhizoids are present. Sporangia are round and smooth. Spores are oval and rough and of different sizes (Plate 62).

Nigrospora sp. Zimm

Colony is grey and fluffy with the reverse dark. Large septate conidiophores rising from large mycelia ends in black round conidia. Hyaline mycelia are also present (Plate 63).

Paecilomyces varioti Bainier

Culture is cottony, white to grey. The myceilia consist of ropes of hyphae. The conidiophores are slender, flask shaped at the base narrowing to the tip which bears the round conidia in single chains (Plate 64).

Penicillium citrinum Thom

Culture is greyish-green with reverse of the culture brown. It shows single branching with phialides grouping together in bunches (Plate 65).

Penicillium chrysogenum Thom

Culture is greyish-green with reverse of the culture brown. It shows single branching e) with the phialides grouping together in bunches (Plate 66).

Penicillium cyclopium Westling

The culture consists of small round bluish colonies with small white margins. The reverse is pale yellowish-brown. The conidiophore is compact with 3 stages of branching. Conidia are globose and smooth (Plate 67).

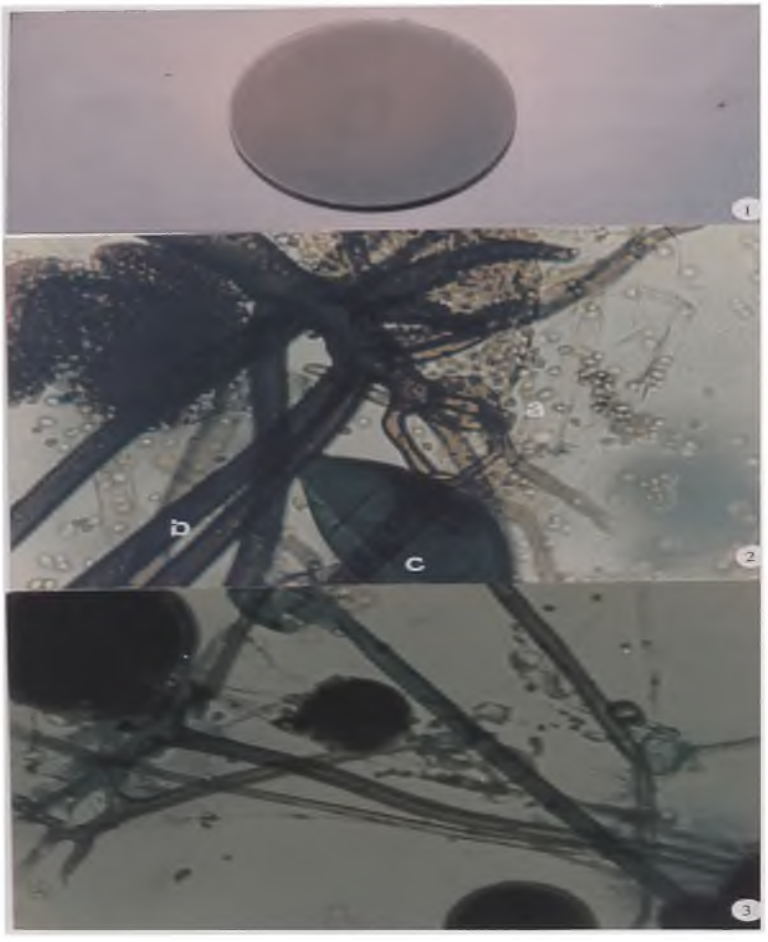

Plate 62. *Mucor haemalis* Wehemer

1) Grey cottony culture on Potato Dextrose Agar.

2) Part of mycelia showing a) rhizoid b) base of sporangium, c) columella, d) conidia. e) → Stolon.

The base of sporangia and rhizoids are initiated at different points on the stolon.

3) Part of mycelia showing smooth, spherical sporangia.

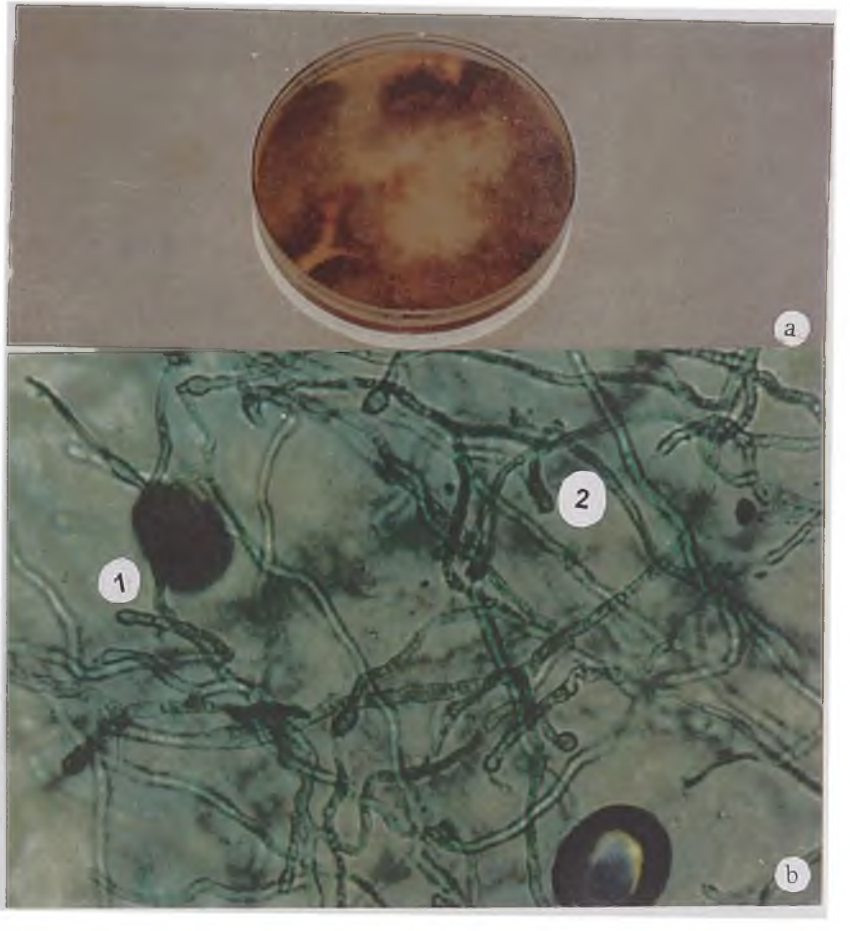

Plate 63 Unidentified fungus resembling *Nigrospora* sp. Zimm

(a) Dark culture

(b) Mycelia showing developing conidiophores with septate hyphae (1) and globose developing spores (2).

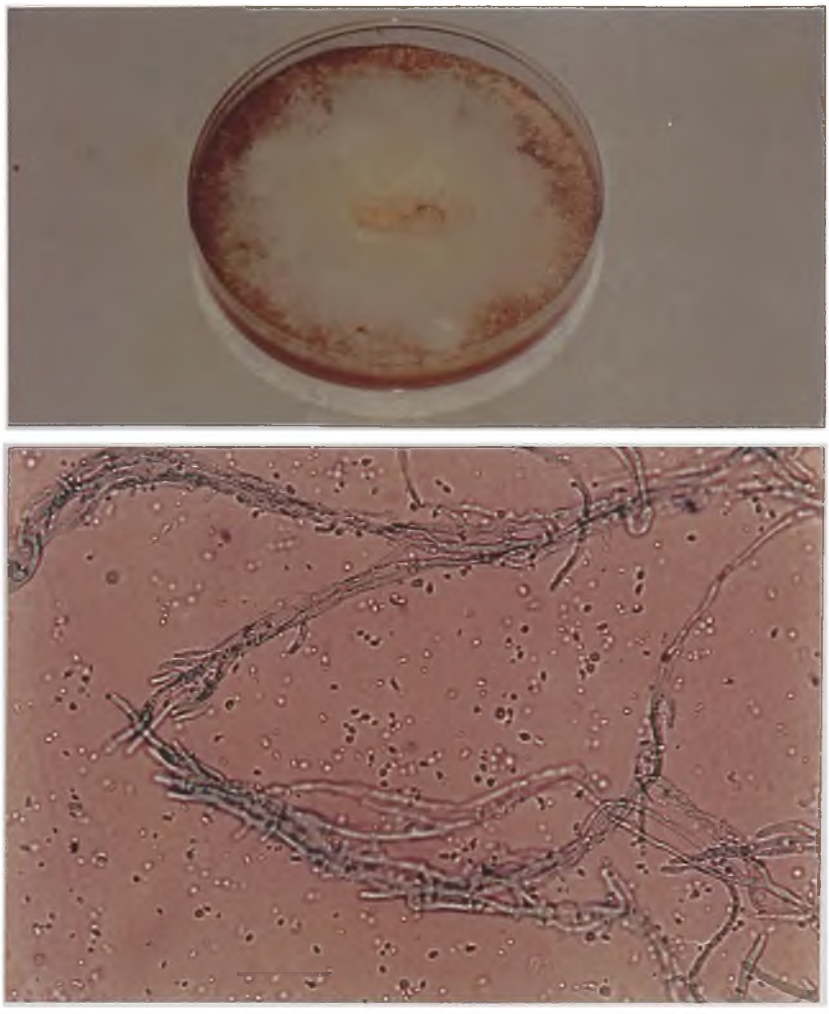

Plate 64 *Paecilomyces varioti* Bainier

a) Two week old cottony culture on Potato Dextrose Agar

b) Ropelike mycelia with conidiophore narrowing at the tip and bearing spherical conidia.

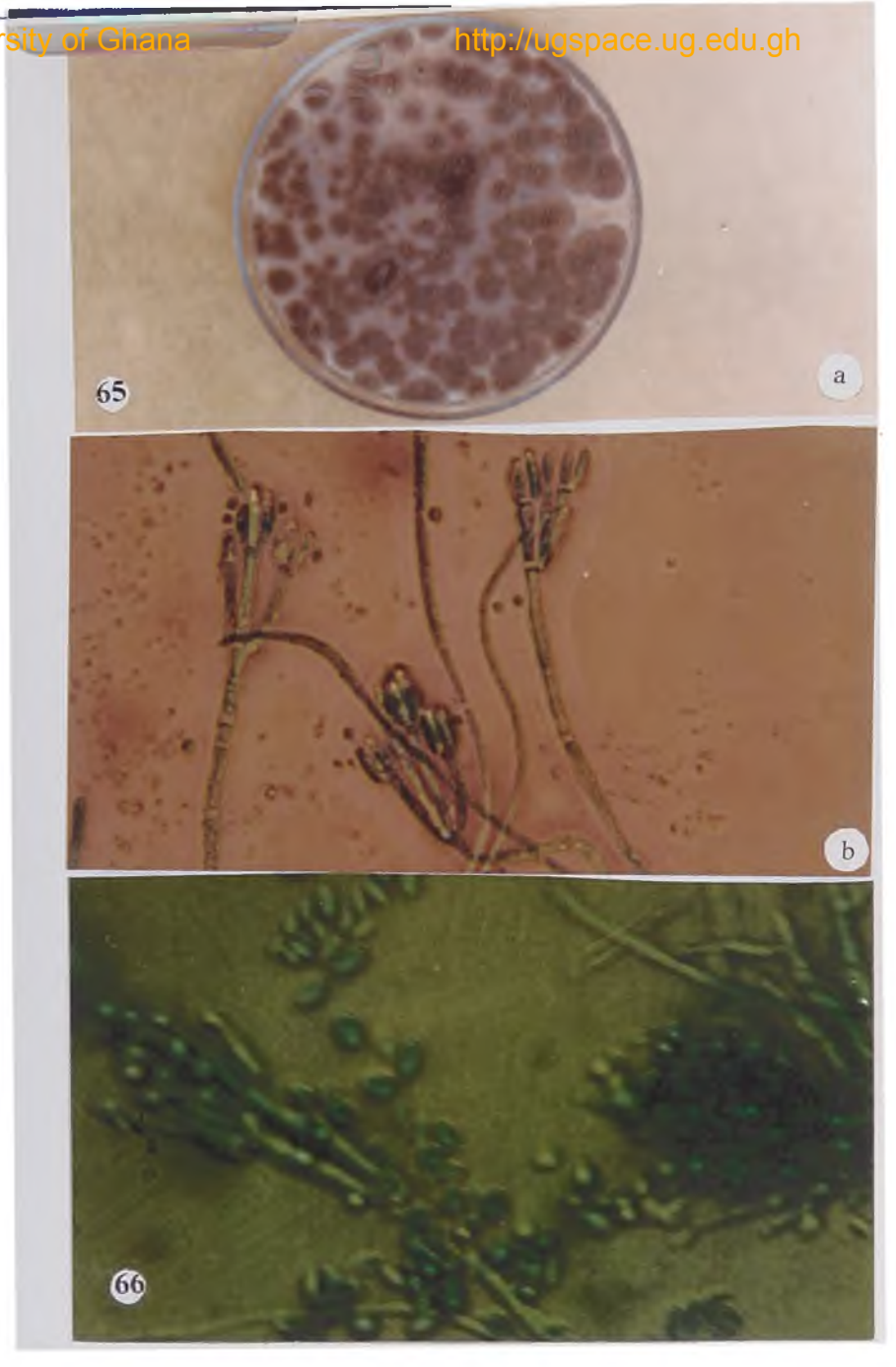

Plate 65 *Penicillium citrinum*

a) Two week old greyish green culture on Czapek Dox Agar

b) Penicillus showing single branching

Plate 66 *Penicillium chrysogenum* Thom

Penicillus show double branching and bear ovoid conidia

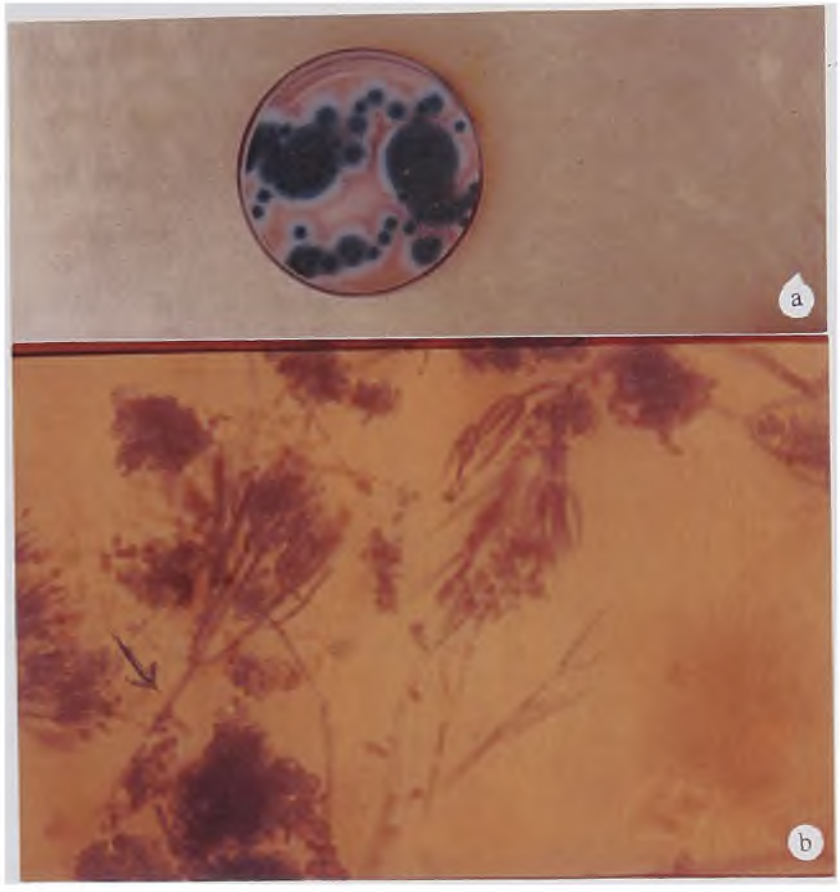

Plate 67 *Penicillium cyclopium* Westling

a) Bluish culture comprising small round colonies

b) Penicillus showing three stages of branching (arrowed).

Penicillium phoeniceum Stoll

The mauve colony is imbedded with white cottony growth covering the mauve and is sporing heavily on PDA. Examination under microscope showed a *Penicilllium* head which has double branching (Plate 68).

Penicillium variable Sopp

Culture is velvety, white to grey with green and orange patches. It has long conidiophores ending in branched metulae. The penicillus is single showing verticillate branching (Plate 69).

Phoma sp. Desm.

Colony is white turning black with white patches. Black pycnidium with a short neck discharging hyaline spores in mucilagenous substance was observed under the microscope (Plate 70).

Pullularia pullulans (De Bary and Low) Berkhout

The culture is dirty white and slimy becoming dark with age. The hyphae are colourless but later become thick, ropelike and twisted, bearing ovate conidia. The presence of some cylindrical structures resembling detached spores are present (Plate 71).

Rhizoctonia sp. *solani*

The culture starts as white, spreading rapidly and then becomes black. The intertwined hyphae are brown with long cells (Plate 72).

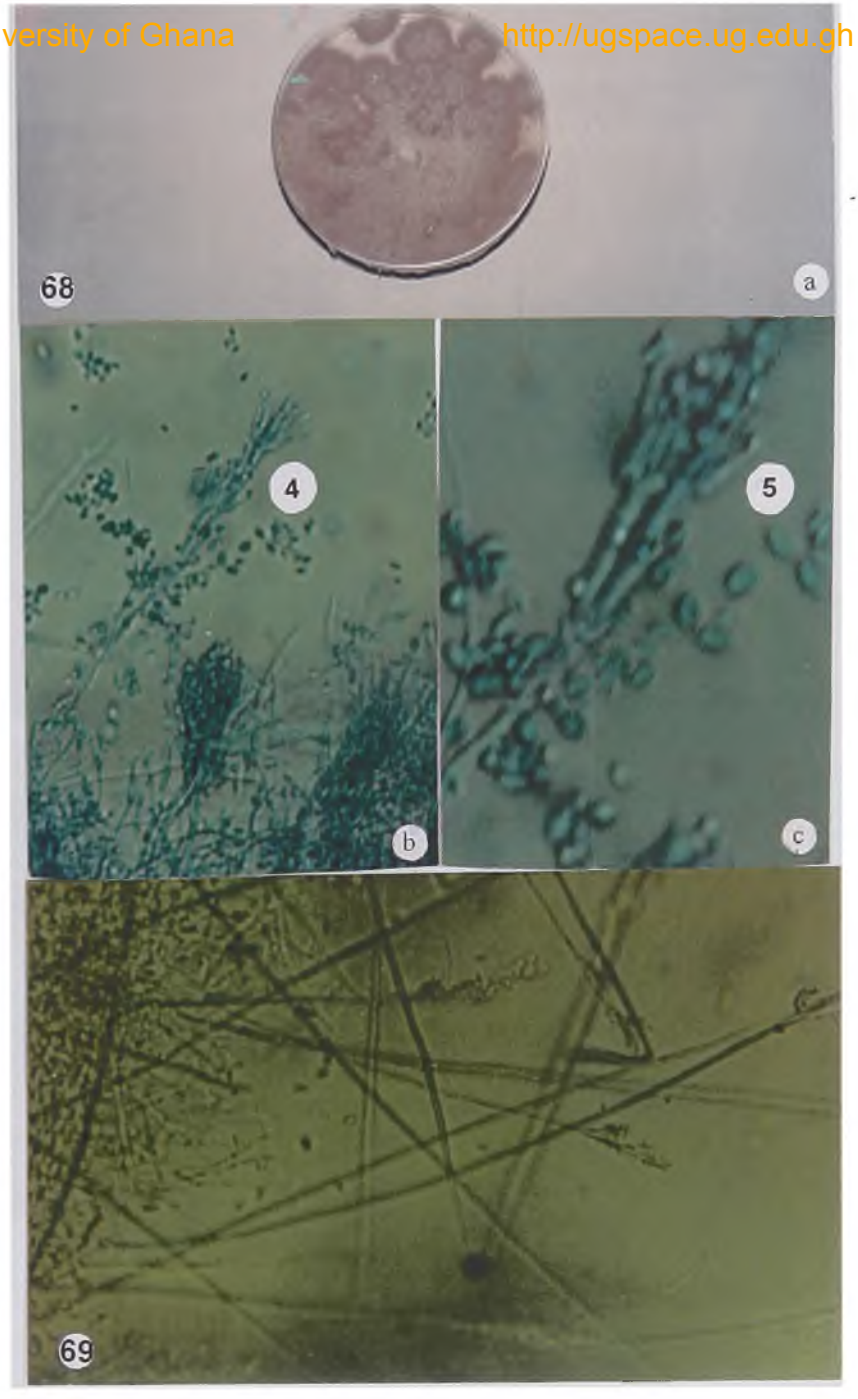

Plate 68 *Penicillium phoeniceum* Stoll

a) Two week old mauve velvety culture on Potato Dextrose Agar

b) Mounted specimen showing penicillin conidia x 400

c) Penicillus showing three stages of branching and diverticillate head. (5) X 800

Plate 69 *Penicillium variable* Sopp

Culture has long conidiophores of various lengths ending in simple penicillus heads.

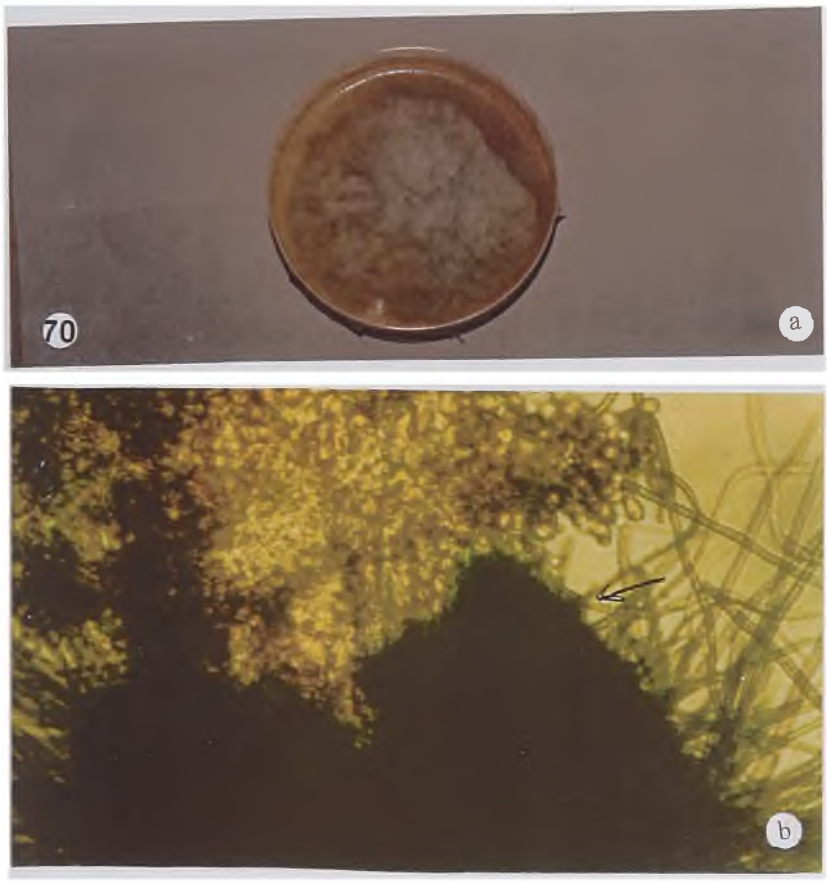

Plate 70 *Phoma* sp. Desm.

a) Two-week old greyish culture

b) Black pycnidum with short neck discharging conidia. X 400

Plate 71 *Pullularia pullulans* Berkhout

Thick colourless, twisted ropelike hyphae with small ovate conidia x 400

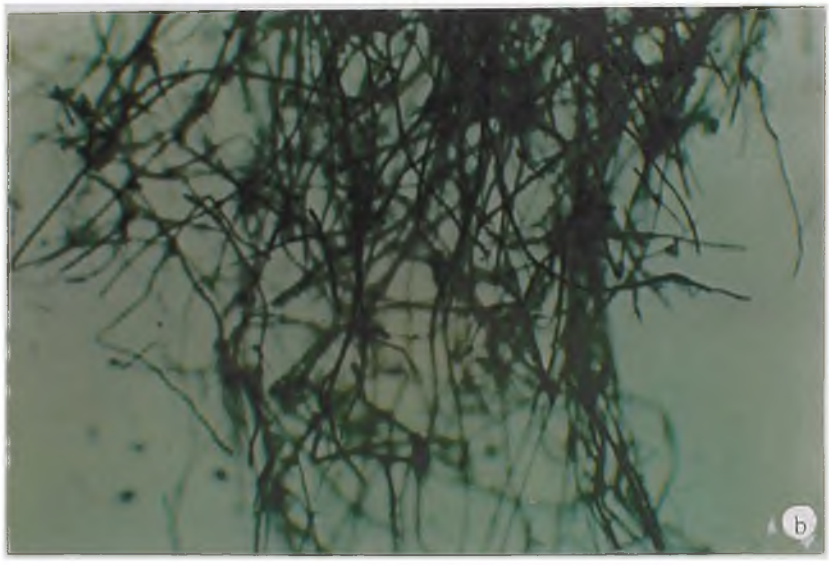

Plate 72 *Rhizoctonia solani*

a) Two week old black culture on Potato Dextrose Agar

b) Intertwining mycelia under at x 400

Rhizopus nigricans Ehrenberg

The culture grows rapidly becoming black in age. The sporangiophores startsfrom the rhizoids. The spores are of various shapes (Plate 73).

Scopulariopsis sp. Bainier

The culture starts as white changes to cream from the middle and then to brown. Under the microscope, there is a septate conidiophore which resembles a penicillus. Some are branched and others are single. The conidia are round and are thick-walled (Plate 74).

Sporendonema ex Fries

The culture forms white a colony which changes to pink in the centre and after 1 Month becomes yellowish. The colony appears fluffy, and rises in the centre. It grows slowly covering a third of the plate after one month. Part of the mycelium appears septate and swollen towards the terminal (like endospores) (Plate 75).

Syncephalastrum racemosum Schroter

The colony grows rapidly and becomes dark resembling *Rhizopus nigricans*. Under the microscope, black heads which resemble *Aspergillus* heads are present. However, the spores are formed in tubular structures (Plate 76).

Unidentified fungus.

The culture is fluffy and grows rapidly, changing from gray to grayish- brown. Features under microscope show that the fungus belongs to the class Zygomycetes and in the order Mucorales. The sporangia are arranged alternately on the stolon and there is a sterile hypha under each sporangium (Plate 77).

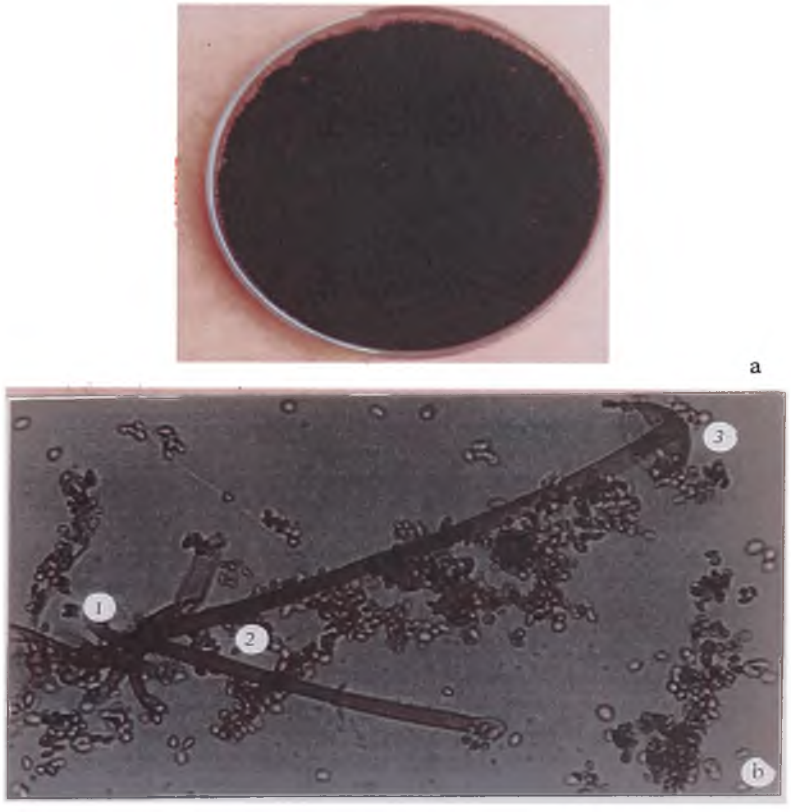

Plate 73. *Rhizopus nigricans* Ehrenberg.

a) Eight day old dark fluffy culture on Potato Dextrose Agar.

b) Conidiophore showing attached stolons (1) rhizoids (2) and base of sporangia (3).

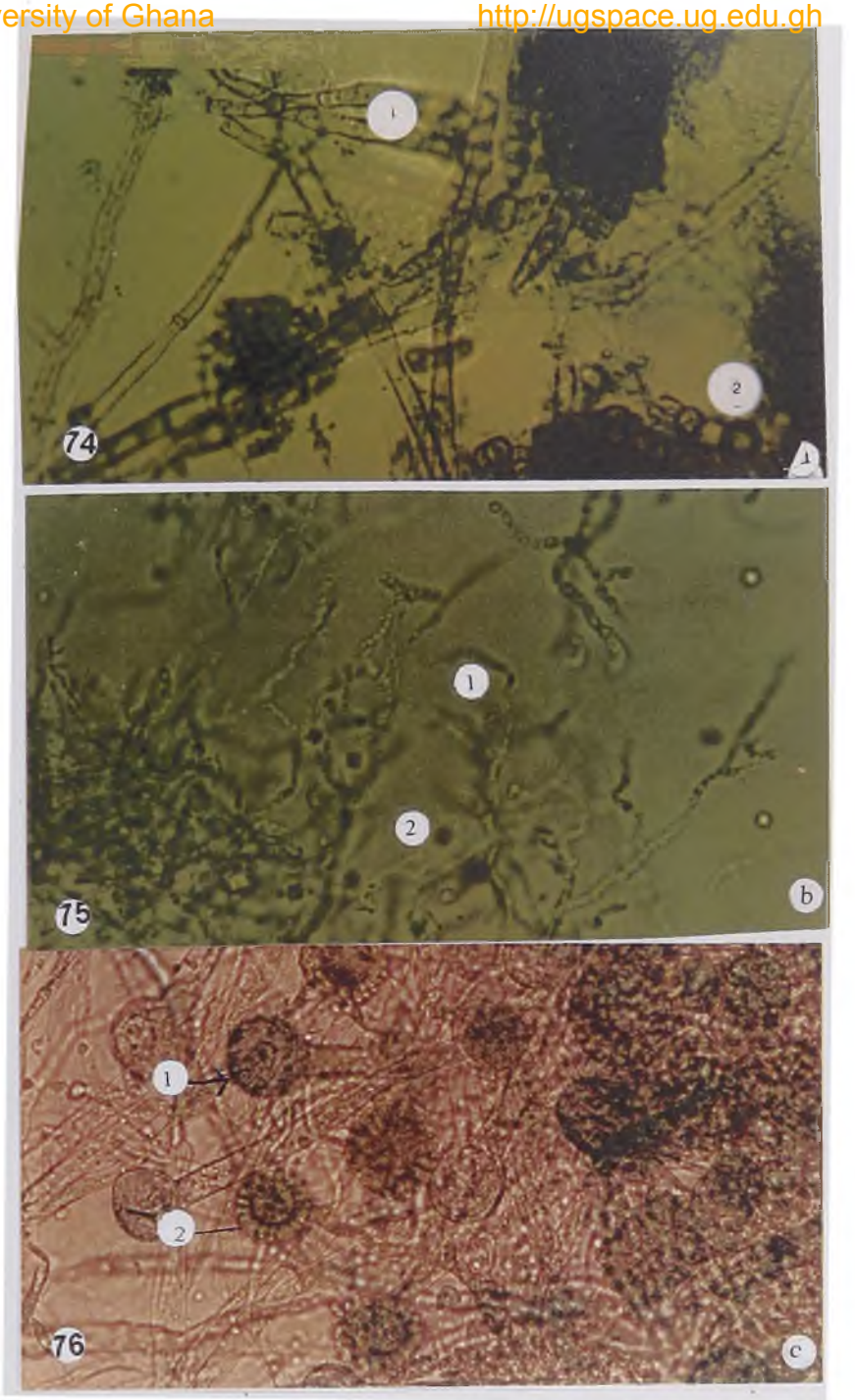

Plate 74 *Scopulariopsis* sp. Bainier
Septate conidiophore (1) bearing large, globose rough conidia (2).

Plate 75 *Sporendonema* sp. ex Fries
Mycelia with septate hyphae and round endospores (2).

Plate 76 *Syncephalastrum racemosum* Schroter
Mycelia under microscope showing
1."Aspergillus-like" globose vesicle 2 .sporing structure

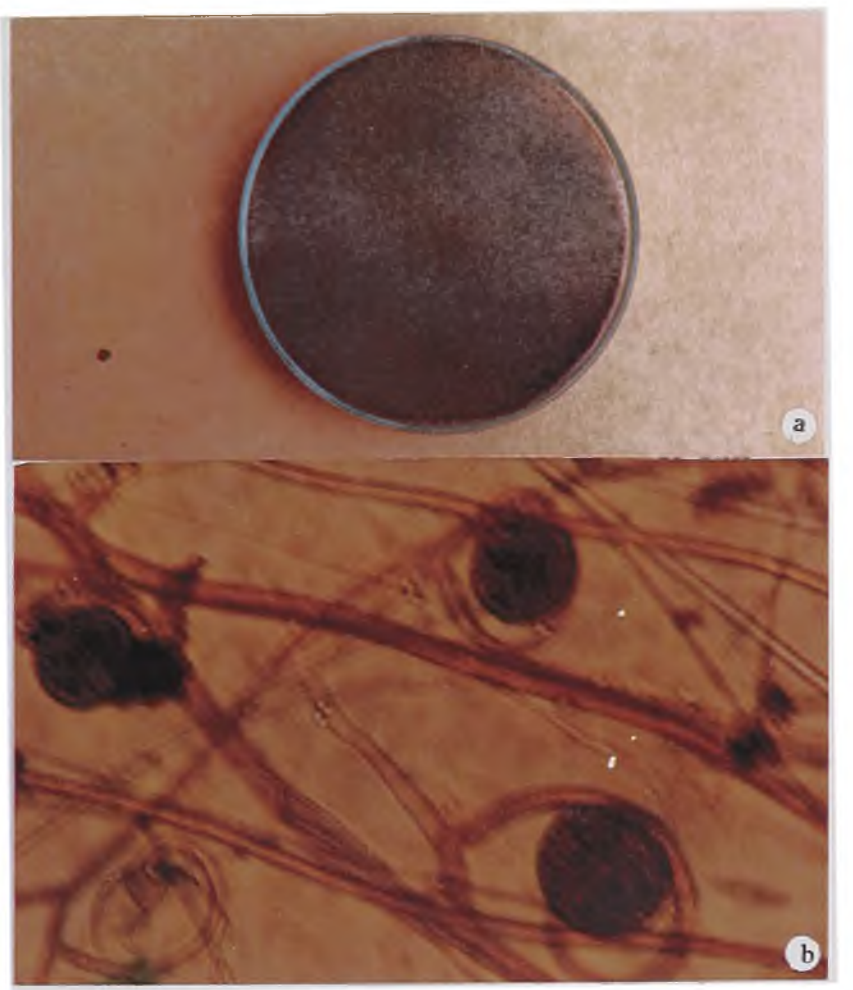

Plate 77. Unidentified fungus.

Stolons showing alternate arrangement of curled sporangiophore x 400.

4.4 DISCUSSION

In order for a surface sterilising agent to be effective, it should be able to get rid of the surface spores without killing the internal fungi. Both sodium hypochlorite and alcohol are good surface sterilising agents but regarding the effect on cocoa beans, sodium hypocholorite residue on the beans after dipping restricted growth and also seeped into the medium giving a fungicidal effect. The beans could not be dipped in distilled water to wash off the residue of sodium hypochlorite for fear of increasing the moisture content of the bean. Dipping in 70 % ethyl alcohol for 5 min appeared to be too long as the alcohol, killed the surface superficially-borne fungi and seeped into the cotyledons killing all internally- borne fungi. Dipping the beans for 1min in 70 % alcohol was just long enough to get rid of the external fungi. It evaporated quickly allowing the internal fungi to grow. It therefore was selected for the subsequent experiment. The fact that some of the fungi appeared only on the unsterilised beans meant that those fungi existed only on the surface of the beans and were removed by dipping in the sterilising agent.

In selecting the culture medium for the isolation of fungi, the rate of growth of fungi and the visibility of the fungal colonies on each medium was considered. The rate of growth was too slow on the moist filter paper. Growth on potato dextrose agar was the fastest as this medium contained nutrients. Isolation was however difficult as the faster fungi covered the slower ones. When growth was considered in terms of the number of beans showing infection by third day, the beans on water agar had the highest number, but colonies were distinct and the

rate of growth was slow. Thus the slower fungi could also be isolated easily. Therefore water agar was selected as the best for isolation.

Fifty eight (58) different fungi have been identified in the study. Forty-eight internal fungi were identified as against the 21 identified by Abitey (1982). Thirteen of the fungi identified by Abitey have been confirmed in this study. They include *Aspergillus chivalieri, A. flavus, A. nidulans, A. niger, A. phoenicis, A. ruber, A. tamarii, A. wentii A. sydowi, A. ustus, Rhizopus nigricans, Mucor pusillus, Fusarium solani, Penicillium citrinum.* In addition twenty-nine fungal species causing internal mouldiness have been recorded for the first time in Ghana. They are *A. amstelodami, A. lutescens, A. itaconicus, A. chivalieri, A. oryzae, A. versicolor, A. fischeri, A. echinulatus, A. pseudo-glaucus, A. parasiticus A. terreus; A. japonicus, A. sulphureus, Penicillium cyclopium, P variable, P. phoeniceum, F. oxysporum, F. moniliforme, F. roseum F. rigidiuscula, F. lateritium, A. proliferans, Absidia corymbifera, Syncephalastrum racemosum, Curvularia lunata, Chaetomium globulosum, M. haemalis, Phoma, Paecilomyces varioti,* and *Byssochlamys fulva.*

Hughes (1952, 1953) and Piening (1962) listed several fungi occurring on cocoa, some of which have been encountered in this study. However, they did not specify which part of the plant and at what stage in the development of cocoa these fungi were found. Therefore as far as internal mouldiness is concerned, the fungi listed above are being recorded for the first time. Of the 31 species identified in Nigeria by Oyeniran and Adejini (1974), 19 have been identified in this study. They are *Aspergillus chivalieri, A. flavus, A. niger, A. fumigatus, A.*

penicilloides, A. glaucus, A. tamarii, A. nidulans, A. ochraceus, A. pseudoglaucus, A. ruber, Geotrichum candidum, Penicillium variable, Fusarium solani, F. oxysporum, Mucor pusillus, Paecilomyces varioti, Rhizopus nigricans and *Absidia corymbifera.*

Internal mouldiness has been found to be either by direct penetration by the fungi during Such as *A. fumigatus* and *Mucor pusillus* or by penetration through cracks which occur the process of drying (Dade 1928, Laycock, 1936; Knapp 1937, de Witts, 1952; Olutiola and Cole 1977, Broadbent and Oyeniran 1968).

Many of the fungi identified in this study have also been reported to be present during fermentation (Dade 1928, Oyeniran 1971). Dade isolated thermophilic fungi- *Aspergillus fumigatus* and *Mucor buntingii* in fermenting heaps. On dried cocoa, he isolated *A. glaucus, A. niger, A. ochraceus, A. tamarii and a Penicillium* sp., some species of *Mucor*, two species of *Absidia* and one of *Circinella*. Maravalhas (1966) found that the type of fungi isolated varied with the crop as well as with the moisture and temperature. He found *A. fumigatus, Mucor pusillus and A. tamarii* growing in fermenting heaps in Ghana. During drying *A. fumigatus* and *Geotrichum candidum* were observed as external fungi on cocoa beans. During storage most conspicuous fungi he found were *Penicillium* sp., *A. glaucus* sp. and *G. candidum*.

The fact that some of the fungi appeared only on the unsterilised beans meant that those fungi existed only on the surface of the beans and were removed by dipping in the sterilising agent. Thorold (1975) identified 192 different fungi growing externally on dried cocoa many of which may not produce mycotoxins

but have varying effects on the flavour of the beans. Some are more detrimental than others. None of the criteria for grading of cocoa includes external fungi (Dand, 1993). When some of the external fungi were cultured in this study, they were found to be viable. In this study, the fungi on the unsterilised beans or the testa were of interest because all internal fungi were also found on the exterior of beans. If the conditions during storage become favourable, these externally borne fungi can grow and cause internal mouldiness. Also, external fungi are important because as workers pour out cocoa or pack emptied sacks, the dust containing fungal spores can cause health problem when inhaled. This danger is mainly from the *Aspergillus* group (Dand, 1993). The effect of radiation in controlling the fungi identified will be investigated in the next chapter.

CHAPTER FIVE

5.0 PREVENTION OF MOULDINESS BY IONISING RADIATION

5. 1 INTRODUCTION

Mouldiness in dried cocoa beans is a major concern of the cocoa industry. In the preceding chapter, fifty-eight (58) species of fungi were isolated from mouldy cocoa beans. Though there may not be any visible fungal growth on the bean, spores of these fungi are present on the beans and are capable of growing to cause internal mouldiness under favourable conditions. It is therefore important to prevent or control mouldiness.

Mouldiness in cocoa beans is controlled by handpicking any visibly mouldy beans during drying and storage. This method is very tedious and not effective when large quantities of beans are involved. The use of ionising radiation has been found to be very effective in controlling micro-organisms. Food-borne pathogens such as *Salmonella* have been eliminated from poultry, red meat and seafood using radiation (Urbain, 1986; Nickerson *et al.*, 1983; Nerkar and Bandekar, 1990). The sensitivity of fungi to radiation has been reported in fruits and spices (Barkai-Golan *et al.*, 1969; Ingram and Farkas, 1977; Farkas *et al.*, 1973; Briggs, 1966).

In this study, the effects of ionising radiation on fungi associated with on cocoa beans have been studied with the following objectives:

1. To study the sensitivity of the different fungal species to radiation.
2. To study the relationship between the population of fungi and the effective dose of radiation.

5. 2 MATERIALS AND METHODS

5. 2.1 Determining the sensitivity of fungal species to radiation.

Freshly dried beans from the Cocoa Research Institute Ghana (CRIG), Tafo were used for this study. Ten dried cocoa beans were placed in each petri dish. Every three of such petri dishes were separately treated with ^{60}Co gamma radiation doses of 1 – 6 kGy at the Radiation Technology Centre of the Ghana Atomic Energy Commission. After irradiation, the Petri dishes were kept in sterilised glass containers at a relative humidity of 90%. The beans were observed for visible fungal growth. The number of beans showing fungal growth were counted and expressed as a percentage of the total being irradiated. The different kinds of fungi were then isolated and identified. Unirradiated samples served as control.

5.2.2 Determining the relationship between effective dose of radiation and fungal population

Two batches of dried cocoa beans, - one batch of freshly dried visibly healthy beans from Cocoa Research Institute, Tafo Ghana (CRIG) labelled "Healthy" with no sign of mouldiness and the other, 'mouldy' beans collected from Tema port were used in this study. The beans were arranged in petri dishes as described above except that in this case there were six Petri dishes per treatment. Half of each batch was placed in Petri dishes containing solidified water agar. The other half was placed in Petri dishes without any medium. The beans were irradiated at the doses of 0 -10 kGy. After irradiation, the beans were stored for seven days at 28 ±0.2 ^{o}C. During storage, those on water agar were observed for growth. The number of colonies appearing were counted and the percent infection determined.

For the Petri dishes without water agar, the number of surviving spores (fungal colony forming units) were determined after 7 days using the Serial Dilution method (Staples, 1973). Briefly 10 beans in each treatment were transferred into separate conical flasks containing 100 ml of 0.1 % peptone solution. The flasks were placed on a shaker for 30 min. One millilitre of the spore suspension obtained was taken through serial dilution, until the desired dilution was obtained, Plating of 1ml aliquots was done using the pour plate method using Potato Dextrose Agar. Plates were incubated at 28 ±0.2 ^{o}C was for 7 days during which they were examined and the colonies of fungi appearing were counted. The dose of radiation giving the least count was selected as the most effective dose.

5.3 RESULTS

5.3.1. Effect of radiation on fungi

The response of the fungi present on cocoa beans to radiation has been presented in Table 1. Generally, the number of fungal colonies as well as the different species of fungi decreased with an increase in the radiation dose applied. In the unirradiated cocoa beans, there were 27 different species present. Twelve belong to the genus *Aspergillus*. A dose of 0.5 kGy did not eliminate any of the species; all the 27 species were present. At 1 kGy, 4 species 3 *Fusarium* and *Trichoderma viride* were eliminated. A dose of 2 kGy eliminated 11 species including three *Penicillium*, *Paecilomyces varioti*, *Syncephalastrum* sp. and *Absidia* sp. At 3 kGy, 12 species were eliminated and at 3.5 kGy, 15 species were eliminated. When the dose was increased to 4 kGy, 19 species disappeared. At 4.5 kGy, 21 species were

eliminated while at 5 and 5.5 kGy, only 3 species, *A. flavus, A. tamarii* and a yeast, *Saccharomyces* sp. were identified. At 6 kGy, only *Saccharomyces* sp. remained.

5.3.2 Effective dose of radiation and fungal population

The number of surviving spores and percent infection determined on healthy and mouldy beans have been presented in Table 15. Both methods used to assess fungal growth showed that there were viable fungal spores on the "healthy" as well as "mouldy" beans but the numbers associated with the mouldy beans were higher. The serial dilution method showed that a dose of 4 kGy reduced the number of survivors from 5.5×10^4 to 1.6×10^1 in the "healthy" beans while in the mouldy beans the number was reduced 4.5×10^{10} to 6.0×10^7. The whole bean culture method also showed that 88.9 % of the visibly healthy beans had fungal growth and 100 % of the "mouldy" unirradiated beans. However, there were more growth per bean on the "mouldy" than on the "healthy" beans. At 5 kGy, no growth occurred on the "healthy" beans but the "mouldy" had 55.5 % (5 out of 9 beans) showing growth and the number of survivors from $4.5 \times 10^{10} - 2.2 \times 10^5$. At 6 kGy, 33.3 % (3 out of 9 beans) of the mouldy beans had fungal growth and at 7 kGy, 11.1 % (1 out of 9) had fungal growth. For the 'healthy' beans, a dose of 5 kGy killed all the fungi while a dose of 8 kGy was needed for the destruction of fungi associated with the mouldy beans.

Table 14. List of Fungi Isolated from Cocoa Beans after Irradiation

Fungi	Dose in kGy										
	0	0.5	1	2	3	3.5	4	4.5	5	5.5	6
Aspergillus flavus	+++	+++	+++	+	++	++	+	+	+	+	
A. tamarii	+++	+++	+	++	++	++	+	+	+	+	
A. wentii	+++	++	+	+	+	+	+	+			
A. panamensis	+++	++	+	+	++	++	+				
A. ustus	++	+	+	+	+						
A. glaucus	+++	+	+	+	+						
A. niger	+++	+++	+++	+++	++	+	+	+			
A. ochraceus	+++	++	++	+	+	+					
A. versicolor	++	+	+	+							
A. nidulans	+	+	+	+	+						
A. candidus	+	+	+	+	+	+					
A. fumigatus	+	+	+	+	+						
Penicillium digitatum	++	++	++								
P cyclopium	+	+	+								
P chrysogenum	+	+	+								
Mucor pusillus	+++	++	+								
Rhizopus sp.	+++	++	++	+	+						
Absidia sp.	+++	++	+								
Curvularia	+	+	+	+	+	+	+				
Saccharomyces sp	+	+	+	+	+	+	+	+		+	+
Fusarium moniliforme	+	+									
F. oxysporum	+	+									
Fusarium sp.	+	+									
Syncephalastrum sp.	+	+	+								
Cladosporium sp.	+	+	+	+	+	+	+	+			
Paecilomyces sp.	+	+	+								
Trichoderma viride	+	+									

+++ More than 50 Colonies per plate; ++ About 30 Colonies;
+ Less than 10 colonies; Blank spaces mean no growth.

Table 15. **The determination of the inactivation radiation dose for fungi present on dried cocoa beans of different microbial quality using two culturing methods.**

Serial Dilution method (No. of survivors)			Whole Beans plating method (% infection)	
Dose (kGy)	**Healthy beans**	**Mouldy beans**	**Healthy beans**	**Mouldy beans**
0	$5.5x10^4$	$45x10^{10}$	88.9	100
4	$1.6x10^1$	$6.0x10^7$	22.2	100
5	0	$2.2x10^5$	0	55.5
6	0	$3.4x10^2$	0	33.3
7	0	$2.3x10^0$	0	11.1
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0

5.4 DISCUSSION

When dried cocoa beans were exposed to radiation the population as well as the different species of fungi decreased with an increase in the dose of radiation applied. Radiation eliminated all the moulds at 6 kGy. The different species responded differently. *Trichoderma viride, Syncephalastrum* sp. and the three species of *Fusarium* were eliminated at 1 kGy and were therefore more radiation sensitive than the two *Penicillium* species *P. digitatum* and *P. chrysogenum* which were eliminated at 2 kGy.

Cladosporium, Curvularia and *Aspergillus niger* which were eliminated at 4.5 kGy were more sensitive to radiation than *A. flavus.*

The *Aspergillus* group were comparatively more radiation tolerant as they required a minimum dose of 3.5 kGy before elimination. In a study on wheat, 3 kGy was required to completely inactivated *Aspergillus, Rhizopus* and *Absidia* (Mohyuddin and Skoropad, 1979). Among the *Aspergillus* group, there were still differences in their sensitivity to radiation. For example, *A. flavus* and *A. tamarii* were still present at 5.5 kGy while the others were not. Ito *et al.,* (1973) also concluded from studies on *Aspergillus* on rice, maize, milo and wheat that the doses for inactivation of spoilage moulds of cereal grains should be 5 - 6 kGy.

Yeast was also present at 5.5 kGy. The effective dose against yeast has been found to be between 6.5 to 20 kGy (IAEA, 1982), and that of most fungi between 2.5 and 6 kGy. The minimum dose required for effective inhibition of post harvest fungi is 1.75 kGy (Sommer and Fortlage, 1966).

Both methods of assessing the rate of fungal infection showed that radiation is effective in eliminating fungal growth from dried cocoa beans (Table 2). The 'healthy' beans, however, required a lower dose than the mouldy ones. In the whole bean culture method, there were more fungal colonies on the mouldy beans and the growth covered the whole bean. The freshly dried beans however, had less number of fungal colonies on them (5.5×10^4) compared to the mouldy ones (4.5×10^{10}) (Table 2). A dose of 4 kGy was enough to reduce the number of colony forming units (cfu) to less than 10 / g of cocoa (3 log cycle reduction). The mouldy beans, however, needed a higher dose of 7 kGy in order to obtain less than 10 cfu. The inactivation dose for the "healthy" beans was 5 kGy and that of the mouldy ones 8 kGy. These two doses ensured that there were no surviving fungi associated with the beans. Both methods of assessing fungal infection confirmed that the inactivation dose for the irradiation of the two groups of beans were influenced by the initial population of fungal spores.

The biological effect of ionising radiation can be due to both direct action with critical cell components and indirect action with molecular entities formed as a result of hydrolysis of water. Ionising radiation is capable of causing chemical changes in micro organisms with the DNA being the most critical target. It is generally assumed that the inactivation of micro organisms by ionising radiation is a result of damage to their DNA. The response of fungi i.e. its resistance to radiation in general depends on; the nature and amount of direct damage produced, the number, nature and life-time of radiation generated reactive chemical entities and the inherent ability of either to tolerate or repair accurately,

the influence of the intracellular and extra cellular environment on the above factors (WHO, 1999). Other factors are radiation dose, the species and strains of fungus, the stage of growth, the concentration or number of organisms present, the chemical composition and the physical state of the medium in which the micro-organism is found, and post-irradiation storage conditions (Thornley, 1963; WHO, 1994, Diehl, 1990). Fungi are capable of repairing many of the different breaks which occur in the DNA strands from radiation damage (Grecz *et al.,* 1983). A number of observations suggest that the inherent differences in the radiation resistance of various species reflect mainly the differences in their capacity to repair radiation damage (Technical IAEA, 1982; Djefal, 1993). It is generally believed that fungi that are radiation sensitive cannot repair double strand breaks whereas radiation-resistant species have some capacity to do so (Grecz *et al.*, 1983).

All moulds were controlled by radiation. The two studies in this chapter have demonstrated that mouldiness in cocoa can be controlled effectively by applying ionising radiation.

CHAPTER SIX

6.0 STUDIES ON THE COMBINATION OF HEAT AND RADIATION ON *ASPERGILLUS FLAVUS* INOCULATED ON DRIED COCOA BEANS

6. 1 INTRODUCTION

In the preceding chapter, ionising radiation was used to effectively control mouldiness in dried cocoa beans. *A. flavus* was one of the three relatively radiation resistant fungi that had to be exposed to a dose of 6 kGy before inactivation. Increasing the dose of radiation may cause the development of off- flavours in the beans and their products. By combining irradiation with other preservation technologies, a lower radiation dose can be used to achieve the required objective and thus preserve product quality.

Researchers have investigated the use of heat and radiation for the control of fungi (Langerak and Canet- Prades, 1979; Mohyuddin and Skoropad, 1970). There are reports on the combination of heat and radiation for controlling fungal infection in stored products where the combined treatment was more effective than radiation alone (Farkas, 1990; Noomhorm et *al.*, 1998). Mild heat followed by low dose radiation has been used for fresh fruits and vegetables to extend shelf-life and preserve product quality (Farkas, 1990; Thomas, 1986). Hot water dipping (a form of moist heat) at 50 ^{0}C for 5 min before irradiation at 1 kGy delayed the incidence of fungal spoilage in grapes (Padwal –Desai *et al.*, 1973). The heat is expected to sensitize the fungi to radiation so that a lower dose of radiation may be used to obtain a synergistic effect (Padwal-Desai *et al.*, 1976).

Before applying this technology on cocoa beans it was necessary to study its effect on *A. flavus in vitro*. *A. flavus* which was found to be radiation

resistant is also known to produce aflatoxin though there seem to be conflicting reports about the production of the toxin in cocoa. In this chapter, therefore, the effect of a combination of heat and radiation to prevent mouldiness in cocoa have been studied using *A. flavus* as a test organism. The effect of post-irradiation storage conditions and the possible production of aflatoxin are also presented.

The objectives of the studies were:

1. To study the effect of moist heat on the spores of *A. flavus*.
2. To study the effect of moist heat and radiation on *A. flavus* spores.
3. To study the effect of moist heat, radiation and short term storage (28 days) on spores of *A. flavus* inoculated on cocoa beans.
4. To study the effect of moist heat, radiation and long term storage (4 months) on spores of *A. flavus* inoculated on cocoa beans.
5. To investigate the possible presence of aflatoxin in stored irradiated cocoa beans.

6. 2 MATERIALS AND METHODS

6.2.1 *In vitro* studies of the combination of heat and radiation on aqueous spore suspension of *A. flavus*

Heat Treatment

A. flavus (NRRL 5906) used was obtained from the Public Health Institute, Bilthoven, the Netherlands. A spore suspension of *A. flavus* was prepared by adding 20 ml sterile solution of Tween 80 (2g/L) to a 7 day old culture of *A. flavus* on Czapek Dox Agar in a 250 ml Erlenmeyer flask. The suspension was manually shaken for 1 min, poured into a sterile centrifuge tube and centrifuged twice at 4000 rpm for 15 min. Each time the pellet was

resuspended in fresh Tween solution and agitated with Vortex mixer. The concentration of the spore suspension was adjusted to 10^7 per ml of water. Heating tubes containing 27 ml of Tween solution each were kept at temperatures of 40 to 60 °C in "Tamson" water baths. A 3 ml aliquot of the spore suspension was added to each heating tube to obtain a concentration of 10^6 spores per ml. Tubes kept at 20 ^{0}C were used as control. Heat was applied to the spores for 2.5, 5 and 10 min.

Irradiation Treatment

Tubes containing 4 ml aliquots of the heat treated spore suspensions were irradiated within 30 min after the heat treatment. The tubes were placed in a cryostat which was kept at 20 ^{0}C and kept rotating throughout the irradiation period to facilitate equal dose distribution. The dose rate was 2.58 kGy/hr as determined using Fricke dosimetry (IAEA, 1977). The doses applied were 0, 0.5 and 1.0 kGy. After irradiation, serial dilutions of the spore suspensions were prepared and 1 ml aliquots placed in Petri dishes with about 9 ml Czapek Dox agar. The plates were incubated at 28 °C for 5 days. The ability of the spore to form colonies was used as the criterion for survival. The number of colonies appearing after 5 days were recorded and the log number of survivors plotted against the radiation dose applied.

6.2.2 Effect of heat and radiation on *A. flavus* inoculated on dried cocoa beans.

Freshly fermented and dried cocoa beans used for studies in this chapter were obtained from the Cocoa Research Institute, Tafo, Ghana. The moisture content of the beans was determined gravimetrically by International Standards Organisations Methods (ISO 2291). Typically, 10 g of ground beans were dried

at 103 °C for 16 hr and the difference between the weight before and after drying was expressed as a percentage of the original weight.

Inoculation of cocoa beans with *A. flavus*

A. flavus inoculum used for subsequent experiments was obtained by inoculating some broken cocoa beans in 500 ml conical flasks by a modified Lillehoj method (1976). The inoculated cocoa was incubated at 28 °C for 10 days. Eight beakers were filled with 600 g of cocoa beans each and *A. flavus* culture in one inoculated flask was mixed thoroughly with beans in 4 beakers. The beakers were covered with aluminum foil to prevent contamination by external fungi. They were kept at 28 °C for 24 hr to allow the fungal spores adhere to the bean surface.

Moist heat treatment of inoculated beans

There was no equipment available for providing moist heat. It was therefore decided that a heat treatment chamber with facilities for moist heat should be built. A method for the heat treatment was therefore developed by modifying conditions in an oven. (Plate 78). The equipment consists of an oven in which was placed a humidifier (D) that sprayed water particles into the environment through the nozzle (B) when the humidifier was off (Plate 78). The Relative Humidity within the oven environment was always <40 % and this was taken as the Dry/Low Humidity. High Relative Humidity (>85) within the oven environment was observed when the humidifier was turned on. Whenever the oven was turned on, the environment within the oven was heated. With the humidifier turned off (i.e. Dry/Low R.H.) dry heat was produced. Moist heat results when the humidifier and the oven were turned on simultaneously. The Dry or moist heat generated inside the oven was circulated by two fans, Fan

(A) fixed on the roof of the chamber and Fan (C) placed close to the heater and humidifier. The R.H. in the oven was measured by placing a psychrometer (E) in the oven.

Moist heat was generated with the equipment at different oven temperatures depending on the oven settings. Some of the cocoa beans in the basket had holes drilled in them into which were placed thermocouples to measure the temperatures within the beans. Thus two temperature readings were taken – one in the oven environment and the other within the cocoa beans. The relative humidity in the oven was also recorded. To evaluate the performance or otherwise of the equipment, the temperature and humidity were recorded whilst the time taken to reach the desired temperature was also noted. The moist heat equipment was evaluated for its performance using a combination of treatments.

6.2.2.1 Heat treatment and irradiation of inoculated beans followed by short storage at 75 & 90 % R.H.

Each batch of inoculated beans was placed into the metal basket (F) of the equipment and subjected to either dry heat (< 40 % R.H.) or moist heat (>85 % R.H.) at 60, 70 or 80 °C respectively for 30 min. Each sample was then placed in polypropylene bag (15 x 30 cm) for irradiation immediately after the heat treatment. The dose rate was 0.055 Gy/min determined by using 3 mm clear Perspex put into the products which were arranged in an aluminum box lined with 5cm thick polystyrene. The beans were irradiated at 1.5 and 3.5 kGy. After irradiation, each sub-sample was placed in Petri dishes. The Petri dishes were placed in polyethylene bags. Half of the sub samples were stored at 75 % and the other half at 90 % R.H for 28 days. The moisture content, microbial count and percent infection were monitored at weekly intervals

during storage. A factorial design was employed with the following factors and levels.

Oven Conditions

- Temperatures 20 °C, 60 °C, 70 °C, 80 °C.
- Relative Humidity Dry (<40 %) Moist (>80 %)
- Radiation Dose 0, 1.5, 3.5 kGy

Storage Conditions

- Storage Humidity 75 %, 95 %
- Storage Time 0, 28 days.

In order to determine the survival of *A. flavus*, 25 g of cocoa beans were added to 100 ml of sterile 0.1% peptone solution in a 250 ml Erlenmeyer flask and shaken in an Orbital shaker at 140 rpm for 30 min. Serial dilutions were made from the spore suspension obtained and 1ml aliquots of each dilution plated using Oxytetracycline Glucose Yeast Extract Agar (OGYE) prepared from 20 g glucose, 20 g agar, 5 g yeast extract powder and 0.5 g oxytetracyline. Counts per gramme of cocoa were calculated and log of the values were plotted against the dose.

6.2.2.2 Heat treatment at 80 ^{0}C, irradiation and long term storage of inoculated cocoa beans at 80 % R.H.

To evaluate the effect of a longer storage period on the treated beans, another study was conducted in which the moist heat at 80 ^{0}C for the duration of 30 min was applied but the doses were 0, 3.5 and 4.0 kGy for better effect. Storage was in woven polypropylene bags measuring 15 x 30 cm instead of petri dishes and at 28 °C and the storage humidity of 80 % R.H for a duration was for 4 months. During storage, the moisture content and number of *A.*

flavus colonies present were determined at monthly intervals and the weight of the beans at two weekly intervals for 4 months The experimental design was factorial with following treatments a) Radiation dose (0,3.5, 4.0) b) Temperature (0, 80 ^{o}C) c) Humidity during heat treatment (<40, >85 %) d) time (0-4 months). The analysis was done by using Stagraphics Software and the means compared using Duncan's Multiple Range Test.

6.2.3 Determination of aflatoxin in artificially - inoculated cocoa beans

The aflatoxin reference standard was obtained from the Food Research Institute, Accra, Ghana. It was a powdered mixture of B_1 G_1 (0.15 μg each) and B_2 G_2 (0.5 μg each). The mixture was dissolved in chloroform to give the concentration of 1.3 μg/ml. All solvents used for the analysis were Analar grade purchased from May and Baker Ltd. Dagenham, England. M-Kiesegel G-Gr. Thin Layer Chromatography (TLC) plastic plates coated with silica gel without fluorescence (Machery Nage and Company Duran Germany) measuring 20 x 20 cm were used for Thin Layer Chromatography.

Treatment of samples

After 4 months storage, extracts from inoculated beans that had been subjected to moist heat at 80 ^{0}C and radiation treatment at 4 kGy and stored at 80 % R.H. above were analysed for the presence of aflatoxin using Thin Layer Chromatography.

In another study, freshly fermented dried beans were inoculated as already described with *A. flavus* isolated from mouldy cocoa beans. After surface sterilising with 70 % ethyl alcohol, the inoculated beans were divided into five batches of 200 g. Each batch was given one of the following treatments:

- Irradiation at 10 kGy, no storage
- No irradiation, no storage
- Storage for 4 weeks followed by irradiation
- Irradiation before storage at 90 % R.H.
- No irradiation but stored.

In addition, two batches of beans which had not been artificially inoculated were stored, one after irradiation, the other without irradiation served as control. The different fungi appearing during storage were identified.

Extraction, Spotting and development of aflatoxin

Extracts from cocoa samples treated above were prepared by a modified method (FAO, 1990). A 50 g sample of ground cocoa was blended with 100 ml methanol and 10 ml distilled water in a warring blender for 3½ mins. To the slurry, 30 ml of distilled water was added and filtered through Whatman's paper (No. 12). A 70 ml aliquot of the clear filtrate was mixed with 20 ml distilled water and 90 ml chloroform in a 250 ml separatory funnel. The bottom layer of chloroform was collected, filtered and dried over anhydrous sodium sulphate in a rotary evaporator. The residue was redissolved in 1 ml chloroform and used for the detection of aflatoxin by using bi-directional thin layer chromatography. There were three extractions per treatment.

Two lines were drawn 2 cm, and 3 cm from the border edge of the TLC plate. Spotting was done with a micro-syringe. Aliquots (1-10 μl) of the standard were spotted on a plate to determine the limit of detection. Aliquot samples ranging from 5 to 25 μl were spotted interspersed with spots of the standard, on the 3cm line and allowed to dry. The plates were developed upside down in a tank containing diethyl either (100 %) to further clean the samples. When the solvent

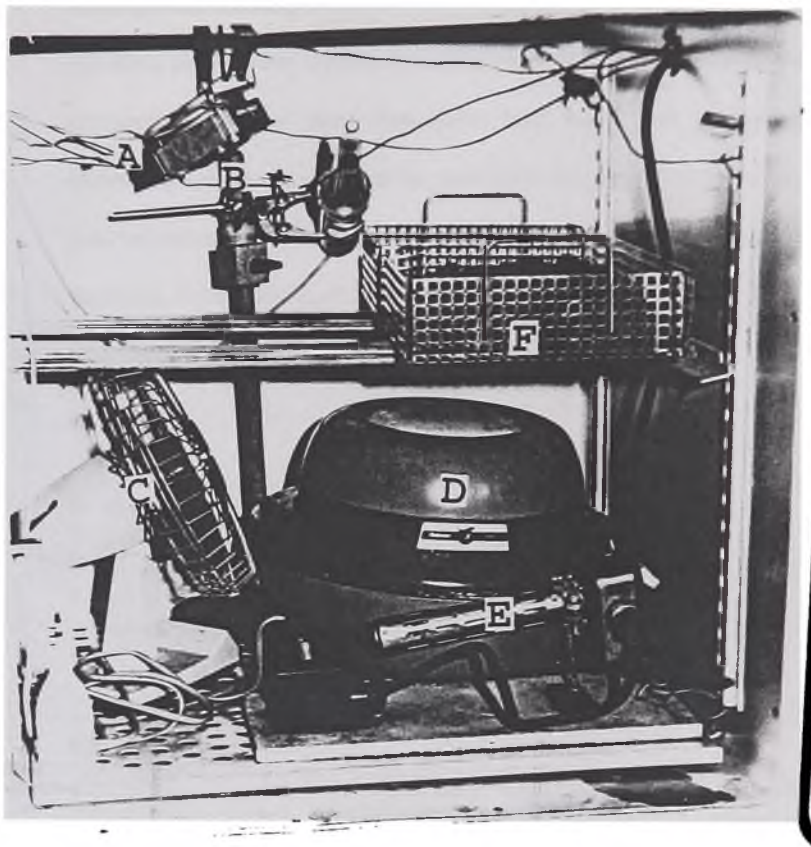

Plate 78 The heat treatment of cocoa in an oven with conditions simulated to provide moist heat.

A.	"Picollo" fan	D.	Humidifier
B.	Psychrometer sensor	E.	Sprayer of humidifier
C.	Table fan	F.	Metal container with beans

front was almost at the edge, the plate was removed and allowed to dry. The 2 cm line was cut off to remove impurities. The plate was then developed normally using chloroform/acetone (90:10) till the solvent was almost at the edge.

Aflatoxin detection

Air-dried plates were exposed to uv/light of 350 nm wavelength. Aflatoxin appeared as blue or green flourescent band on a dark background. The chromatographs were obtained by photographing the bands in the dark with polariod camera. The Rf values were determined and compared to those of the standards. Concentration of the toxin was determined visually by comparing the intensity of fluorescence and then using the formula below

$$\mu g/kg = \frac{SxYxV}{X \times W} \quad \text{where}$$

S= µl of aflatoxin standard equal to sample
Y= Concentration of the aflatoxin standard in ug/ml
V= µl of final dilution of extract
X =µ l of sample extract giving a spot intensity equal to S
W= mass of sample represented by the final extract in g.

6.3 RESULTS

6.3.1 Effect of the combination of heat and radiation on aqueous spore suspension of *A. flavus*

Results of application of moist heat only at different temperatures and durations to spore suspensions of *A. flavus* are presented by fig 6. Applying heat up to 55 ^{0}C for duration of 2.5 min was ineffective in reducing in the number of surviving spores of *A. flavus*. Increasing the temperature from 55 to 58 ^{0}C gave a rapid reduction of 3.2 log cycles (decrease from 5.2 to 2) which was significant ($p<0.05$). Heating between 58 and 60 ^{0}C caused a gradual reduction from 2 to less than 1 log cycle. Heat applied for 5 min resulted in 1.2

log cycles reduction between 50 and 55 °C. A rapid decrease of 3 log cycles was obtained at 55 and 58 °C, and at 59 °C the surviving spores were reduced to less than 1 log cycle. The heating for 10 min gave results similar to that of 5 min up to 58 °C but at 59 °C, the spores were totally eliminated. In general, heat treatment below 50 ^{0}C did not have any effect on *A. flavus* spores. In the range of 50 – 60 ^{0}C, the heating for 10 min gave the best results and was lethal at 59 ^{0}C. Fig. 7, Tables 16 and 17 summarise the effect of heat for 2.5, 5 and 10 min durations and radiation on the survival of *A. flavus* spores. In Fig. 7, the application of radiation alone (20 ^{0}C) was more effective in reducing the number of survivors than heating alone (0 kGy) at all the temperatures and durations applied. In general, the combined treatment gave a better reduction than heat and radiation applied separately for all the durations. Heat treatment up to 52 ^{0}C followed by a radiation dose of 0.5 kGy resulted in a gradual reduction .Then there was a significant reduction of 2.5 kGy. Heat treatment at 55 ^{0}C combined with either 0.75 kGy or 1 kGy radiation eliminated all spores and gave a 5 log cycle reduction .Heating at 52 ^{0}C followed by 1 kGy gave a synergistic effect. Total inactivation of fungi was attained at 55 ^{0}C with an irradiation dose of either 0.75 or 1 kGy radiation.

When *A. flavus* spores were heated at 40-50 °C for 5 min. before irradiation, the temperature of heat treatment did not have much effect on the number of surviving spores. Heating at 52 °C resulted in a rapid significant reduction in the number of spores, a synergistic effect was evident. This effect continued and at 53 °C, heating in combination with radiation the dose of 0.5, 0.75 or 1 kGy resulted in complete inactivation of the spores.

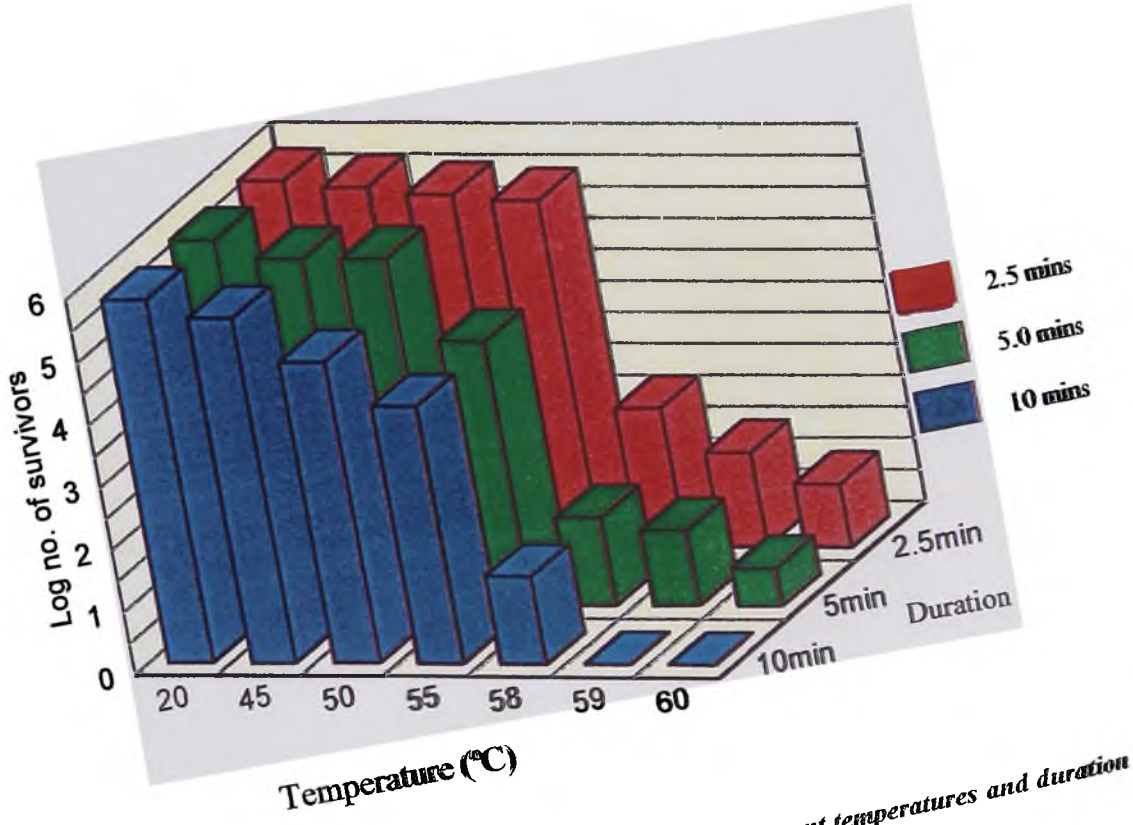

Figure 6. The effect of moist heat applied at different temperatures and duration on the survival of spores of Aspergillus flavus.

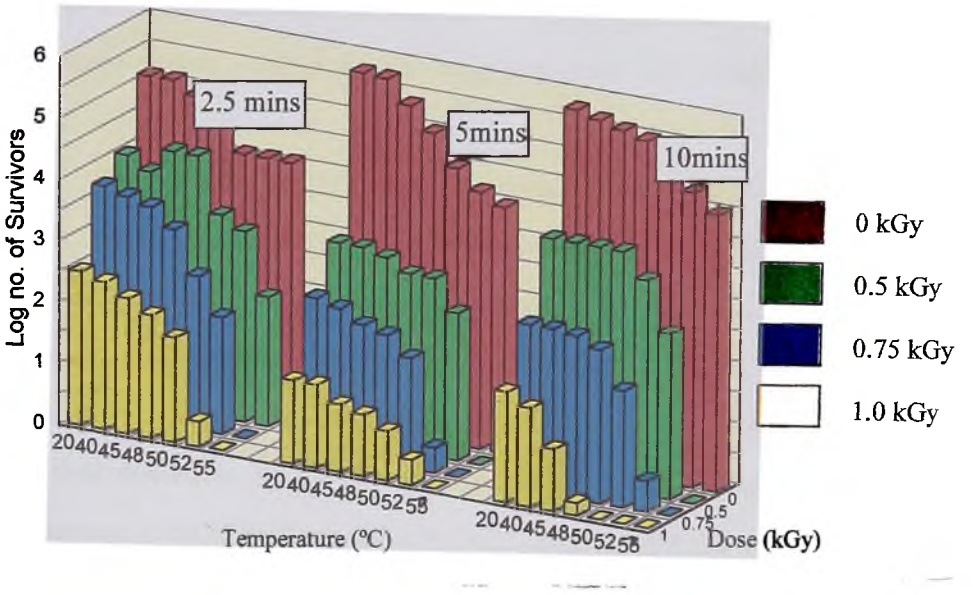

Figure 7. ***The effect of moist heat applied at different temperatures and duration combined with radiation on the survival of spores of Aspergillus flavus.***

When heating was followed by a dose of 0.5 kGy (green) synergistic effect and total inactivation occurred only at 53 ^{0}C. At 0.75 kGy radiation, significant reduction started when heating was done at 52 ^{0}C, giving 4.5 log cycle reduction. At 1kGy a synergistic effect started at 48 ^{0}C. Heating at 50^{0}C for 10 ^{0}C followed by radiation at 1 kGy totally inactivated of the spores.

The analysis of variance of the data presented in fig 7 showed a high significant difference ($p>0.01$) for the interaction of heat and radiation (Appendix). The mean log number of survivors of *A. flavus* presented in Table 16 showed a significant difference from 50 ^{0}C when heating lasted 2.5 min. For the durations of 5 and 10 min, significant decrease in survivors started at 48 ^{0}C.

The D_{10} values (the dose that reduces the population of organisms to 10 %) obtained are presented in Table 17. This complements observations in fig. 8 and Table 16. The D_{10} values gives the slope of the inactivation curve when the log of the number of survivors is plotted against dose on a linear scale. The regression coefficient was 0.9807 to 0.9992. From 50 ^{0}C, there was a general decrease in the D_{10} value with an increase in heating time and temperature. At 50 ^{0}C heating for 2.5 min, the D_{10} was 0.49; at 5 min heating the D_{10} was 0.27 and for 10 min heating the D_{10} was 0.20.

Fig. 8 shows the temperature and humidity variation during the heat treatment at 80 ^{o}C at low (<40 %) and high humidity (>85 %). The cocoa beans reached the required temperature in less than 10 min in all trials. The variation in the temperature during treatment was about 2 ^{0}C after the required temperature had been reached. During the low humidity heat treatment, the temperature in the bean reached equilibrium within the oven after 15 min. The

Table 16. Effect of heat (at different temperatures and durations) and radiation on the survival of *A. flavus* spores (mean ± S.E)

Duration (min) / Temperature (^{0}C)	2.5	5	10
20	3.78 ± 0.53 a*	2.99± 0.94 f	3.35 ± 0.87 k
40	3.62 ± 0.55ab	2.91± 0.95f	3.25 ± 0.89 k
45	3.56 ± 0.60 ab	2.71± 0.92f	3.13 ± 0.96 k
48	3.38 ± 0.64 b	2.57± 0.85g	2.84 ±1.11 m
50	2.95 ± 0.54 c	2.39± 0.76 g	2.45 ±1. 07 n
52	2.51 ± 0.65 d	1.86± 0.89 h	1.98 ± 1.09 o
53	—	0 .99 ± 0.99 i	1.12 ± 1.22 p
55	1.51 ± 0.96 e	----	------
Std.Err.of Diff.	0.26	0.32	0.37

**Each figure is a mean of 4 pairs of 2 replicates per treatment.*
Means followed by the same letters are not significantly different ($p < 0.05$)

Table 17. The effect of combination of heat and radiation on the survival (D_{10}) of *A. flavus* spores. in aqueous medium

Temp. °C	2.5min. heating*		5 min. heating		10min. heating	
	D_{10}	r	D_{10}	r	D_{10}	r
20	0.47	.9686	0.28	.9858	0.25	.9906
40	0.46	.9696	0.24	.9948	0.24	.9986
45	0.50	.9788	0.28	.9919	0.28	.9897
48	0.54	.9329	0.23	.9863	0.19	.9867
50	0.49	.9899	0.27	.9992	0.19	.9928
52	0.33	.9630	0.22	.9821	0.20	.9807
53	—	---	0 .12	.9664	0.11	.9866
55	0.19	.9829	---	----	---	---

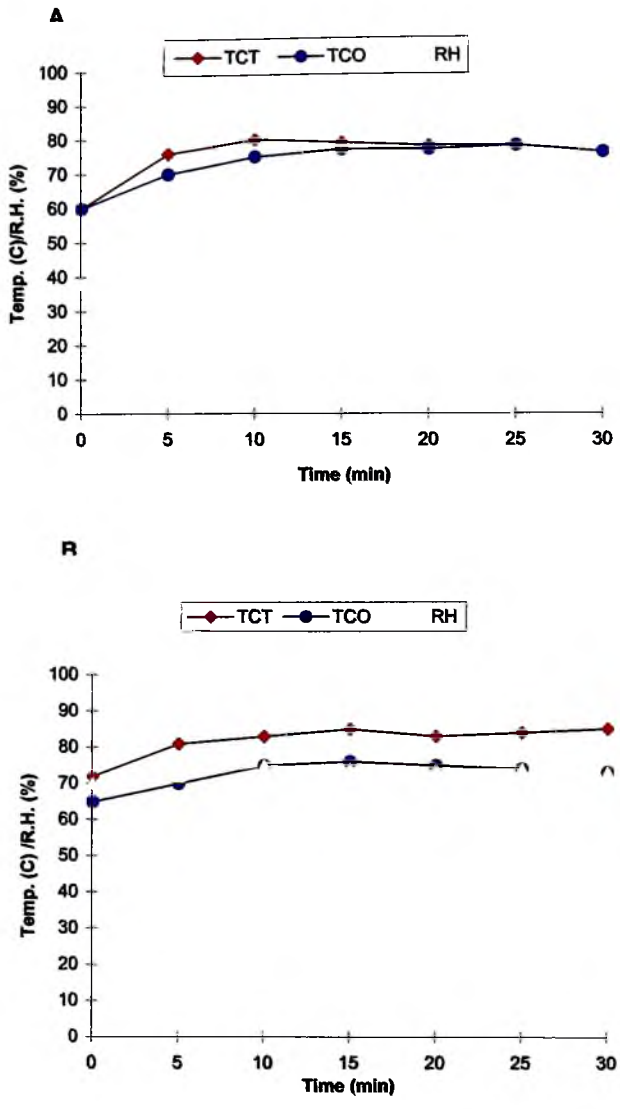

Figure 8. Temperature and relative humidity changes the moist heat equipment during the development of the method of heat treatment at 80 ^{0}C low humidity (A) and high humidity (B).

TCT	Thermocouple in the Tray
TCO	Thermocouple in the Oven
RH	Relative Humility

beans receiving moist heat were however in equilibrium in 10 min.

6.3.2 Effect of heat and radiation on *A. flavus* inoculated on dried cocoa beans.

Table 18 summarises the moisture content of the beans determined before and immediately after moist heat treatment. The moisture content of beans that were subjected to moist heat ranged between 7.0 and 7.59 and those for dry heat between 7.15 and 7.53. The moist heat applied before irradiation did not significantly change the moisture content of the beans.

Fig. 9 shows the effect of treatment at 60, 70 and 80 °C and radiation on the survival of *A. flavus* spores after 28 days storage at 75 % RH. The microbiological determination of the survival of *A. flavus* immediately after the combined treatment at 60, 70 and 80 °C showed that the combination of heat treatment and radiation gave better reduction in the spores than either heat or radiation applied alone (Fig 9). For example, 3.5 kGy radiation at 20 °C reduced the number of survivors from 6.47 to 3.33 (3.4 log cycles). Heat only (0 kGy) at 80 °C reduced the survivors from 6.57 to 5 (1.57 log cycles) with dry heat and to 3.21 (3.36 log cycles) with moist heat. Moist heat at 80 °C followed by radiation at 3.5 kGy reduced the cfu to 1.2 (5 log cycles reduction). It was also evident that moist heat was better than dry heat. Storage at 75% R.H. for 28 days did not affect the number of surviving spores

The effects of the various factors (Temperature, Radiation dose, Storage humidity) influencing survival of *A. flavus* spores based on mould counts have been summarised in Tables 19 as follows.

Table 18. The moisture content of Cocoa Beans after Combination treatment of heat and radiation

Heating Temp. (°C)	Dose (kGy)	Moisture Content
20 Dry	0	7.15 ± 0.08
	3.5	7.51 ± 0.04
	4.0	7.53 ± 0.10
20 Moist	0	7.28 ± 0.46
	3.5	7.15 ± 0.02
	4.0	7.00 ± 0.07
80 Dry	0	7.02 ± 0.02
	3.5	7.15 ± 0.02
	4.0	7.23 ± 0.09
80 Moist	0	7.24 ± 0.03
	3.5	7.25 ± 0.37
	4.0	7.59 ± 0.07

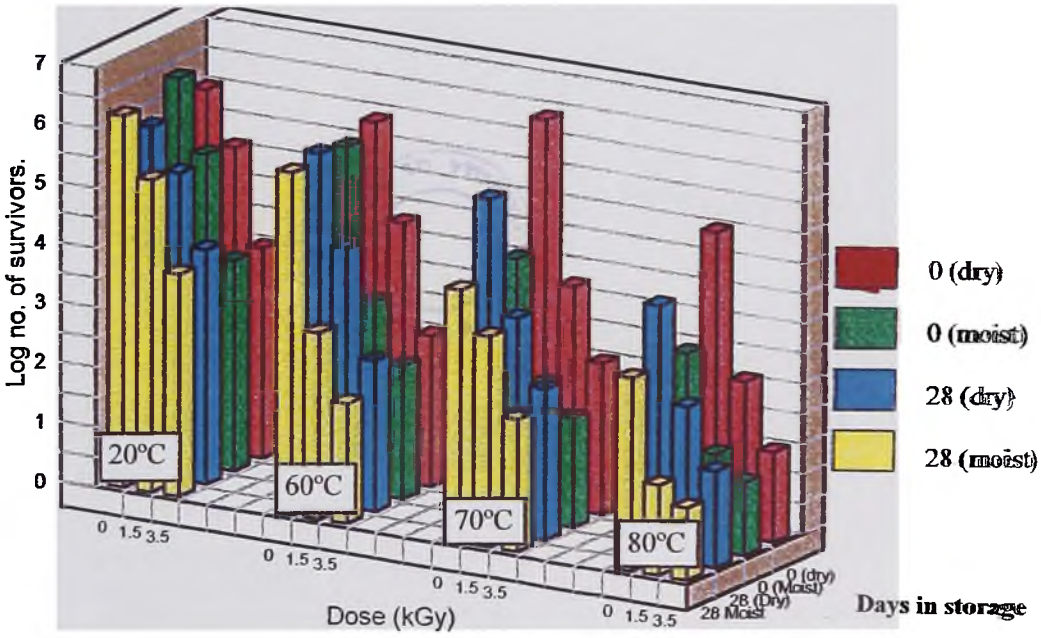

Figure 9. The effect of combined treatment of heat and radiation on the survival of A. flavus inoculated on cocoa beans at 20, 60, 70 and 80 ^{0}C and stored at 75% R.H.

Table 19. The separate effects of a) temperature, b) heating humidity, c) radiation and d) storage humidity on the survival of *A. flavus* on cocoa beans stored at 28°C and at 75% and 90% R.H.

a) Temperature (°C)		b) Humidity during heating	
(%R.H.)			
20	3.48 d	<40	3,32 e
60	2.79 b	>85	2.48 f
70	2.98 c	S.E.D	0.08
80	2.35 a		
*S.E.D	0.11		

c) Radiation (kGy)		d) Storage Humidity (% R.H.)	
0	4.95 i		
1.5	2.30 h	75	2.38 k
3.5	1.45 g	90	3.42 l
S.E.D	0.09	S.E.D	0.08

Mean Values in the same column followed by the different letters are significantly different.
**S.E.D - Standard error of the difference between beans.*

- There were significant differences *(P<0.05)* between the mean log number of survivors. Heating at 80 °C was the most effective temperature.
- Moist (>85 % R.H.) heat was significantly *(P<0.05)* more effective in reducing the number of spores than dry heat (<40 % R.H).
- The difference between the effect of the doses were significant *(P<0.05)*. A dose of 3.5 kGy reduced the number of surviving spores by 3.5 log cycles.
- The humidity under which the beans were stored had an effect on the survival of the spores. The difference between 3.42 and 2.38 was significant *(P<0.05)*.

The interactions between the type of heat applied, temperature, the radiation dose have been summarised in Table 20a. At each temperature selected for the heat treatment, the combined effect of moist heat and radiation gave better lower reduction in the number of spores of *A. flavus* spores *(P<0.01)*. The best control was obtained at 80 °C moist heat followed by 3.5 kGy radiation. Table 20 b summarises the effect of the interaction of storage humidity, temperature and dose. The beans heated at 60 °C, 70 °C and 80 °C and stored at 75 % R.H. gave better control of *A. flavus* spores than at 90 % *(P<0.05)*. The best effect was 80 °C heating followed by 3.5 kGy of radiation and storage at 75 % R.H. The comparison of shelf life of samples stored at 90 % R.H. based on 10 % infection showed that heat alone extended shelf life up to 5 days while heat followed by radiation was more effective in preventing mouldiness than either heat or radiation applied alone at all temperatures

Table 20 a. The interactive effects of humidity during heat treatment temperature of treatment and radiation on the survival of *A. flavus* spores inoculated on cocoa beans and stored for 28 days.

Humidity during Heat Treatment			40%			85%
Radiation Dose (kGy)	0	1.5	3.5	0	1.5	3.5
Temp. (^{0}C)						
20	5.67a*	2.94d	1.64f	5.88a	2.950	1.80f**
60	5.23b	2.78de	1.81f	5.05b	1.00h	0.91h
70	5.96a	2.93d	2.02f	1.84c	1.22gh	1.84f
80	5.20b	2.22ef	1.48fg	2.74d	1.75f	0.75h

**Figures with the same letters are not significantly different (P< 0.05).*

***Figures are log n of number of surviving spores of A. flavus*

Table 20 b. The interactive effects of radiation, temperature and storage humidity on survival of *A. flavus* inoculated on cocoa beans and stored for 28 days.

Radiation Dose (kGy)	0		1.5		3.5	
Storage Humidity	75 %	90 %	75%	90%	75%	90%
Temp. (^{0}C)						
20	5.43b*	6.12a	2.41ef	3.48d	0.90g	2.54e
60	4.47c	5.81ab	1.05g	2.74e	0.69	2.02f
70	4.50c	5.38b	1.81f	2.960e	1.1 0g	2.13f
80	3.52d	4.41c	1.85f	2.13f	0.84g	1.39g

**Figures with same letters are not significantly different (P< 0.01). **S.E.D: Standard error of the meanof temperature/Radiation/Storage humidity*
****Figures are log n of number of surviving spores of A. flavus*

Table 21. The shelf life (days) of cocoa stored at 90 % RH after combination of heat and radiation based on 10 % infection.

Temp.	Humidity		Dose(kGy)		
^{0}C	%		0	1.5	3.5
20	dry	40	9	21	21
	moist	85	10	21	24
60	dry	40	14	18	>28
	moist	85	10	21	>28
70	dry	40	14	22	>28
	moist	85	14	24	>28
80	dry	40	12	21	>28
	moist	85	19	>28	>28

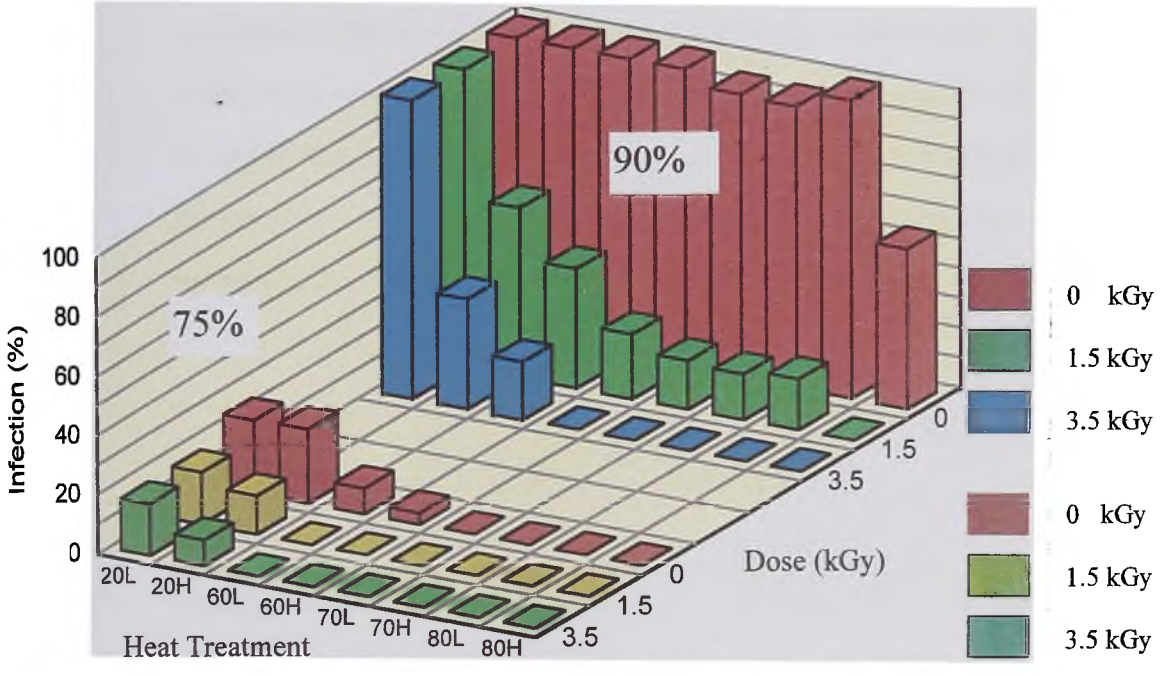

Figure 1 *Percent infection of cocoa beans after inoculation with A. flavus spores and treatment with heat and radiation before storage at 75% and 90% R.H. for 28 days.*

(Table 21). The growth of *A. flavus* was delayed for 28 days or more in samples treated with moist heat at 80 °C followed by 1.5 kGy and in all samples treated with heat at 60, 70 and 80 °C followed by 3.5 kGy radiation.

Fig. 10 represents the percent infection of cocoa beans inoculated with *A. flavus* spores, treated with a combination of heat and radiation and stored at 75% a 90 % R.H. for 28 days. Even though, there were viable spores of *A. flavus*, treated beans stored at 75 % R.H. did not show any visible growth throughout the 28-day period except on the 28th day when there seemed to be some growth on the control, those treated with radiation only and those heat treated only at 60 °C. The rest did not show any infection. At 90% R.H. storage (Fig.10), the following treatment combination did not show any infection throughout the 28-day period;

- 60 °C heat dry followed by 3.5 kGy radiation,
- 70 °C heat (moist & dry) followed by 3.5 kGy radiation,
- 80 °C heat (moist & dry)followed by 3.5 kGy radiation,
- 80 °C moist heat followed by 1.5 kGy radiation.

The rest showed infection but those treated with moist heat always showed less infection than those that received dry heat.

6.3. 2 Effect of Heat treatment, irradiation and long term storage on spores of *A. flavus* inoculated cocoa beans

Results of the effect of the combination of moist heat at 80 ^{0}C for 30 min followed by irradiation and storage at 80 % R.H. for 4 months on the survival of *A. flavus* are presented in Fig. 11 and Table 22. The relationship between the storage humidity and moisture content of beans are also presented in Figs. 12 &

13. The log number of *A. flavus* spores surviving after moist heat treatment at 80 °C and radiation at 3.5 and 4 kGy and storage at 80 % R.H. have been summarised in Fig. 11. The different radiation doses applied have been grouped on the X-axis according to heat treatment as follows:

- 20 L - <40 % R.H. dry, no heat (Control).
- 20 H – no heating, moisture only at >85 % RH.
- 80 L – dry heat (<40 % RH) only at 80 °C.
- 80 H – moist heat (>85 % RH) supplied at 80 °C.

The beans that were heat treated (80 °C) before irradiation had less number of surviving spores of *A. flavus* than those that received only radiation (Fig.11). Those that were exposed to moist heat (>85 %) before irradiation had less number of fungal colonies than those that were treated with dry heat. The number of surviving spores therefore decreased in the following order 20 °C <40 %, 20 °C >85 %, 80 °C <40 %, 80 °C >85 %. Moist heat applied alone at 80 °C was more effective than dry heat at 80 °C. It reduced the number of fungal colonies by 4 log cycles from (10^6 to 10^2) while the count for dry heat remained at 10^6. When moist heat at 80°C was applied before irradiation at 4 kGy, the number of colony forming units was reduced to less than 10 per gramme of cocoa.

The statistical analysis of the number of log number of survivors (fungal colonies) of *A. flavus* showed a significant difference for temperature, relative humidity and dose ($p<0.01$) (Appendix 11). The storage time did not cause any significant change in the colony counts. All the means compared within each parameter were significantly different (Table 22).

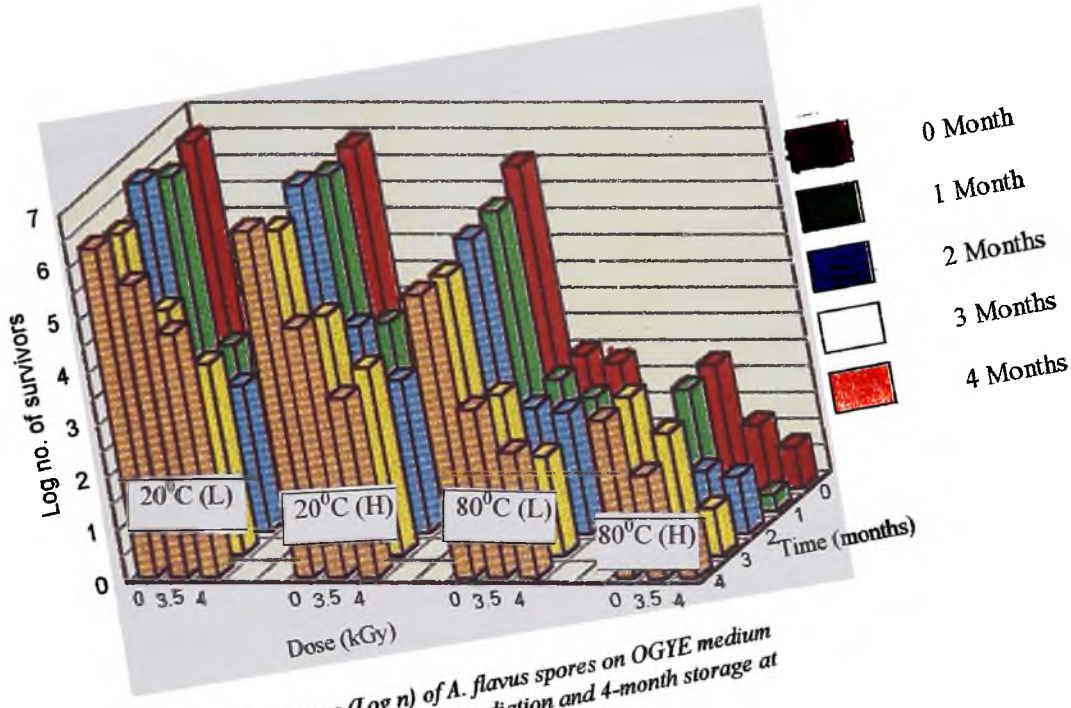

Figure 11 *The number of survivors (Log n) of A. flavus spores on OGYE medium after combined treatment of heat and radiation and 4-month storage at 28°C, 80% RH.*

During the 4 month storage period, the storage humidity was accidentally changed from 80 % to 65 % R.H. This caused about 0.6 % general decrease in bean moisture content in all the treatments in the first month. When the humidity was reverted to 80 % R.H. the moisture content of the beans also increased and there was equilibrium within a month (Fig. 12). This slight change in the moisture content of the beans was also reflected in the change in weight the samples in storage (Fig. 13). During the 1st month (4 weeks) of storage the change in weight was negative coinciding with the reduction in moisture content. The increase in weight was followed a similar pattern till it reached equilibrium within a month (Figs. 12 & 13).

6.3.3 Detection of aflatoxin in artificially - inoculated cocoa beans

The lowest quantity of the reference standard that could be detected by TLC was 2 µl (2.6 ng). When the extract from inoculated cocoa beans that had been treated with heat and radiation and stored at 80% R.H for 4 months were analysed, no aflatoxin could be detected. In the second study, no aflatoxin was detected in all the samples analysed after 10 days of storage at 90 % R.H. After 4 weeks of storage, aflatoxin B_1 was detected in extract of cocoa beans that were inoculated but not irradiated before storage which was not removed by irradiation. (Table 23, Plate 79). The reference standard travelled by the point of origin and separated into 4 fluorescent bands which were identified from their colours and Rfs as aflatoxin B_1, B_2, (blue) G_1 and G_2 (green). Though present in the chromatograph, aflatoxin B_2 did not appear clearly when photographed (occupies space between aflatoxin B_1, & G_1). The Rf values for aflatoxin B_1 was 0.86, and the sample extracts also have Rf value of 0.86 (Table 24).

Table 22. **Multiple Range Test for the Means Number of Survivors of *A. flavus* on cocoa beans after heat treatment and radiation and storage at 80% R.H.**

(a)	**Temperature**
^{0}C	Survivors
80	2.55 g
20	4.62 h
(b)	**Relative Humidity**
%	Survivors
85	3.08 a
40	4.07 b
(c)	**Dose (kGy) Survivors**
4	2.44 c
3.5	3.05 d
0	5.24 e

Figures that are followed by different letters are significantly different.

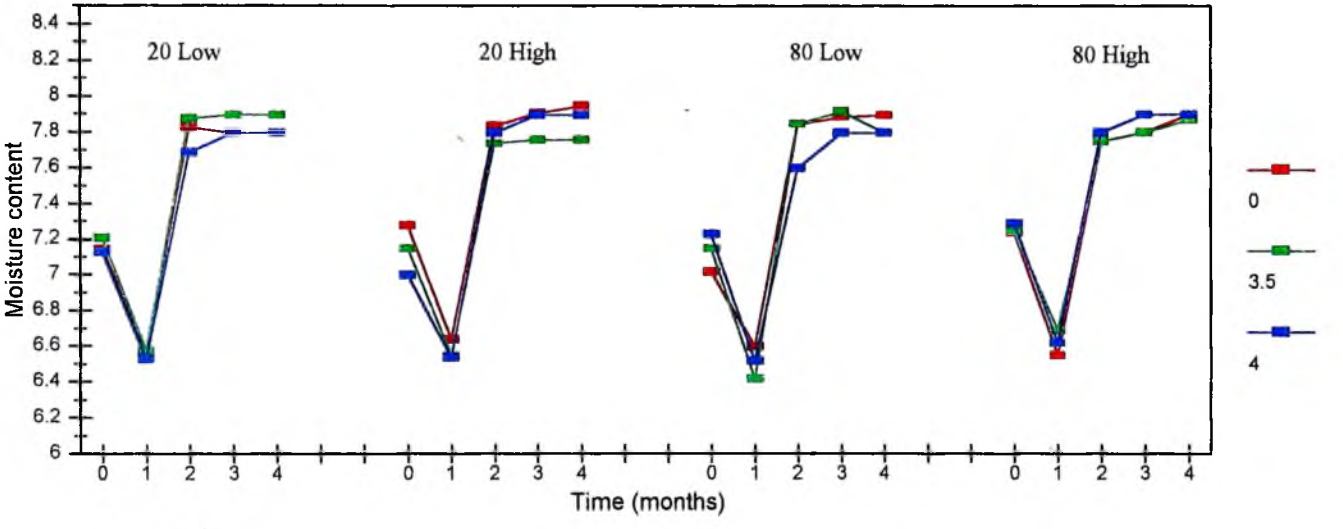

Figure 12 The moisture content of cocoa beans after heat and radiation treatment and during storage at 28°C for four months.

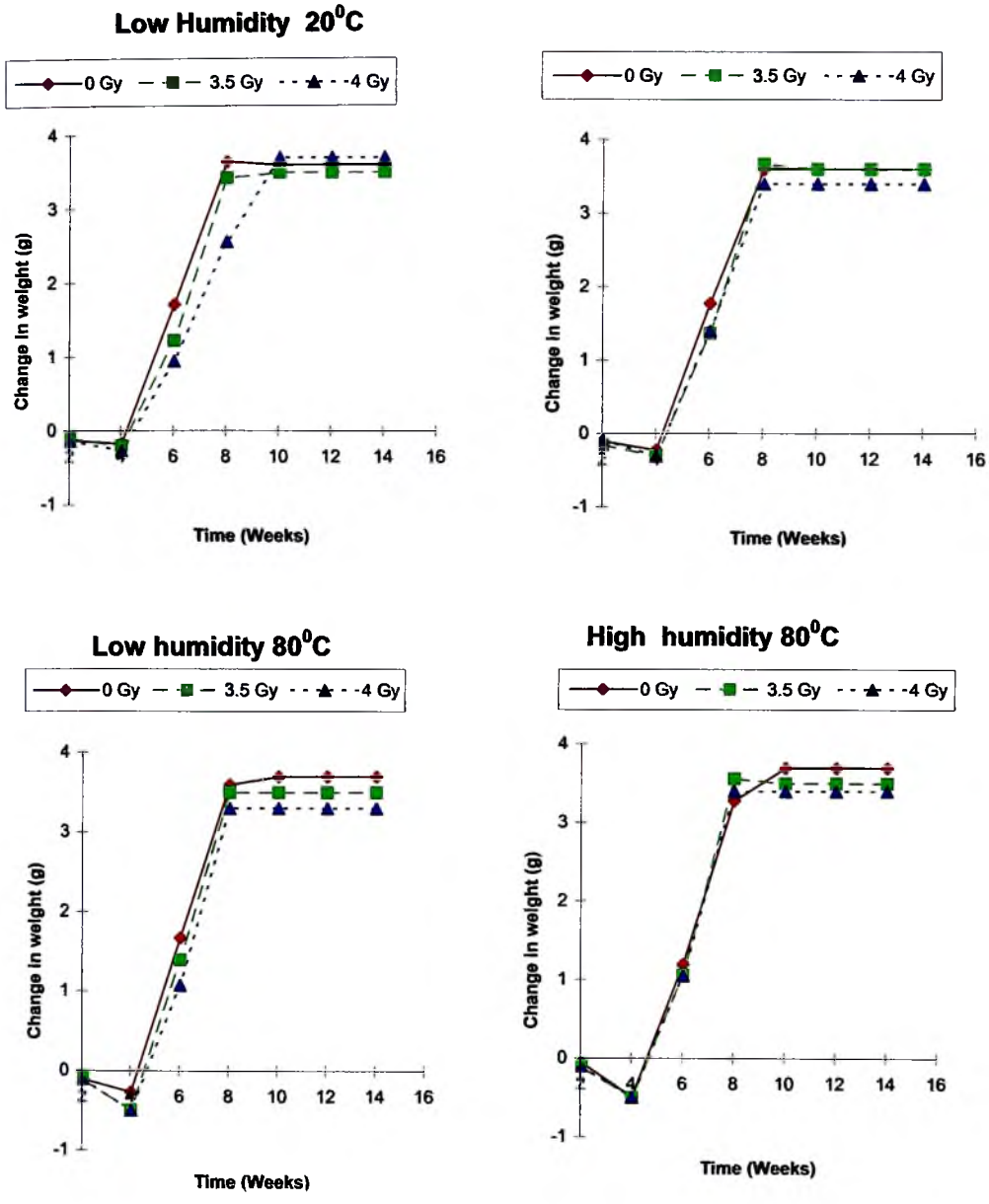

Figure 13. The change in weight of irradiated beans during storage at 28 °C and 65% and 80% relative humidity for 4 months.

Table 23. The detection of aflatoxin from cocoa extract from samples subjected to various treatments using Thin Layer Chromatography

Treatment of Beans	Period of detection		
	0 day	10 days	4 weeks
Inoculated with *A. flavus*			
1. No irradiation, no storage	-		
2. Irradiation, no storage	-		
3. Storage then Irradiation		-	+
3b. Storage then Irradiation			
4. Irradiation before storage		-	
5. No irradiation, before storage		-	+
Non-inoculated beans			
6. Irradiation before storage			-
7. No irradiation, no storage			
Moist heat + radiation + 4 months storage at 80% RH			

- toxin not detected Blank spaces mean no samples analysed
\+ toxin detected

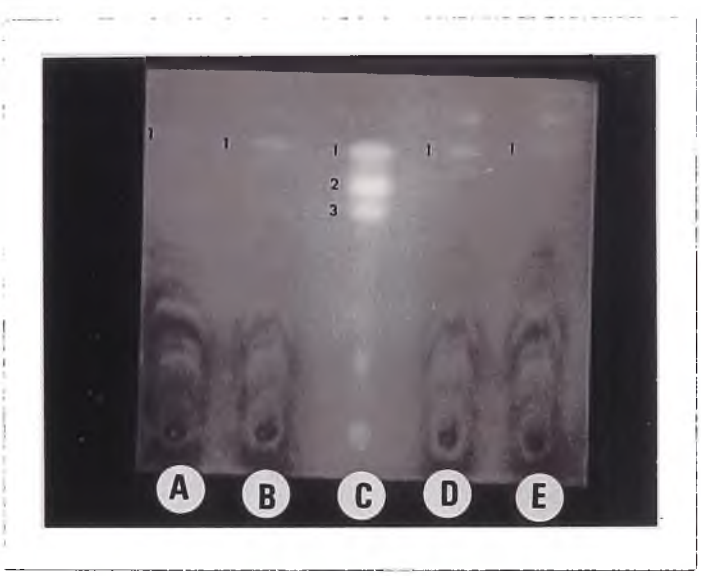

Plate 79 Chromatographs of extracts from cocoa beans inoculated with *A. flavus* and variously treated.

A & D No irradiation but storage for 4 weeks
C Standard reference
B & E Inoculated, storage for 4 weeks before irradiation

1 Aflatoxin B_1
2 Aflatoxin G_1
3 Aflatoxin G_2

Table 24. The Rf values of aflatoxins present in the reference standard and the unknown samples.

Aflatoxin	Rfs			Average	S.D
	Rep 1	Rep 2	Rep 3		
B_1	0.86	0.88	0.83	0.86	0.03
G_1	0.78	0.81	0.78	0.80	0.02
B_2	0.68	0.78	0.74	0.76	0.02
G_2	0.68	0.71	0.67	0.68	0.02
Sample 1	0.86	0.88	0.83	0.86	0.03
Sample 2	0.86	0.88	0.83	0.86	0.03

Sample 1 Extract from inoculated cocoa after 4 weeks storage

Sample 2 - Extract from inoculated cocoa after 4 weeks storage and irradiation

$Rf = \frac{X}{Y}$ where X = distance from spotted point to the aflatoxin band.

Y = distance from spotted point to the solvent front

When the intensities of the fluorescence of the 25 µl of the unknown sample was compared visually to those of various concentrations of the reference standard, they were found to be similar to that from the spot of 2.5 µl. which was 1 µ g / kg of aflatoxin B_1

ug/kg of unknown sample $= \frac{2.5\mu L \times 0.5\mu g \times 100\ \mu l}{25\mu l \times 100\mu l \times .05\ kg.} =$ 1µg/kg.

The uninoculated sample that became mouldy in storage contained *A. flavus, A. candidus, A. niger, A. carbonaceous, Mucor* sp., *Fusarium* sp. and *Penicillium cyclopium*.

6.4 DISCUSSION

Heat treatment below 50 ^{0}C did not have any effect on *A. flavus* spores. In the range of 50 – 60 ^{0}C, heating for 10 min gave the best results and was lethal at 59 ^{0}C. The response of *A. flavus* to heat *in vitro* was similar to that obtained by Langerak and Canet-Prades (1979) who observed that the spores of *Penicillium expansum* were not much affected by heat till 49 ^{o}C, after which the survival of the spores decreased further with an increase in temperature. Results obtained when heat was applied alone agrees with the findings of Padwal-Desai *et al.* (1976) on non-germinating spores of toxigenic strain of *A. flavus.* It was evident from the study that the temperature and the duration of the heat treatment influenced the effectiveness of the combined treatment on the spores of *A. flavus.* Padwal-Desai (1976), also reported that the temperature of heat was more effective than the duration as shown in the results of the current study.

Moist heat treatment was more effective in reducing the number of spores of *A. flavus* than radiation or heat applied separately. The best combination for each heating time were:

- 2.5 min – 55 ^{0}C & >0.75 kGy ;
- 5 min - 53 ^{0}C & >0.5 kGy ;
- 10 min – 50 ^{0}C & 1.0 kGy.

The choice of one may depend on the economics of the treatment. A D_{10} value of 0.4 kGy was reported for irradiated aqueous suspension of *Aspergillus parasiticus* (NRRL 3145) (Sharma *et al.,* 1980). For several fungi, mild heat has been found to sensitise them to radiation resulting in synergism (Ben-Arie and Barkai-Golan, 1969; Poisson and Cahagnier, 1969). The synergistic effect obtained agrees with the findings of Padwal-Desai *et al.* (1979), on heat treatment before irradiation of non-germinating *A. flavus* spores. The synergism of the combination of heat and radiation could be attributed to the fact that heat acts on the molecular structure of the cells, slowing down enzymatic activity and eventually denaturing the enzymes of the cells and producing a lethal effect at higher temperatures (Grecz *et al.,* 1967). Radiation damages the DNA causing a lethal or sublethal effect. Applying these two processes therefore enhances lethality. Enzymes are coagulated and hydrated readily. In addition, heat is transferred more readily in humid air (Hawker *et al.,* 1952). The results of the *in-vitro* studies demonstrated that the combination of moist heat and radiation is effective in killing *A. flavus* spores in a water suspension. What remains to be done is to determine whether or not a combination of moist heat and radiation can effectively destroy *A. flavus* spores.

The results of the *in vitro* studies had to be demonstrated *in vivo* spores of *A. flavus* inoculated on cocoa beans. The equipment designed to provide moist heat was efficient as there was no difficulty in providing dry or moist heat. Temperatures tried were reached in less than 10 min and subsequently maintained unless the door was opened. The beans were not heated over long period. Thirty

minutes heat treatment was enough. Moist heat treatment did not have any significant effect on the moisture content of the treated beans. The beans only received a fine spray of moisture which dried off before storage. Of all combinations of treatments tried, moist heat (>85 % R.H.) gave better results than dry heat treatment (<40 % R.H.). This is because there was better penetration of the beans with the moist heat (Hawker *et al.*, 1953).

In chapter 5, radiation alone inactivated *A. flavus* at 6 kGy. The combination of moist heat and radiation gave better results than the two processes applied separately. Although none of the combinations gave total inactivation of *A. flavus* spores, the effect was synergistic. The best combination was moist heat at 80 ^{0}C for 30min followed by 3.5 kGy radiation which reduced the log number of survivors by 6 log cycles (6.5 to 1.2). Fungal growth was suppressed at 75 % R.H. and it was difficult to assess the survival of *A. flavus* on the beans. Storage at 90 % R.H. gave conditions more favourable for fungal growth. All surviving spores grew and therefore offered better assessment of the treatments applied.

The cocoa beans stored at 90 % had a mean moisture content of 8.5 % after 28 days storage, Scott (1928) and Theimer (1958) reported that the cocoa becomes mouldy if the moisture content exceeds 8 %. It is interesting to note that in the *in-vitro* study, *A. flavus* in suspension could be totally inactivated (5 log cycles) by applying moist heat at 50 ^{0}C for 10min followed by 0.5 kGy radiation and yet on cocoa, heat treatment at 80 ^{0}C for 30 min followed by 3.5 kGy radiation was needed before a 5-log cycles reduction could be obtained. *A. flavus* in suspension was more sensitive to the combination of heat and radiation than

when on the product (Mohyuddin and Skoropad, 1970). This preliminary study, confirmed the observation in the *in-vitro* study that the type of heat and the temperature at which heat treatment is applied are very important. The combination of moist heat at 80 ^{0}C followed by 3.5 kGy gave the best synergistic effect and was therefore selected for the long – term study. The moist heat did not have any significant effect on the moisture content of the beans. The post irradiation storage humidity was found to have an effect on survival of *A. flavus.*

The survival of *A. flavus* determined immediately after the combined treatment of heat and radiation indicated that the combined treatment was more effective in reducing the number spores of *A. flavus* than either heat or radiation applied separately. This confirmed the finding of the *in-vitro* study and that of the short – term study on the beans. Both *in-vivo* studies also showed that moist heat applied was more effective than dry heat. Moist heat applied at 80 ^{o}C for 30 min followed by 4 kGy radiation gave the best result. There was no visible growth of *A. flavus* throughout the 4 month storage period. van Kooij 1978, working on cocoa, observed that cocoa irradiated at 4 kGy and stored at 28 ^{o}C and 80 % R.H. was free from fungal growth for a period of 9 months. The increase in weight followed a similar pattern till it reached equilibrium within a month.

In studying the effect of radiation on fungi, the influence of post-irradiation storage conditions namely relative humidity (RH) and packaging were also noted. The cocoa treated with radiation alone or heat and radiation were stored at the relative humidities of 75, 80 and 90 %. Those stored at 75 % R.H. and 90 % R.H. were in open Petri dishes while those stored at 80 % were in

woven polypropylene bags. The initial moisture content of the stored beans was about 75 %. Those stored at 90 % relative humidity had mean moisture contents of 8.5 % after 28 days, and there was high incidence of mouldy beans in the control. Reports by Scott (1928) and later by Theimer (1958) showed that stored cocoa becomes mouldy if the moisture content exceeds 8 %. This explains why there was a high percentage of mouldy beans. Water was absorbed and this increased the moisture content and made water available for germination of *A. spores* that were on the beans causing mouldiness. At low humidity, water is bound to the solute molecules so that there is less free water available for growth (Northolt *et al,* 1977). The initial moisture content of the beans stored at 75 % R.H. was not in equilibrium with the moisture in the ambient humidity, therefore the moisture content of the beans decreased with storage time from 7.5 to 6.5 % in the control. When the beans were stored at 80 % R.H. in polypropylene bags, there was no visible growth for the 4 months period. Work done by Henderson and Pixton (1978) attributes the hydroscopicity of cocoa beans to the fact that they are dead and therefore there is free movement of moisture in and out of the bean through the testa, according to the ambient humidity, till there is equilibrium. Oyeniran (1979), working on the influence of the storage environment on the quality of commercial cocoa, found that the moisture content of the beans changed from 6.9 % in the course of 3 months to 6.5, 7.1, 7.4, 8.5, 9.2, 11.7 and 17.98 % when stored at relative humidities of 65, 71, 75, 81, 84, 90 and 98 % respectively. He observed that internal mouldiness occurred at R.H. between 75 % and 98 %. This observation has been confirmed in the current

investigation. The optimum storage humidity after the combination of moist heat and radiation should be below 75 % RH. The microbiological analysis of samples stored under 75% R.H. showed that the spores of *A. flavus* were viable but the storage humidity of 75 % suppressed growth. .It is necessary to package before irradiation to prevent recontamination after the treatment as radiation does not have any residual effect. The polypropylene packaging used therefore served the dual purpose of packaging and preventing recontamination. This would seem to be better for this purpose than the jute bag which is more porous and would allow greater exchange of moisture and air, between the storage environment and the internal content of the bag.

Aflatoxin was not detected in cocoa stored at 80 % RH after treatment even though they were inoculated with toxigenic *A. flavus* before treatment with heat and radiation. This is because storage humidity of 80 % RH. did not promote growth of the fungal spores and no toxin was produced. In the second study, when the moisture level was favourable (90 % R.H.) however, the fungal conidia on the unirradiated, stored samples had the chance to grow and produced aflatoxin. The calculated concentration of the samples was 1 μg/kg. This was below the maximum permissible limit of 5 μg/kg for aflatoxin B_1 in cocoa. Hilmy and Chosdu (1994), working on ground nutmeg, found that in nutmeg kept below 85 % R.H. growth of mycelium and toxin production were inhibited by a dose of 3 kGy. The production of aflatoxin in nutmeg began 25 days of incubation at 97 % R.H. and after 45 days at 91 % R.H. Storage humidity is therefore important for the production of aflatoxin. The type of aflatoxin and the amount produced

depends on genetic, nutritional and several environmental factors such as the strain of fungus, the composition of the medium (Davis and Diener, 1968; Schroeder,1966), the presence of Zinc (Maggon *et al.*, 1977), temperature (Schroeder and Hein, 1967; Stultz and Krumperman, 1976; Frank *et al.*, 1971); moisture level (Lopez and Christensen, 1967; Hilmy and Chosdu, 1994), oxygen concentration and duration of incubation (Landers *et al.*, 1967; Maggon *et al.*, 1977).

The fact that no aflatoxin was detected after 10 days could mean that either the toxin was formed but the quantity present could not be detected by Thin Layer Chromatography or no toxin was formed at all. The sample irradiated before storage did not contain any toxin because the fungal spores were killed by radiation. The fungus however, was able to grow and produce the toxin in the unirradiated sample. Emam and Faraq (1994) reported that a dose of 3 kGy inhibited the growth of fungi and prevented aflatoxin production in semi dried Egyptian dates.

The aflatoxin that had already been produced was not destroyed by applying radiation. This is in agreement with observations reported by other workers that mycotoxins already formed in food are resistant to radiation. Wurakit (1987) found no significant difference between the aflatoxin B_1 concentrations of irradiated and unirradiated groundnuts when a dose of 6.4 kGy was applied after storage. Fenell (1966), working on groundnuts, observed no reduction in toxicity of aflatoxin B_1 after irradiation. Using a bacterial test methodology, Temcharoen and Thilly (1982) reported that an aflatoxin –contaminated groundnut meal

required a high dose of 50 kGy to eliminate the toxicological effect of the aflatoxin. Doses as high as 180 kGy have been reported to degrade only 10 % of aflatoxin in a dry environment (Aibara & Miyaki, 1970). Patel (1989) reported that simultaneous treatment with hydrogen peroxide and gamma radiation resulted in a synergistic inactivation of aflatoxin B_1. van Dyck *et al.,* (1982), irradiating an aqueous solution of aflatoxin B_1 with a dose of only 20 kGy, however, reported an almost complete degradation of the toxin.

No aflatoxin could be detected from beans that were neither inoculated with *A. flavus*, nor irradiated but naturally became mouldy in storage (due to fungal spores already present on the beans). This was probably because even though *A. flavus* happened to be included in natural infection there were other fungi present which competed with *A. flavus* in growth. In such a scenario, *A. flavus* may not be able to produce enough aflatoxin to be detected as in the case where only *A. flavus* was present. There is also the probable reason that the *A. flavus* strain present was not the toxigenic type, in which case no toxin could be produced. The presence of potential toxigenic species on food products does not always mean that these products contain mycotoxins. This is because the environmental conditions for fungal growth are not necessarily the same as those for toxin production. Prevention of fungal growth, however, is a good practical approach to protecting foods from mycotoxin contamination.

CHAPTER SEVEN

7.0 THE EFFECT OF RADIATION ALONE OR HEAT COMBINED WITH RADIATION ON THE QUALITY OF DRIED COCOA BEANS

7.1 INTRODUCTION

In determining the quality of cocoa beans, the flavour, colour and mouldiness are among the criteria considered by the United Kingdom Cocoa, Chocolate and Confectionery Alliance (Minifie, 1989). For good flavour and colour, a number of conditions must prevail during fermentation. These include a temperature of about 50 ^{0}C for several days and periodic stirring of the heap to facilitate aeration. These conditions enhance the development of the desired colour of the beans and the flavour precursors. The final chocolate flavour is developed during roasting and depends on the presence of flavour precursors (Minifie, 1989).

Mouldiness causes tainting of the beans and this, together with the roasting, determine the final taste of the product. In another investigation, several species of fungi associated with mouldiness were isolated from cocoa and identified. Cocoa butter, like other fats, undergoes changes during storage which result in the production of an unpleasant taste and odour on rancidity. It is important, therefore, to ascertain whether the radiation treatment or its combination with heat enhances rancidity.

The super cooling and contracting properties of cocoa butter are valuable as they affect the moulding of the chocolate block and also its keeping quality. Because flavour, colour and taste of cocoa butter are important quality parameters in

the cocoa industry, any treatment given to the beans prior to processing must be evaluated for their effect on these quality parameters.

In the preceding chapters, radiation only or heat in combination with radiation were applied successfully to prevent mouldiness. Earlier work has been carried out on the effect of radiation only (Takyi and Amuh, 1979), up to 2 and 5 kGy on cocoa beans. In the present study the effect of radiation and a combination of heat and radiation on the quality of cocoa beans have been investigated. The quality parameters considered with respect to cocoa butter were the melting point, saponification value, unsaponifiable matter, free fatty acids, peroxide value and solidifying (cooling) curve. The effect of radiation on organoleptic properties of cocoa powder following heat and radiation treatments was also determined.

7.2 MATERIALS AND METHODS

7.2.1 Analysis on cocoa butter

Samples of dried cocoa beans weighing 100 g each were exposed to radiation doses of 0 to 6 kGy from a Cobalt-60 gamma irradiator. The irradiated and unirradiated samples were all dehulled and ground separately using a laboratory grinder. Cocoa butter was extracted using Petroleum ether in a soxhlet extractor. The fat was dried using a rotary evaporator (IUPAC, 1974). Part of each sample was sent to the Cocoa Processing Laboratory for analysis. The following determinations were made on the cocoa butter; the slip (melting) point, saponification value, unsaponifiable matter, peroxide value and % free fatty acid (FFA) according to methods specified by Pearson, (1976). The relationship

between radiation and storage rancidity (using peroxide values and free fatty acids) was determined by irradiating freshly dried cocoa (1994 sample) and cocoa stored for 1 and 2 months (1993 sample).

7.2.1.1 Determination of fatty acid using gas chromatographic analysis

The Chromatographic analysis was done at Ghana Standards Board, Accra. Chromatographic separation of the fatty acids was accomplished using a standard steel column (6ft OD 1/2') G.P. 5% CDEG5 - PS on 100 120 Supel Coport. Operating conditions were as follows: The initial and final temperatures were 70 ^{0}C and 190 ^{0}C respectively and the programme temperature rate was 20 ^{0}C per min. The initial programming time was 69 min while the final time was 75 min. The injector and detector were at 250 ^{0}C. The volume of samples injected was 1 µl. The flow rate of nitrogen was 20 ml/min.

Extraction of fatty acids from cocoa

One hundred gramme batches of freshly dried cocoa were irradiated at doses 0-10 kGy after which fat was extracted from each sample using the method described earlier. To facilitate gas-chromatographic identification and relative quantification of the individual fatty acids, the fatty acids had to be esterified. The esterification mixture was prepared by adding 100 ml methyl alcohol to 7g of zinc chloride melted in a porcelain crucible and cooled. The mixture was stirred to obtain a complete salt solution and 2 g zinc powder was added. The cocoa butter was liquefied by heating and 2 g was weighed into a glass ampoule to which 2 ml of esterifying mixture was added. The ampoule and its content were shaken before sealing. The sealed ampoules were dried in an oven (100 ^{0}C) for 12 hours. There

were two layers in each ampoule. The lower layer was used for the chromatographic injection. The seal of the ampoule was broken just before drawing an aliquot of sample with a micro-syringe for analysis. A known quantity of standard with know fatty acid was injected first and the peaks identified using the table provided. The same aliquot / volume of each sample including the control were injected. The peaks of the different samples were identified and compared. The quantities were compared using the area under the curve.

7.2.1.2 Determination of cooling (solidifying) curves of cocoa butter

After treatment with moist heat at 80 ^{0}C for 30 min as described in the previous chapter, the samples weighing 600 g each as well as the control were pre-roasted at 100 ^{0}C for 30 min to facilitate removal of the shell from the nibs. The nibs were sieved to get rid of pieces of shells and embryos. The samples were heated at 140 ^{0}C for 60 min. After grinding nibs into fine powder, the cocoa butter was extracted with hexane. The cooling curves of the different samples were determined using the method of IUPAC 11 B5 (1974). The butter was cooled in an iced water mixture at 17 ^{0}C for a period of 90 min. The temperature was taken at 1 min intervals. A graph of the temperature against the time was plotted. The minimum and maximum temperatures and the time taken to reach those temperatures were recorded. A temperature quotient ratio was calculated for each sample and the values compared.

7.2.2 Organoleptic test on cocoa powder

After the extraction of cocoa butter using the presser method (Minifie, 1989), the cake which resulted was ground and pulverised on a pair of revolving cylinders at 13 to15 ^{0}C to obtain cocoa powder. Sub samples for the organoleptic test were obtained by mixing the prepared 3.5 g cocoa powder, 1.5 g of crystallize sugar and 50 ml of water at 60 ^{0}C and mixed with a vortex mixer. Drinks prepared from the sub samples were coded. Each of the 22 laboratory panelists was given 6 coded cocoa drinks to evaluate for the taste, colour and odour by grading them using a scale of 0 – 10 (0 = very bad; 10 = very good). These grades were then ranked from 1 to 6 according to the quick rank test by Kramer (1956). The results were analysed statistically using analysis of variance.

7.3 RESULTS

The results of analysis made on cocoa butter are presented in Table1. Data on the irradiated samples compared favourably with the factory specifications (Appendix 14, Table 25). The values for free fatty acid were between 0.43 and 0.51% which was within factory standard of 1.75 %. The Slip point value which ranged between 34.1 and 34.7 ^{0}C was also within the limit of 32 – 35 ^{0}C. The determined saponification values ranged between 188.02 and 189.67 which compares favourably with the standard set at factory (188 to 189.0).

The free fatty acid determined during storage of the samples presented in Figs. 14 & 15 showed that the percent free fatty acid did not vary with the dose applied. The analysis of variance of the old cocoa (1993) showed that differences

within the doses as well as between the different months of sampling were significant ($p< 0.05$) (Appendix 15). The significant differences between the mean doses did not follow any particular trend (Table 26) and all determinations made were within the acceptable limit of 1.75 %. In the 1994 samples of cocoa which was freshly dried, the FFA values were relatively low but followed a similar trend. All determinations made before storage after 1 month were below 0.30 % (Fig 15). The second month's values were quite significant from the rest, reaching about 1 % (Appendix 16 and Table 27). Generally, percentage FFA was not influenced by radiation.

The Peroxide values followed the same trend as the percentage FFA (Figs.16 & 17). The analysis of variance for 1993 cocoa (Appendix 17) shows a significant difference between the different months. Comparison of the means showed that the difference between the doses did not follow any trend. (Table 28). For example, the mean peroxide value for the control was 26.83 similar to that of 5 kGy 26.67 (Table 28). The analysis of variance for the fresh sample showed significant difference for the dose, the months, and the month and dose interaction ($p<0.05$) (Appendix 18). The differences within the dose did not follow any particular trend and was only attributed to the individual samples (Table 29). The peroxide values increased with storage time in all the determinations. It was generally observed that there was no relationship between radiation dose and the peroxide value. Therefore the application of radiation or a combination of heat and radiation did not cause rancidity of the treated beans.

Table 25. The effect of radiation on some properties of cocoa butter (Mean ±SD).

Dose (kGy)	Free fatty Acid (%)	Slip point ^{0}C	Saponification value meq/kg fat	Unsaponifiable Matter
0	0.43 ±0.01	34.6 ±0.28	188.53 ±0.38	0.25 ±0.01
1	0.43 ±0.01	34.4 ±0.14	188.51 ±0.37	0.25 ±0.01
2	0.50 ±0.02	34.6 ±0.22	188.25 ±0.16	0.24 ±0.01
3	0.45 ±0.02	34.8 ±0.08	188.15 ±0.05	0.24 ±0.01
4	0.50 ±0.02	34.7 ±0.16	188.17 ±0.21	0.23 ±0.01
5	0.46 ±0.01	34.5 ±0.08	189.92 ±0.47	0.28 ±0.03
6	0.51 ±0.02	34.7 ±0.08	188.60 ±0.30	0.29 ±0.01
Factory Standard	0- 1.75	32 - 35	188.0 - 196.0	0 - 0.35

Values are means of four replicates

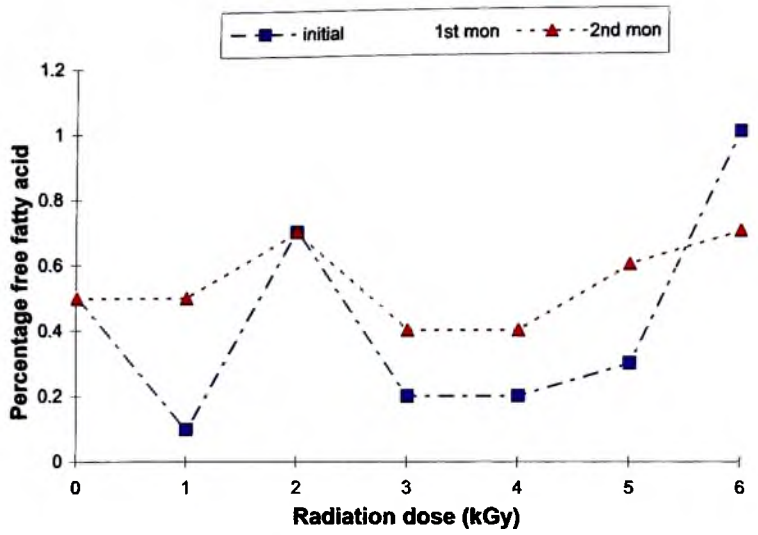

Figure 14. Free fatty acid content of cocoa beans after heat and radiation treatment and storage at different periods in 1993.

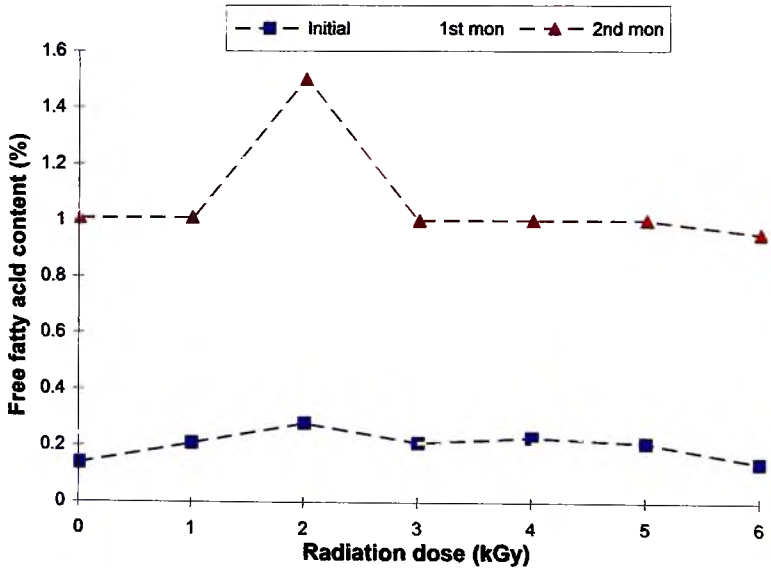

Figure 15. Free fatty acid content of cocoa beans after heat and radiation treatment and two months of storage in 1994

Table 26. Effect of radiation and storage period on percent free fatty acids in cocoa butter - 1993 sample.

Dose (kGy)	0	1	2	3	4	5	6
Mean FFA (%)	0.66 b	0.60ab	0.94d	0.71c	0.54a	0.71c	0.091d
Month	0	1	2				
Mean FFA (%)	0.42 e	0.6g	0.55 f				

Figures marked with same letters are not significantly different (P<0.05)
SED between doses =0.02; SED between storage time=0.07

Table 27. Effect of radiation and storage period on percent free fatty acids in cocoa butter - 1994 sample.

Dose (kGy)	0	1	2	3	4	5	6
Mean FFA (%)	0.45bc	0.51d	0.46 b	0.43a c	0.44 c	0.46 b	0.42a
Month	0	1	2				
Mean FFA (%)	0.21 e	0.24 g	1.03f				

Mean values marked with same letters are not significantly different (P<0..05)
SED between doses =0.02; SED between storage time =0.02

The gas chromatographic qualitative analysis of cocoa butter from irradiated as well as unirradiated cocoa showed the presence of palmitic (16:0), stearic (18:0) and oleic (18:1) acids in all the samples.

The results of the study on how cocoa butter cools have been summarised in Fig.18 and Table 30. The cooling curves of cocoa butter from the cocoa treated with heat only (80 ^{0}C, 0 kGy); radiation only (4 kGy, 20 ^{0}C) and those treated with combination of heat and radiation (80 ^{0}C, 4 kGy) were all similar (Fig.18). The fat cooled rapidly from 40 to 20 ^{0}C within 13min. Thereafter, there was a lag phase (13 –37th min) with minimal change in temperature as the fat solidified. Then the temperature rose again to 25 ^{0}C before it started to decrease again. The minimum temperature recorded was 18 ^{0}C and the maximum 27 ^{0}C (Table 30). The calculated temperature quotient ranged between 0. 21 and 0.24 ^{0}C/min. This showed that the treatments given did not have any adverse effect on the quality of the cocoa butter.

Results of organoleptic evaluation of cocoa powder have been presented in Table 31. For a sample to be significantly different, the sum should be outside the range of 58 and 96 (Table 31). For acceptability, the mean score of a sample should be above 5. There was no significant difference between the samples treated with radiation alone, or heat and radiation and the untreated samples for colour and taste. The rank sum of the samples treated at 80 ^{0}C, high humidity and 4.0 kGy showed some differences which were not statistically significant (Appendix 19).

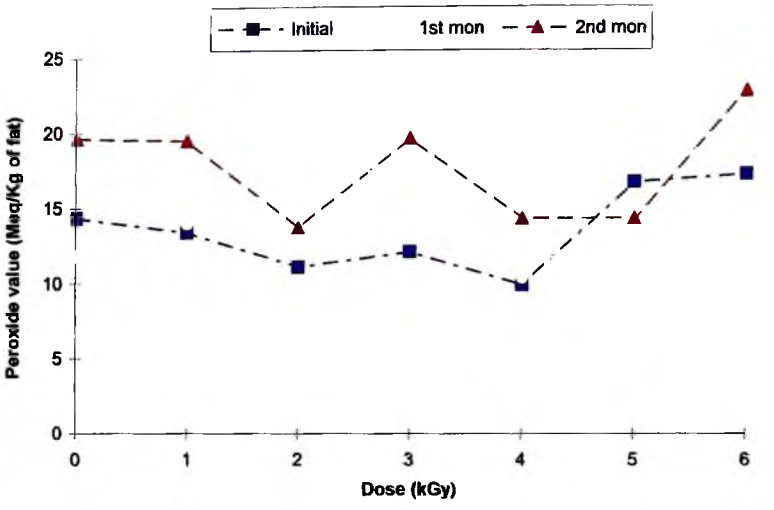

Figure 16. Peroxide value (Meq/kg) of cocoa butter prepared from cocoa stored after irradiation in 1993.

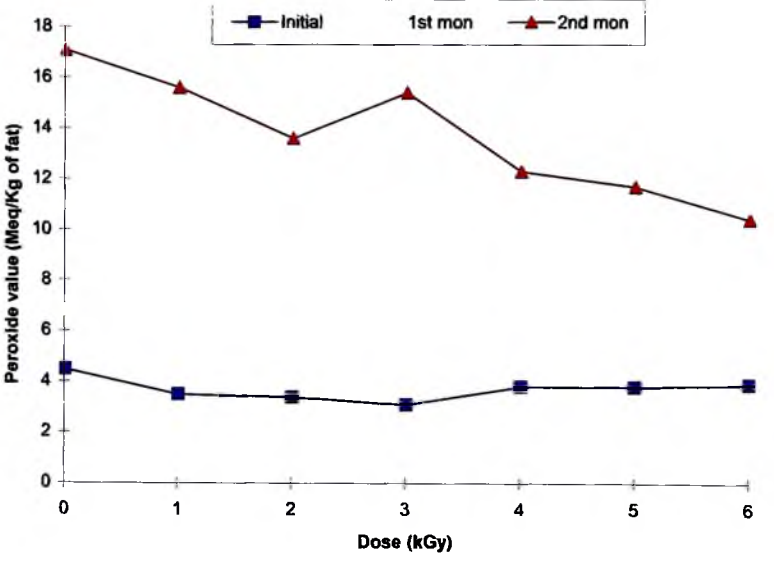

Figure 17. Peroxide valve (Meq/Kg) of cocoa butter prepared from irradiated cocoa beans after different periods of storage in1994.

Table 28. Effect of radiation and storage period on percent peroxide value of cocoa butter -1993 sample.

Dose (kGy)	0	1	2	3	4	5	6
m e a n p. value	26.83 d	22.95 b	21.75 b	20.94 a b	19.23a	26.67d	24.15c
Month	0	1	2	9			
Mean p/value	16.21g	11.81f	17.69g	47.03h			

Figures marked with same letters are not significantly different
SED between mean doses =2.35; SED between storage time = 1.77

Table 29: Effect of radiation and storage on percent peroxide values of cocoa butter - 1994 sample.

Dose (kGy)	0	1	2	3	4	5	6
Mean p. value	11.67p	6.17m	7.22n	8.30o	6.70m	6.49m	6.30m
Month	0	1	2				
Mean p. value	3.63u	6.82v	15.18w				

Mean values marked with the same letters are not significantly different at (P>0.05).
SED between mean doses =0.673; SED between mean doses = 0.434

Table 30. Temperature quotients of cooling curves of cocoa butter after combined treatment of heat and irradiation

Treatment		Minimum Temp.(C)	Minimum Time	Maximum Temp.	Time (t)	ΔT	Δt	ΔT/Δt °C/min
20 L	0	18	36	22	53	4	17	0.24
20 L	4.0	18	39	22	57	4	18	0.22
80 L	0	18	35	22	54	4	19	0.21
80 L	4.0	18	36	22	54	4	18	0.22
80 H	0	18	35	22	53	4	18	0.22
80 H	4.0	18	37	22	54	4	17	0.24

L=Low Humidity = <40 % RH H= High Humidity = >85 % RH

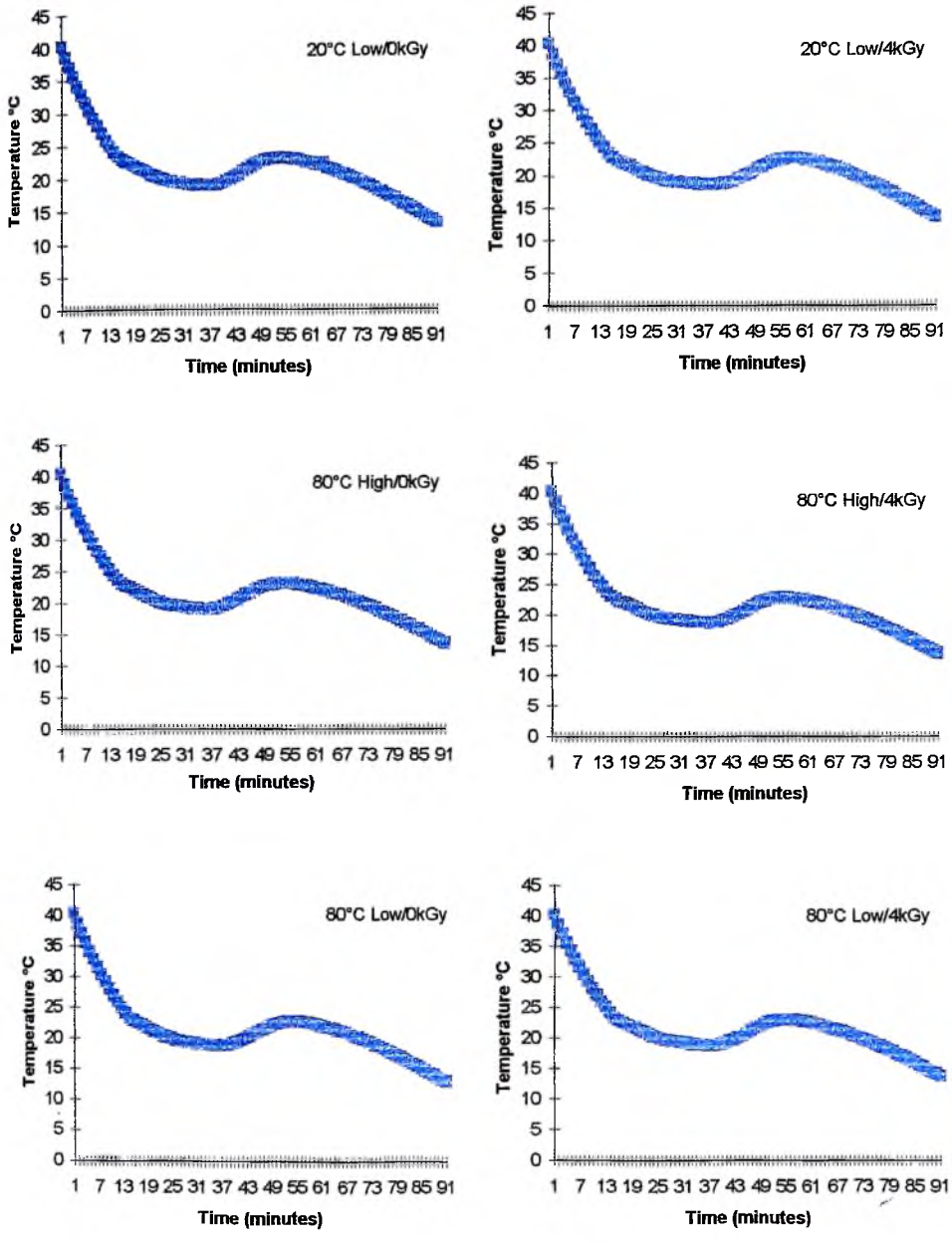

Figure 18. The cooling curves of cocoa butter prepared from beans after combined treatment of low and high humidity, heat and radiation.

7.4 DISCUSSION

The Codex Alimentarius Commission gives the slip point of cocoa butter between 30 to 34 ^{0}C and the melting point between 31 to 35 ^{0}C (Dand, 1993). The Cocoa Processing Company does not only use a range between 32 to 35 ^{0}C for slip point but also for other values. This implies that all cocoa butters are not the same. The slight differences observed between the samples for slip point, saponification value, unsaponifiable matter would be attributed to the fact that they are different samples rather than to changes caused by radiation alone or heat and radiation because no trend of change with dose was observed. All determinations were within the limits set in the factory.

As indicated earlier, changes occurring during storage may lead to rancidity. In this investigation, the determination of free fatty acids and peroxide values were used to determine rancidity. The changes in these values for the irradiated samples were similar to that of the control (unirradiated). This means that the exposure of the beans to radiation or combination of heat radiation did not cause rancidity of the cocoa butter. The increase in the % FFA and peroxide values observed with time were storage effects. The formation of FFA is known to be slow at first and increases with storage. High levels of FFA occurs in cocoa that is either stored for a long time in a too hot or too humid environment or as a result of the activity of micro-organisms. Lipase which does not only result from microbial actions is also present when the natural raw cocoa breaks down triglycerides into the separate groups of fatty acids. Their presence in large quantities may give a rancid flavour to the products (Dand, 1993). Takyi et *al.*,

Table 31. Sensory evaluation of cocoa drink from irradiated cocoa

Treatment				Taste				Flavour				Colour			
Temp. °C	Hum %	Dose kGy	Code	Position	Mean Rank	Standard Deviation	Rank Sum	Position	Mean Rank	Standard Deviation	Rank Sum	Position	Mean Rank	Standard Deviation	Rank Sum
20	low	0	T	2	3.20	0.98	70.5	5	3.84	1.37	84.5	4	3.59	0.92	79
80	low	0	X	5	3.77	1.52	83	4	3.30	1.34	72.5	3	3.45	1.25	76
80	high	0	V	1	3.16	1.61	69.5	3	3.25	1.52	71.5	5	3.61	1.34	79.5
20	low	4	B	4	3.55	1.35	78	2	3.16	1.62	69.5	6	3.70	1.77	81.5
80	low	4	Y	3	3.20	1.68	70.5	1	3.00	1.58	66	1	3.23	1.35	71
80	high	4	M	6	4.11	1.36	90.5	6	4.45	1.16	98	2	3.41	1.42	75

22 panel members tasted 6 samples of cocoa drink and graded them between 0 and 10 (= very good); those grades were ranked from 1(= high, good) to 6 (= low bad) using Kramers's Ranking Test
$P \leq 0.05$ Rank *sum 58 –96 not significant.*

(1979) analysed cocoa beans after irradiation at dose 0, 0.1, 0.2, 0.5, 2.0 and 5.0 kGy. They found no significant differences in respect of reducing sugars, total fats as determined by iodine value, free fatty acid value, saponification value, refractive index, slip point and specific gravity among other determinations.

According to Minifie (1989), the composition of fatty acids in cocoa butter are as follows: Myristic (14:0) 0.1 %; Palmitic (16:0) 25.8 %; palmitoleic (16:1) 0.3 %; stearic (18:0) 34.5 %, oleic (18:1) 35.3 %; linoleic (18:2) 2.9 % and arachidic (20:0) 0.1 %). Qualitative chromatographic analyses showed the presence of palmitic, stearic and oleic acids in the control (unirradiated) as well as irradiated samples. The above observations had been confirmed by several group of workers. Prawato (1989) reported that fatty acids of cocoa contain C14, C16, C 16:1, C 18, C 18:1, C 18:2 C 18:3 and C 20. Of these he found out that C16, and C18 and C18:1 were most predominant. Erickson *et al,* (1973) also reported that the composition of fatty acids are palmitate (26 %), stearate (34 %), oleate (35 %), linoleate (3 %), arachidic (1 %) and trace amount of several acids.

The similarity of the cooling curves determined on cocoa butter from beans that were heat treated before irradiation meant that these treatments did not cause any significant change in the cocoa butter which can offset solidification during processing. Similar results were obtained by Keinart (1973) on Ghana cocoa.

In this study neither radiation treatment nor heat treatment followed by irradiation had adverse effect on organoleptic evaluation of cocoa powder. These

results agree with van Kooij's (1978) findings that beans irradiated at 4 kGy were still acceptable to the panel.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL DISCUSSION

In chapter 3 of this study, results obtained from both surveys conducted at the farmers' level estimated that less than 2 % cocoa beans lost per year (1.73 % & 1.66 %) in the Tafo District. In the questionnaire approach, 51 % of the farmers mentioned mouldy beans as a cause of loss, suggesting that mouldiness is a problem in the cocoa industry in the Tafo District. In terms of percentages, however, mouldy beans was estimated to be 0.16 % of marketable beans per year, close to 0.13 % obtained in the bean collection approach. No matter how small the quantity, it is important that good sorting out is done before marketing in order to have high quality beans. It was observed that 20 out of 24 farmers discarded 0.42 % in the minor cocoa season as against 9 farmers discarding 0.014 % in the major season. Mouldiness therefore appeared to be more of a problem in the minor season than in the major cocoa season.

The majority of farmers lost between 1 and 5 kg per season. The other causes are damage by rodents, insects, germination, over drying, flat bean, under-developed bean, and blackpod disease. The information obtained in the survey could be extrapolated to cover the loss in the Tafo District as farmers were selected from all the societies in the district.

At the buyers' level, all the buyers confirmed the earlier observation that mouldy beans occurred more in the minor season than in the major season.

Seventy-five percent of the buyers said they did not do any sorting for defective beans during redrying and that all sorting out is done by the farmers before the beans are purchased. Three of the buyers sorted out the defective beans during redrying. During the major season when there is adequate sunshine, the occurrence of mouldy beans was found to be negligible (0.00002 %). From the discussion above, sorting out and removal of mouldy beans are done mainly at the farmers' level. Cocoa purchased in Ghana is therefore of high quality since grading is done during which subgrade beans are rejected.

At the port, it was observed that some of the internally mouldy beans could not be detected at the farmers' level and so may come through to the port. In this study, cocoa arriving at the Tema Port during the major season of 1992 and minor season of 1993 were examined using the cut test and then graded again before shipment. Mouldiness was again found to vary with the season, confirming earlier finding at the farmers and the buyers' levels, that there is more incidence of mouldy beans in the minor than in the major season. The percent mouldy beans were 0.5% and 0.8% during the major and the minor seasons respectively.

The quantity of mouldy beans contained in exportable cocoa at Tema Port was estimated from the data collected. Out of 4,518.24 T of defective beans estimated for the year, mouldy beans was 603.83 T, accounting for 0.56 % of marketable beans. The average for 5 years was 0.69 %. In order to determine how much of our cocoa goes mouldy, the determination at the farmers' level, buyers

level and at the port could be added to give an estimated quantity of mouldy beans produced annually or the quality of the beans in each consignment.

The current study was carried out using questionnaire and bean collection approach at the farmers' and buyers' levels and the cut test at the port. Dharmaputra *et al., (*1999), carried out similar surveys on postharvest handling of cocoa beans at the farmer, trader and exporter levels in South Sulawesi, Indnesia. They also conducted interviews, but in addition applied both the cut test and the direct plating method at the farmer, trader and exporter levels to determine mouldiness. Whereas in the current study, the percentage of mouldy beans determined at all levels were below the recommended tolerable limit of 4 %, in South Sulawesi, during the two seasons, the percentage of mouldy beans at farmers' level was lower than 4 %, while those from some samples at trader and exporter levels were higher than 4 %. However, based on the direct plating method, all of the samples at trader and exporter levels were mouldy. Our study did not include the direct plating method to allow comparison of results.

The current criteria used to assess mouldiness the cut test provides an assessment of the beans from which the analyst infers certain characteristics of the cocoa. Even though the percentage determined may change with subsequent checks, the cut test is still used to provide an initial assessment of quality, because it is very quick, requires little equipment or training, and is very common in cocoa trade. It however has some limitations: Interpretations of results may differ as it is subjective. There are no criteria about what type eye-sight or type of light should be used. The test does not directly measure the true status of the cocoa. It

does not measure the extent of mouldiness or the type of fungi present. Non detection of mouldy beans during the test does not mean that the sample is free of mouldy beans. Despite these shortcomings, the method is still accepted by Food and Drug Administration of U.S.A (Dand, 1993). The cut test still is part of the quality requirements of the Biscuit, Cake Chocolate and Confectioners Alliance (BCCCA) of the United Kingdom and is recognised by those involved in the production and distribution of cocoa world wide.

The level of mould contamination of the beans could also be determined in the laboratory by culturing samples of the beans, observing them and determining the percentage of beans that are mouldy. Though more effective than the cut test, this method is more time consuming. Ideally, it should be possible to test every single bean. Unfortunately, no such method is available. Biotechnologists are developing tests that can rapidly detect contaminating micro-organisms and toxins produced by them. The tests make use of DNA probes or monoclonal antibodies and ensure the safety of products being marketed (ABA leaflet, 1995). It is hoped that such a method could be developed for the grading of cocoa.

The current methods of grading is such that it is impossible to detect all mouldy beans and so these defective beans are not discarded but sold. Assuming that a method is developed in future which is able to detect internal moulds and therefore all mouldy beans are removed before export, then in terms of revenue the potential loss to the country from cocoa shipped through Tema Port in 1992 could be as follows;

The estimated weight of mouldy beans = 603,82T

If the price of cocoa/T	=	$989
Minimum revenue lost from sales		
in 1992 of cocoa from Tema	=	$603.82 x 989
	=	$597,177.98

Since the estimated weight is based on the current cut-test which only gives an indication of percent mouldy beans, the actual loss of revenue due to mouldy beans is likely to be much higher than $597,177.98 calculated. The problem of mouldiness should therefore be given attention.

Mouldiness in Ghanaian cocoa was first reported by Bunting (1928) and Dade (1928) who identified some fungi causing internal mouldiness. Other workers mentioned mouldiness occurring during fermentation, drying and storage (Knapp, 1937; Maravalhas, 1966; Rohan, 1963). Abitey, (1982), studied the level mouldiness in exportable cocoa at Tema port using a laboratory method. He found the average percent infection in minor season higher (6.4 %) than that of the major season (3.9 %). His observation has been confirmed by this study.

The problem of mouldiness has been reported by workers in other cocoa growing countries (Oyeniran, 1971, 1973, 1979; Broadbent and Oyeniran, 1968; Hansen, 1975; Powell and Wood, 1959). Manufacturers consider mouldiness in cocoa as the second worse fault on cocoa. The slightest fungal infection within the bean limits its use. Chocolate made from a lot with more than 3 % mouldy beans as measured by the cut test has a musty taste (BCCCA, 1996), some of the fungi may produce mycotoxins. Some may not be pathogenic but may have varying effects on flavour. Also the dust produced by external moulds may be

harmful to workers in the cocoa industry (Dand, 1993). The fact that the industry considers mouldy beans the 2nd worse defect means that it is important and their presence should be minimised as much as possible.

In chapter 4, fungi occurring on cocoa in Ghana have been up-dated. The most prevalent species (25) were in the *Aspergillus* genus, five in *Penicillium*, and eight *Fusarium*. Forty seven fungi caused both external and internal mouldiness. Twenty-nine fungal species causing internal mouldiness are being reported for the first time on cocoa in Ghana. They are; *Aspergillus amstelodami, A. penicilloides, A. itaconicus, A. oryzae, A. versicolor, A. lutescens, A. fischeri, A. echinulatus, A. parasiticus, A. japonicus, Penicillium cyclopium, A. pseudo-glaucus, A. sulphureus, Penicillium variable, P phoeniceum, Fusarium oxysporum, F. roseum, F. moniliforme, F. lateritium, F. rigidiuscula, Paecilomyces varioti, Syncephalastrum racemosum, Curvularia lunata, Phoma* sp., *Chaetomium globulosum, Absidia corymbifera , Byssochlamys fulva, A. proliferans and Mucor haemalis.* One specie resembling *A. ochraceus* was isolated, but in view of the current concern expressed worldwide on ochratoxin A, further work using modern methods would be required for confirmation of its identity.

Work done on fungi on cocoa in Ghana was started by Bunting (1928) and Dade (1928). They identified six species of *Aspergillus namely A. flavus, A. glaucus, A. ochraceus, A. tamarii, A. niger and one Penicillium* sp. Abitey (1982) who studied the degradation of the testa of cocoa by *Aspergillus* species identified 21 species of fungi. All the previous workers concentrated on internal fungi. This

study however considered both external and internal fungi because the objective was to find control measure for all fungi associated with cocoa beans.

The main objective in chapters 5 & 6 was to ascertain whether radiation alone or radiation in combination with heat treatment could effectively control fungi *in-vitro* and on dried cocoa beans. The fungal population was found to decrease with an increase in the radiation dose. These studies confirmed that, when a population of micro-organisms is irradiated with a low dose, a few of the cells are damaged or killed. With increasing radiation dose the number of survivors decrease as it does with increasing heat treatment. Different species and different strains of the same species respond differently to the same in-activation dose.

When ionising radiation is absorbed by biological material it may act directly on the nucleic acid (which is the critical target) by ionising or exciting it, thereby initiating a chain of events that leads to biological changes e.g. mutations or death. This is the dominant process when dry spores of spore forming micro-organisms such as fungi are exposed to radiation (Diehl, 1990). Indirect effects occur when the excited molecules interact with other molecules or atoms in the cell particularly whether to produce free radicals which in turn, can diffuse far enough to damage the DNA (Diehl, 1990). The effect of radiation on micro-organisms (e.g. fungi) depends on the type of radiation, dose, dose rate, the species of organism, inoculum size, growth phase, the medium in which the fungus is grown, the temperature during radiation, the presence or absence of oxygen. (IAEA, 1982). In fungi, the radiation sensitivity is measured by

determining the dose required to prevent growth using the end point method. The lowest dose giving no survival is the in-activation dose for the number of spores inoculated (Diehl, 1990).

It was therefore concluded from the first series of experiments that radiation alone could control mouldiness in cocoa and for effective control of fungi in cocoa radiation dose of at least 6 kGy should be applied.

Even though a dose of 6 kGy was very effective, it is always desirable to apply lower doses of radiation to eliminate the possibility of affecting the organoleptic (taste, odour, etc.) quality of the product. The essence of applying heat before radiation was to sensitize the fungi so that a lower dose of radiation could be applied. Thus in Chapter six, a series of experiments in which heat was applied before radiation are described. It was observed that even though heat and radiation effectively controlled *A. flavus in vitro* and also *in vivo* on the beans, the effective radiation dose in the combination was influenced by the type of medium the spores were in at the time radiation was applied. This has also been observed by other workers (Djefal, 1993; IAEA, 1982). It was also observed in this study that, spores irradiated in aqueous medium required a dose of 0.75 kGy after heating for 2.5 min at 55 °C or 5 min heating at 53 °C followed by 0.5 kGy or 10 min heating at 50 °C and 1.0 kGy to completely inactivate *A. flavus*. However, the dry spores inoculated onto dried cocoa beans required a relatively higher dose of 4 kGy after moist heat treatment at 80 °C for 30 min before complete inactivation could be obtained. Spores of *A. flavus* in the wet state were more sensitive to radiation than those in the dry state. The specification of medium is

important because the inactivation dose of the same micro-organism differs considerably in different media. Many researchers have reported that complex aqueous medium provides more protection than a buffer solution (Diehl, 1990; WHO 1993). In dry medium such as dried cocoa beans, the reaction of water is minimised and therefore a higher dose of radiation is needed for inactivation. Hilmy and Chosdu (1994), examining the effect of post-irradiation humidity of 75-97 % on growth of *A. flavus* on ground nutmeg reported that the effect of radiation varied with different relative humidities and media.

Powell and Wood (1959), observed that when isolated beans were stored in an ambient humidity of 82 % R.H., the beans were in equilibrium at a moisture content above 8% in a matter of days while cocoa in free standing bag may take several days. Grecz *et al.,* (1976) found that the moisture content of the beans stored at 28 °C and 80 % R.H. was constant during the first month and then increased from 5 to 8 % during the following 3 months. In this study, the moisture content of cocoa stored at 28 °C and 80% R.H. decreased from an initial moisture content of 7.3 % to 6.5 % in the first month when the relative humidity was accidentally lowered to 65 %. However when it was reverted to 80 %, the moisture content increased from 6.5 % to 7.8 % after 1 month and equilibrated. In an investigation on cocoa by Amoako -Atta *et al.,* (1981) cocoa beans placed in a heap at 28 °C and 80 % RH equilibrated between 6 and 8 days.

Irradiation does not leave any residue on the product. It was therefore necessary to include packaging to prevent re-contamination after irradiation. The jute sack which is the usual packaging material for cocoa was found to be too

porous for exchange of air etc. between the environment and the content of the bag. Woven polypropylene has been found to perform better in this regard than jute sack in a previous study (Appiah, 1982). The woven polypropylene bags used in packaging irradiated cocoa did not only prevent recontamination by fungi but also restricted the movement of moisture and delayed equilibrium time. The percentage change in weight during storage of the bagged cocoa was less than (1 %), indicating that woven polypropylene bag is a good packaging material for cocoa. The longer equilibrium time in the bags agrees with the findings of Powell and Wood (1959).

In this study it was found that initial moisture content of the bean, the relative humidity of the environment during storage and the types of packaging influenced the effectiveness of radiation in controlling fungi in storage. For long term storage, the irradiated beans should be stored in a 65 % R.H. environment to prevent the beans moisture content from exceeding 8 % in storage; for periods shorter than 4 months, beans could be stored in an environment of 80 % R.H.

In Chapter seven, the effect of radiation on some quality parameters of cocoa was ascertained in a series of experiments. The melting point, saponification value and unsaponifiable matter of cocoa butter determined at the Cocoa Processing Company were within the standard set in the factory, indicating that exposing the beans had not adversely affected these quality parameters.

The fatty acid composition of cocoa butter from irradiated as well as unirradiated cocoa separated into palmitic, stearic and oleic acid. Further studies have to be carried out to determine the relative quantities of these fatty acids after

irradiation of cocoa beans. Exposure of cocoa to radiation or a combination of heat and radiation did not have any effect on the rancidity of the extracted cocoa butter.

The cooling curves for the samples were similar. The temperature quotient of the control and that of the samples treated with moist heat at 80 °C and 4 kGy were 0.24 °C/min. This meant the heat and radiation treatment did not have any effect on the rate at the cocoa butter solidified.

When cocoa drink prepared from cocoa powder from irradiated beans were subjected to organoleptic (sensory) evaluation by 22 panelists, there was no significant difference between the control and the samples treated with heat and radiation for colour, flavour and taste. The application of radiation only or in combination with heat did not affect the quality of cocoa exposed to the treatments.

From the discussions above, it is technologically feasible to prevent and control mouldiness in dried cocoa beans by applying either radiation only or a combination of heat and radiation without significantly affecting the quality of cocoa exposed to the treatments. One may, however, like to know whether irradiation of cocoa beans is practically feasible and whether it could be considered in the Ghanaian context.

Cocoa is still the most important cash crop in Ghana. The two major problems of the cocoa industry are mouldiness and insect infestation. Mouldiness is controlled by hand picking and keeping the moisture content of the beans below 7.5 %. The current use of the cut test to assess mouldy beans is only an estimate and allows a lot of mouldy beans to pass undetected. Should a new method of

assessing and detecting the presence of internal fungi replace the cut test or should the need arise to reduce the microbial counts to a minimum required level, there will be the need to apply an alternative method of controlling or preventing mouldiness in cocoa.

Cocoa becomes heavily infested with insects if the cocoa is not periodically fumigated during storage. Huge sums of hard currency are spent annually on the importation of fumigants for that purpose. Fumigation of food with various chemicals such as ethylene dibromide (EDB), methyl bromide and ethylene oxide is either prohibited or is being increasingly restricted in most advanced countries for health, environmental and occupational safety reasons. Although Phostoxin which is currently being used for fumigation of cocoa beans is very effective in controlling insects, there is concern about development of resistance by the insects against the fumigant.

Irradiation can be applied to cocoa for dual purposes, controlling both insects and fungi. The safety and effectiveness of irradiation as a food preservation method have been recognised by the Codex Alimentarius Commission when it adopted a Codex General Standard for Irradiated Foods in 1983 and its associated Code of Practice and recommended both for acceptance by all member countries in 1984. The regional Plant Protection Organisations operating within the framework of the International Plant Protection Convention have endorsed irradiation as an effective quarantine treatment of fresh agricultural products since 1991 (ICGFI, 1996). Irradiation has the advantage of not leaving any residue. With the increasing prohibition and restriction of chemical

preservatives, irradiation is likely to play a more prominent role in food trade in the near future

In Ghana, it has been established that mouldiness and insect infestations are two of the most important causes of loss in the cocoa industry. The other causes are damage by rodents, insects, germination, over drying, flat bean, under-developed bean, and blackpod disease. The effective dose for the disinfestation of cocoa beans against insects has already been established at the laboratory scale in Ghana (Amoako - Atta, 1979). The results in Chapters 5, 6 & 7 of this study have also demonstrated that it is technologically feasible to prevent and control fungal infection in dried cocoa beans either applying radiation only or in combination with heat without adversely affecting the quality of the beans. Pilot scale experiment on irradiation of cocoa beans to study the economic feasibility and transfer the technology to end-users is currently underway. Standard for irradiated foods (GS 210), the regulation which allows the commercialisation of the technology, is now in place. Discussions on the acceptance of irradiated food in world trade is still in progress. It is hoped that when commercial irradiation becomes a reality in Ghana, this study will contribute immensely towards the application of the process as a phytosanitary measure to replace fumigation in exported cocoa. It may also enhance local storage of cocoa beans either for processing into secondary products or in anticipation of better market prices with minimal storage losses.

8.2 CONCLUSION

These studies on cocoa beans have led to a number of conclusions.

- The % loss of cocoa beans at the farmers' level was estimated to be less than 2 % per year (1.73 % & 1.66 %) in the Tafo District with the majority of farmers losing 1-5 kg of beans per year.
- Cocoa beans lost due to mouldiness was quantified at the farmers' level using two methods. Using the questionnaire approach, mouldiness came out as the second, most important cause of loss accounting for 0.16 % of farmers marketable yield. In the bean collection approach the loss was 0.13 % in the Tafo District. The quantity of mouldy beans at the buyers level was negligible (0.00002 %). Further surveys covering other areas will have to be carried out in order to obtain the estimated in the country.
- The quantity of mouldy beans at the Tema port for 1992/93 period was 603.82 T which was 0. 56 % of the exportable cocoa. The average for a period of 5 years was 0.69 %. Although the quantity lost seem small the study is still important in view of the negative effect of mouldiness on processed cocoa.
- Mouldiness did not seem to be a problem at the buyers' level or at the Tema port. The analyses showed that the Ghanaian exportable cocoa is of a high quality. The formula derived could be used to estimate the quantities of cocoa beans showing various defects and to advise farmers accordingly. Even though mouldiness at the port is very low it is important to maintain

the quality because of the effect of mouldy beans on the products manufactured from them.

- In the isolation of fungi from cocoa beans, dipping in 70 % ethyl alcohol for 1 min was found to be the best surface sterilising method. Culturing on water agar medium gave the best results. External fungi were found to be as important as internal ones as they contribute to internal mouldiness. Twenty nine fungal species have been added to the list of fungi known to occur on cocoa in Ghana.

- The investigation has shown that it is technologically feasible to prevent and control mouldiness in dried cocoa by applying either radiation alone or a combination of heat and radiation. The sensitivity of the fungi appeared to vary from species to species and within species. The effect was found to increase with an increase in the dose of radiation.
- The moisture content before, during and after irradiation influenced the effect of radiation. *A. flavus* required different inactivation doses in different media. *A. flavus* spore suspension was completely inactivated *in vitro* by applying heat at 55 ^{0}C for 2.5 min and 0.75 kGy, 53 ^{0}C for 5 min and 0.5 kGy radiation or 50 ^{0}C for 10 min and applying radiation at 1.0 kGy. However, the dried cocoa beans inoculated with *A. flavus* required a dose of 6 kGy or a combination of heat at 80 ^{0}C for 30 min and a radiation dose of at least 3.5 kGy to control mouldiness.
- No aflatoxin was detected in cocoa beans inoculated with *A. flavus* and exposed to 10 kGy radiation before storage; whereas the inoculated beans that

were stored without irradiation became mouldy and produced aflatoxin. Thus, use of irradiation to control mould growth also prevents production of aflatoxin. Irradiation did not destroy the already formed aflatoxin.

- Application of radiation only or in combination with heat did not significantly affect the sensory quality of the beans thus exposed. Radiation can therefore be applied to cocoa beans to extend its shelf life and to reduce losses.

8.3 RECOMMENDATIONS

- The information obtained in the survey could be extrapolated to cover the loss of cocoa beans in the Tafo District. Similar surveys could be carried out in a representative number of districts to cover the Eastern Region. In order to obtain the quantity of beans lost by farmers in the whole country, it is recommended that similar experiments be conducted in representative districts in a number of regions. To determine the quantity of discarded beans at the buyers' level, should similar surveys should be conducted in the representative buying centres in the country.
- It is being recommended that the data which is routinely collected at the port before shipment be analysed as in this study so that there will be information about the quality of cocoa beans exported annually. This will be useful information which could help in evaluating the trend of mouldiness in our cocoa. Through this analysis it will be possible to trace where farmers are not meeting the required standard or where standard is falling and advise farmers to improve upon their performance.

- Further work need be done on bulk storage of irradiated cocoa beans for period longer than 4 months. Good quality beans should be used when irradiating beans for storage as irradiation does not decontaminate bad quality beans of aflatoxins.
- For effective results the treatment must be accompanied by good manufacturing practices. It is important that healthy beans with moisture content of about 6 to 7 % are used.
- It is being recommended that for long term storage, the irradiated beans should be stored in a 65 % R.H. environment to prevent the beans moisture content from exceeding 8 % in storage; for periods shorter than 4 months, beans could be stored in an environment of 80 % R.H.

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APPENDICES

Appendix 1: Cocoa growing Districts and Societies in the Eastern Region of Ghana.

Number	Districts	Number of societies
1	Suhum	30
2	Nkawkaw	34
3	Kibi	19
4	Koforidua	16
5	New Tafo	16
6	Nankese	21
7	Nsawam	17
8	Ekuase	26
9	Anyinam	21
10	Kwahu Tafo	14
11	Akim Oda	38
12	Ofoase	20
13	Asamankese	24
14	Akyiase	23
15	Kade	37

Appendix 2: Societies and number of cocoa farmers inthe TAFO DISTRICT

Number	Societies	Number of farmers
1	Adjapoma	226
2	Addai Nkwanta I	265
3	Addai Nkwanta II	423
4.	Asikasu	293
5	Effakrom	138
6	Kukurantumi I	349
7	Kukurantumi II	359
8	New Tafo I	767
9	Old Tafo	585
10	Anyinasin	218
11	Asafo	408
12	Maase	276
13	New Tafo II	379
14	Osiem	443
15	Sokode Juaso	143
16	Tontro	206
	Total	5,483

Appendix 3: Questionnaire on the survey on discarded beans on the farmer's level

1.(a) Name of Village. .. .. Date.... .. ..
(b) District... .. .. (c) Religion. .. . ..

2. Farmers Name... . ... Sex... .. ....
Age.. ... .

3. (a) Size of Farm. . . ..(Acres/Ha)

(b) Average yield of load/bags) . ..

4. Cocoa Beans losses.

(a) Do you sometimes throw away some of your cocoa?(Yes/No)

(b) At what stage (i) when drying? .. ..

(ii) During storage? . ...

(c) Why do you throw away the beans?

(i) Mouldy.. . .(ii)Insect damage? . .

(iii) Rodent damage?.... . .(iv)..... ...
(v)

Otherreasons(Specify) . .. .

(d) How much do you throw away or pick out before marketing duriny one season? . . ..

(e) (Estimate as No. of bags or American tins fulls)

(f) What do you do with beans you pick out? . .

(i) Throw away?. .. . .

(ii) . .. .

(g) Who buys the discarded beans? .. . .

(h) What do they use the beans for? . ..

. ...

5. (a) How do you convey your beans to the buying centre?

.. . . --- .

Appendix 4: FORMS FOR DISCARDED BEANS

Name of farmer:...................... Season:..............

Village :....................... Total Yield..........

Size of Farm :......................

Date	Quantity Discarded	Quantity (Weight) of cocoa by Weevil	Moulds	Rodents	Others
TOTALS					

of damage observed in the discarded cocoa beans expressed as percent of the total discarded bean per season.

TYPES OF DAMAGE	MAJOR	MINOR
Insect	4.9	12.80
Rodent	44.33	18.25
Mouldy	1.4	10.48
Black pod-infected	3.28	46.75
Under developed	23.28	2.87
Flat	10.51	2.45
Germinated	8.14	3.21
Slaty	4.58	3.07

Appendix 6: Analysis of Variance of Various Defects at Farmers Level for Major and Minor Seasons

(a) Mouldy beans

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Season	1	5.6719	5.6719	5.6719	18.00	0.000
Error	46	14.4979	14.4979	0.3152		
Total	47	20.1898				

(b) Insects infested beans

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Season	1	3.9331	3.9331	3.9331	9.91	0.003
Error	46	18.2545	18.2545	0.3968		
Total	47	22.1876				

(c) Blackpod beans

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Season	1	102.40	102.40	102.40	5.32	0.026
Error	46	885.48	885.48	19.25		
Total	47	987.89				

Appendix 7: The Weight and No. Of Bags of Cocoa Shipped During 1992/93 Main Season from Tema Port

NAME OF VESSEL	WT. (TONS)	BAGS
Konings	4000	64,000
St. Blare	500	8,000
Therese Dalmas	1050	16,800
Ned Van Cloon	100	1,600
Christian Maersk	250	4,000
Pacific Marie	300	4,800
Charlotte Maersk	200	3,200
Veronique Delmas	800	12,800
Clara Maersk	125	2,000
Thalassini Axia	100	1,600
Sherbres	2050	32,800
Medlloyd V. Dielman	200	3,200
Cornilia Maersk	500	8,000
Yolanda	250	33,600
Torm SP.	500	8,000
Christian Maersk	250	4,000
Theresa Dalmas	500	8,000
Althi	367	58,800
Naasven	325	5,200
Theresa Dellmas	600	25,600
Esmaralda	200	3,200
Christian Maersk	550	8,500
Medlloyd	100	1,600
V. Delmas	200	32,000
Cornelia Maesk	250	4,000
Tano River	250	4,000
Sirius Delmas	500	8,000
Shebro	350	5,600
Tarkwa	100	16,000
Pacific Marie	750	12,000
M.V. Pos Europe	50	800
Christine Maersk	30	4,800
Sessil air	1150	18,400
Cold Hilla	4350	69,600
Therese Delmas	1555	24,880
Levant Gracht	3000	48,000
George Lyras	200	3,200
Tano	345	5,520
V.Delmas	500	8,000
Fionia	50	800
Volta River	1650	26,400
Ketira	250	4,000
Shebro	950	15,200
Parnam Mary	1200	19,200
Molande Delmas	700	11,200
B. Gracht	1500	24,000
Ub Poineer	200	3,200
Torm Freya	500	8,000
J. Delmas	2000	32,000
G. Diel. 4193	50	800
T. Delmas	800	12,800

Appendix 8: The Quality (% Average Defects) of Cocoa Beans Shipped During 1992/93 Main Season

SHIP	M	G	S	W	OD	TM	TS	AOD	Purity	F/G
Alblasg2	0.40	0.43	2.45	0.47	0.01	0.50	2.45	0.90	96.29	97.59
Alblasgr	0.41	0.48	2.58	0.40	0.03	0.40	2.58	0.87	96.18	97.38
Altai	0.72	0.85	2.77	0.15	0.22	0.72	2.77	1.21	95.34	97.07
Altai2	0.38	0.29	2.33	0.24	0.02	0.38	2.44	0.71	96.63	97.57
Archin	0.73	0.80	2.55	0.10	0.05	0.73	2.55	0.95	95.78	97.38
Archined	0.78	0.63	2.62	0.22	0.04	0.78	2.62	0.87	95.64	97.34
Axia	0.67	0.88	2.67	0.00	0.05	0.67	2.67	0.95	95.72	97.33
Blaize	0.78	0.80	2.56	0.31	0.12	0.75	2.48	1.04	95.65	97.51
B'gracht	0.72	0.74	2.66	0.22	0.13	0.71	2.58	1.08	95.54	97.29
Charlott	0.52	0.62	2.26	0.53	0.06	0.52	2.22	1.03	96.06	97.46
Chastine	0.34	0.64	2.56	0.11	0.32	0.54	2.46	0.98	96.09	97.50
Clara	0.57	0.96	2.40	0.00	0.09	0.57	2.49	1.10	95.86	97.51
Clara2	0.57	0.10	2.57	0.43	0.00	0.57	2.57	0.57	96.33	97.43
Cloon	0.62	0.57	1.88	0.78	0.05	0.55	1.40	1.57	96.30	97.93
Cloon2	0.48	0.57	2.31	0.24	0.04	0.48	2.31	0.89	96.65	97.69
Corinthi	0.48	0.31	2.70	0.37	0.06	0.48	2.70	0.74	95.81	97.33
Cornel	0.69	0.80	3.29	0.09	0.31	0.87	3.09	1.19	95.13	96.71
Corneli	0.10	0.00	2.90	0.23	0.00	0.20	2.90	0.23	96.67	97.10
C'Maersk	0.36	0.42	2.81	0.14	0.05	0.36	2.81	0.61	96.21	97.02
Delmas	0.74	0.81	2.76	0.14	0.09	0.76	2.77	1.01	95.50	97.04
Delmas3	0.62	0.64	2.78	0.26	0.17	0.64	2.79	1.05	95.57	96.80
Demas6	0.34	0.49	2.62	0.23	0.05	0.38	2.56	0.76	96.33	97.34
Diemen	0.24	0.92	2.98	0.18	0.24	0.52	3.23	1.31	95.68	96.55
D'srus	0.50	0.56	2.64	0.72	0.00	0.50	2.69	1.21	95.58	97.46
Echo	0.33	0.58	2.38	0.43	0.04	0.33	2.41	1.01	96.31	97.56
Esmera	0.89	0.84	2.71	0.00	0.06	0.93	2.73	0.90	95.44	96.84
Esmeral	0.76	0.68	2.34	0.18	0.03	0.73	2.25	0.93	96.12	97.66
Europe	0.33	0.57	2.77	0.30	0.00	0.33	2.77	0.90	95.90	97.23
Foinia	0.23	0.57	1.67	0.00	0.00	0.00	0.23	1.67	97.57	98.33

M = mouldy

S = slaty

G = germinated

W = weevil –infested

O = other defects

TM = total mouldy

TS = total slaty

AOB = all other defects

SHIP	M	G	S	W
Freya	0.60	0.75	2.78	0.27
Freya2	0.31	0.31	2.65	0.21
George	0.91	0.73	3.12	0.00
Gold	0.34	0.52	2.31	0.20
G'Alisa	0.37	0.34	2.51	0.51
G'hilla	0.46	0.57	2.47	0.42
G'Varda	0.34	0.33	2.52	0.22
Helga	0.20	0.47	2.33	0.33
Hilla	0.51	0.44	2.56	0.36
Jezerd	0.53	0.48	2.94	0.03
Kehia	0.51	0.63	2.92	0.24
Koggegr	0.52	0.51	2.76	0.15
Koning	0.45	0.83	2.42	0.01
Koninsg	0.54	0.84	2.36	0.02
L'gracht	0.49	0.69	2.65	0.22
Maashav	0.83	0.72	3.23	0.19
Maersk	0.54	0.59	2.76	0.23
Maersk4	0.32	0.44	2.75	0.25
Maesk	0.76	1.21	2.66	0.21
Maru	0.74	1.00	2.72	0.08
Nedlloy	0.62	0.57	1.88	0.33
Nedloyd	0.73	0.45	2.93	0.28
Ocean	0.39	0.54	2.65	0.24
Oril	0.72	0.85	2.77	0.15
Panama	0.61	0.61	2.70	0.19
Panama2	0.29	0.24	2.65	0.29
Parkere	0.58	0.56	2.58	0.31
Pioneer	0.49	0.53	2.50	0.13
Pol	0.35	0.35	2.53	0.48
P'maru2	0.38	0.53	2.32	0.15
Shebro	0.90	0.85	2.82	0.20
Shebro2	0.67	0.57	2.80	0.27
Shebro5	0.59	0.66	2.58	0.32

OD	TM	TS	AOD	PURITY	F/G
0.04	0.61	2.73	1.07	95.59	97.25
0.02	0.31	2.65	0.54	96.50	97.29
0.00	0.91	3.12	0.73	95.25	96.88
0.22	0.35	2.65	0.94	95.58	97.31
0.01	0.41	2.47	0.81	96.27	97.51
0.00	0.38	2.52	1.00	96.26	97.48
0.06	0.38	2.54	0.63	96.49	97.43
0.00	0.20	2.33	0.77	96.67	97.67
0.06	0.51	2.48	0.97	96.12	97.43
0.06	0.53	2.84	0.57	95.73	97.04
0.25	0.49	2.92	1.11	95.49	97.03
0.04	0.52	2.76	0.69	96.07	97.22
0.22	0.49	2.34	1.09	95.68	97.58
0.23	0.59	2.36	1.13	95.01	96.92
0.07	0.53	2.62	0.99	95.93	97.33
0.08	0.79	3.39	1.02	95.03	96.42
0.03	0.54	2.76	0.84	95.91	94.13
0.14	0.36	2.66	0.77	96.14	95.94
0.02	0.76	2.44	1.59	95.11	97.17
0.09	0.75	2.71	1.18	95.38	97.31
0.10	0.62	1.88	1.02	96.50	98.05
0.05	0.73	2.93	0.77	95.90	97.07
0.02	0.37	2.64	0.71	96.20	97.35
0.22	0.72	2.77	1.21	95.34	97.07
0.07	0.62	2.72	0.84	95.84	97.33
0.05	0.29	2.68	0.58	96.57	97.33
0.10	0.59	2.53	0.91	95.90	96.59
0.19	0.55	2.56	0.88	95.94	97.38
0.06	0.42	2.51	0.78	93.20	93.72
0.09	0.48	2.36	0.65	96.45	97.42
0.07	0.90	2.82	1.08	95.19	97.12
0.11	0.65	2.76	0.95	95.68	97.20
0.13	0.59	2.53	1.07	95.88	97.36

SHIP	M	G	S	W
Shebro6	0.43	0.60	2.41	0.45
Sherbro	0.66	0.88	2.65	0.05
Sherbro3	0.38	0.38	2.44	0.13
Sirius	1.06	1.39	2.64	0.07
Sirius3	0.39	0.35	2.86	0.27
Sissilir	0.22	0.55	2.85	0.17
S'Delmas	0.42	0.43	2.74	0.24
Tana	0.38	0.63	2.71	0.29
Tana2	0.35	0.49	2.63	0.34
Tano	0.79	1.06	2.61	0.14
Tano2	0.60	0.69	2.74	0.25
Tarkwa	0.68	1.03	2.66	0.12
Tarkwa2	0.45	0.39	2.54	0.34
Tarn	0.42	0.40	2.67	0.28
Tdelmas	0.32	0.59	2.63	0.15
Therese	0.54	0.73	2.74	0.02
Therese3	0.44	0.44	2.45	0.17
Therese4	0.44	0.50	2.27	0.47
Tormsp	0.51	0.50	2.51	0.14
Veroniqu	0.46	0.54	2.74	0.41
Volta	0.64	0.64	2.73	0.37
Volta2	0.43	0.44	2.45	0.38
V'Delmas	0.52	0.68	2.68	0.24
Yolande	0.38	0.51	2.63	0.22
Yolande2	0.54	0.57	2.72	0.28
Yolande4	0.51	0.62	2.63	0.48
Y'Delmas	0.37	0.39	2.48	0.17
Average	0.52	0.60	2.61	0.24

OD	TM	TS	AOD	Purity	F/G
0.09	0.51	2.38	1.02	96.22	97.40
0.11	0.67	2.63	1.00	95.87	97.32
0.11	0.39	2.59	0.55	96.41	97.33
0.04	1.07	2.69	1.35	1.06	1.39
0.08	0.47	2.80	0.67	96.17	97.14
0.38	0.25	2.85	1.02	95.88	97.06
0.10	0.44	2.80	0.70	96.07	97.15
0.07	0.38	2.71	1.01	95.92	97.29
0.01	0.35	2.63	0.84	96.25	97.37
0.10	0.79	2.61	1.31	95.29	97.39
0.16	0.60	2.72	1.06	95.70	96.11
0.07	0.67	2.63	1.28	95.47	97.27
0.03	0.40	2.55	0.73	96.40	97.44
0.03	0.51	2.72	0.72	96.13	97.28
0.16	0.30	2.64	0.88	93.84	95.02
0.29	0.65	2.62	1.06	95.60	97.27
0.04	0.45	2.39	0.64	95.86	96.46
0.06	0.44	2.26	0.96	96.26	97.78
0.16	0.51	2.44	0.79	96.34	97.00
0.03	0.46	2.74	0.91	95.85	97.25
0.15	0.64	2.76	1.15	95.53	94.23
0.11	0.49	2.44	0.82	96.18	97.45
0.30	0.51	2.69	1.10	95.70	97.34
0.15	0.39	2.64	0.86	96.15	97.27
0.07	0.52	2.74	0.84	95.94	119.76
0.02	0.57	2.59	1.09	95.75	97.33
0.05	0.37	2.41	0.64	96.56	97.48
0.09	0.54	2.59	0.93	94.82	96.30

Appendix 9: Shipment of Cocoa from Tema Port During Minor Season of 1993

Shipment No.	Ship	Weight	No. of bags
1	Alblasgracht	1429	22,864
2	Sherbro	1500	24,000
3	Veronique	100	1,600
4	Yolande Deli	900	14,400
5	Keta Logoon	5000	80,000
6	Ursus Delmas	3350	53,600
7	Francois De Sale	1150	18,400
8	Christian Maersk	500	8,000
9	MV Keta Logoon	1009.8	16,156.8
		14938.8	239,020.8

Appendix 10: Analysis of variance of survival of *A. flavus* on cocoa beans after combined treatment on heat and radiation and storage for 1 month at 75 and 90% R.H.

Source of Variation	dif	Sum of squares	Mean squares	Variation
Temp	3	7.7628	58761	38.073*
Heat hum	1	8.44202	202	124.213**
Rad	2	106.82812	41406	785.91
Lin	1	98.14005	98.14005	1444.003**
Quad	1	8.68807	8.68807	127.834**
Humstor	1	13.09385	13.093	192.659**
Temp. hum	3	3.96542	1.32181	19.449*
Temp. rad	6	2.58023	0.43004	6.327
Dev. lin	3	1.68363	0.56121	8.257
Dev. Quad	3	0.89661	0.29887	4.397
Heat hum. rad	2	0.61085	0.30542	4.494*
Dev. lin	1	0.61051	0.61051	8.983
Dev. quad	1	0.00034	0.12100	0.005
Temp humstor	3	1.18882	0.39627	5.831
Heathum humstor	1	0.12100	0.12100	1.780
Rad. humstor	2	0.07042	0.03521	0.518
Lin. dev	1	0.07031	0.07031	1.035
Quad. dev	1	0.00010	0.00010	0.002
Temp.humtemp.rad	6	3.88683	0.64781	9.532*
Dev. dev. lin	3	1.92456	0.64152	9.439
Dev. dev. quad	3	1.96227	0.65409	9.624
Temp.humtemp.humstor	3	0.25977	0.08659	1.274
Temp. rad.humstor	6	0.68883	0.11481	1.689
Dev. lin. dev	3	0.44131	0.14710	2.164
Dev. quad. dev	3	0.24752	0.08251	1.214
Heathum.rad.humstor	2	0.35607	0.17803	2.620
Dev. lin. dev	1	0.06125	0.06125	0.901
Dev. quad. dev	1	0.29482	0.29482	4.388
Residual	6	0.40778	0.06796	
Total	47	150.26285	3.19708	

* Significantly different at $P \leq 0.05$

** " " " " 0.01

Temp Temperature Lin Linear

Heat hum Humidity during heat. Quad Quadratic

Rad Radiation Humstor Storage humidity

Appendix 11: Analysis of variance survival of *A. flavus* on cocoa beans after heat and radiation treatment and storage at 28°C, 80% R.H/. for 4 months

Source of variation	Sum of square	d.f	Mean square	F-ratio	Sig. lev.
Main effects	169 7943	7	21.22429	36.253	.0000
Store time	2.79667	4	.699168	1.194	.3246
Temp.	65.22923	1	65.22922	111.416	.0000
Humidity	14.72131	1	14.72130	25.145	.0000
Dose	87.04716	2	43.52358	74.341	.0000
RESIDUAL	29.85820	51	.5854550		
TOTAL(CORR.)	199.6525	59			

Appendix 12: Analysis of variance suvival of A. flavus on cocoa beans kept at 20°C, 80% R.H. for 4 months

Source of variation	Sum of square	d.f	Mean square	F-ratio	Sig. lev.
Maineffects	54.46176	7	7 780251	33.868	.0000
Storetime	3.430567	4	.857642	3.733	.0192
Temp.	.000053	1	.000053	.000	.9881
Humidity	51.03114		5103114	.000	.9881
Dose	87.04716	2	43.52358	74.341	.0000
RESIDUAL	29.85820	51	.5854550		
TOTAL(CORR.	199.6525	59			

Appendix 13: Analysis of variance of suvival of *A. flavus* on cocoa after heat treatment at80°C. and irradiation and storage at 28°C, 80% R.H. for 4 months

Source of variation	Sum of square	d.f	Mean square	F-ratio	Sig. lev.
Main effects	67.285987	7	9.612284	67 459	.0000
Storetime	1.134980	4	.283745	1.991	.1349
Humidity	29.521920	1	29.521920	207.184	.0000
Dose	36.04716	2	18.314543	128.531	.0000
2-Factor interactions	4.7719400	2	2.3859700	16.745	.0001
RESIDUAL	2.8498200	20	.1424910		
TOTAL(CORR.)	74.907747	29			

Omissing values have been excluded

Appendix 14: Standard specifications for Cocoa Processing Company Ltd

PRODUCT		COCOA BUTTER
PARAMETERS		
1.	Free fatty acids (as oleic acid)	1.75 (max)
2.	Clarity (using Nephelometer)	10.0% (max)
3.	Iodine Value	35.0-40.0
4.	Refractive Index	1.4560-1.4570
5.	Saponification Value	188.0-196.0
6.	Unsaponifiable Matter	0.35% (Max)
7.	Slip Point	32.0 35.0°C
9	Taste	mild

Appendix 15. Analysis of Variance for percentage free fatty acid determined in cocoa stored in 1993.

Source of Variation	df	SS	MS	Variance ration	P
Dose	6	1.08298	0.18050	5.35	0.00
Month	3	6.84011	2.28004	67.52	0.00
Dose X month	17	1.75901	0.10347	3.06	0.05
Residual	27	0.91174	0.03377		
Total	53	10.52943			

Appendix 16: Analysis of variance for free fatty acid in cocoa (irradiated) butter stored in 1994.

Source of variation	df	SS	MS	Variance ratio	P
Dose	6	0.39773	1.85	0.006629	0.125
Month	2	6.417336	896.66	3.208678	0.001
Dose x month	12	0.035917	0.64	0.002993	0.616
Residue	28	0.100197	0.003578		
Total	48	6.593244			

Appendix 17: Analysis of variance for peroxide values in irradiated cocoa butter- 1993 samples.

Source of variation	df	SS	MS	Variance ratio	P
Dose	6	397 44	66.24	3.01	0.021
Months	3	10375.30	164.62	164.62	0.001
Dose/month	18	687.82	38.20	1.73	0.097
Residual	28	616.67	22.02		
Total	55	12577.57			

Appendix 18: Analysis of variance of peroxide values derrived from cocoa butter from irradiated cocoa-1994

Source of variation	df	SS	MS	Variance ratio	P
Dose	6	147.064	34.511	15.46	0.001
Months	2	1141.468	570.734	360.04	0.001
Dose/month	12	342.443	20.204	12.75	0.001
Residual	27	42.800	1.585		
Total	47	1551.002			

of variance data on organoleptic
n of cocoa powder after heat and
trements

a. Colour

Source of variation	df	Sum of Squares	Means sqaure	Variation (F)
Judges	21	201.51	9.60	0.73
Treatment	5	34.50	6.90	0.525
Resudual	105	1379.33	13.14	
Total	110	1413.84	12.85	
Grand Total	131	1615.34		

b.Taste

Source of variation	df	Sum of squares	Mean square	Variation (F)
Judges	21	170.37	8.11	0.625
Treatment	5	83.81	16.76	1.290
Residual	105	1363.99	12.99	
Total	110	1445.80	13.16	
Grand Total	131	1618.17		

c. Flavour

Source of variation	df	Sum of squares	Mean square	Variation (F)
Judges	21	363.73	17.32	1.062
Teatment	5	172.15	34.43	2.111
Residual	105	1712.84	16.31	
Total	110	1884.99	17.14	
Grand Total	131	2248.72		

Appendix 20: Colour Measurement of cocoa extract by spectrophotometer

concentration (%)	Wave length (nm)				
	310	320	450	400	420
100	<2	<2	1.40	0.52	0.41
50	1.85	1.47	0.66	0.30	0.25
40	1.78	1.34	0.65	0.57	0.44
30	1.45	1.02	0.46	0.20	0.16
20	1.08	0.76	0.34	0.15	0.13
10	0.76	0.50	0.21	0.10	0.09

21a: Fungi Occuring on Dried Cocoa Beans

Fungi	Unsterilised Bean	Alcohol Sterilised	Testa Removed Unsterilised
A. penicilloides	+	+	+
A. amstelodami	+	+	+
A. chivalieri	+	+	+
A. itaconicus	+	-	+
A. nidulans	+	+	+
A. flavus	+	+	+
A. oryzae	+	+	+
A. fumigatus	+	+	+
A. wentii	+	+	+
A. tamarii	+	+	+
A. effusus	+	-	-
A. versicolor	+	+	+
A. fischeri	+	-	-
A. ruber	+	+	+
A. echinulatus	+	-	+
A. pseudoglaucus	+	+	+
A. ochraceus	+	+	+
A. niger	+	+	+
A. glaucus	+	+	+
A. terreus	+	+	+
A. phoenicis	+	-	-
Penicillium variable	+	+	+
P. cyclopium	+	+	+
P. chrysogenum	+	-	-
P. luteum	+	+	+
P. phoeniceum	+	+	+
Fusarium nivale	+	-	-
F. moniliforme	+	-	-
F. oxysporum	+	-	+
Fusarium lateritium	+	-	-
F. rigidiuscula	+	-	-
F. solani	+	+	+
Fusarium sp	+	-	-
Fusarium roseum	+	+	-
Absidia corymbifera	+	+	+
Rhizoctonia	+	-	+
Sporendonema	+	-	+
Paecilomyces varioti	+	+	+
Geotrichum candida	+	+	+
Pullularia	+	-	-
Trichoderma viride	+	-	-
Mucor pusillus	+	+	+
B. theobromae	+	+	+
Rhizopus nigricans	+	+	+
Penicillium digitatum	+	+	+
Saccharomyces	+	-	+
Nigrospora	-	-	+
	+	-	-
Syncephalastrium			

after Different Treatments

Appendix 21b : Fungi occuring on Dried Cocoa Beans after Different Treatments

FUNGI	UNSTERILISED BEAN	ALCOHOL STERILISED	TESTA REMOVED UNSTERILISED	TEST ALONE
Chaetomium	+		+	
Verticillium	+			+
A. parasiticus	+	+		+
A. sydowi	+	+		+
A. lutescens	+	+	+	
A. sulphureus		+		+
A. japonicus	+	+	+	+
scopulariopsis	+			
P. citrinum	+	+		+
P. digitatum	+			+
C. lunata	+	+		+
C. globulosum	+	-	+	
Chaetomium sp.	+	+		+
Byssochlamys fulva	+	+	+	
Mucor sp.	+			+
Mucor haematis	+			+

Appendix 22. The Mean Moisture Content of Cocoa Beans Taken at Different Times after Combined Treatment of Heat and Radiation

Temp.	Dose (kGy)	Initial moisture content (MC)	Final MC at 75%	Final MC at 90%	Temp.	Dose (kGy)	Initial Moisture	Final MC at 75%	Final MC at 90%
20°C Low	0	7.6 ± .12	6.56	-	20°C High	0	7.39 ± .08	5.87	-
	1.5	7.65 ± .13	6.55	-		1.5	8.0 ± .36	6.14	8.9
	3.5	7.97	6.71	-		3.5	7.74 ± .42	5.42	9
60°C Low	0	7.58 ± .17	5.98	-	60°C High	0	8.41 ± 0.46	6.95	8
	1.5	7.85 ± .1	6.15	8.15		1.5	7.98 ± .39	6.35	8.7
	3.5	6.99 ± .03	6.72	8.54		3.5	8.73 ± .42	5.86	8.3
70°C Low	0	7.1 ± .12	6.33	-	70°C High	0	8.05 ± 0.53	6.88	8.1
	1.5	7.32 ± .13	6.32	9.8		1.5	7.25 ± 0.22	6.64	8.4
	3.5	7.6	6.54	8.95		3.5	7.50 ± 0.22	6.71	8.65
80°C Low	0	8.72 ± .12	7.2	-	80°C High	0	7.87 ± 0.48	7.30	9.0
	1.5	8.23 ± .13	6.64	8.26		1.5	8.13 ± 0.34	6.47	8.43
	3.5	7.92 ± .19	6.41	9.41		3.5	7.87 ± 0.28	6.35	8.41

Low = 40% relative humidity during heat treatment

Hight = >85% relative humidity

Beans were too mouldy, therfore no moisture content was determined.

Appendix 23 The moisture content of cocoa beans after heat and radiatiion treatment and during storage at 28°C for 4 months.

Temp. °C	Dose applied (kGy)	Moisture content of beans after: Initial Moisture content	1 month (65%rh)	2 months (80%)	3 months (80%)	4 months (80%)
	0	7.15 ± 0.08	6.57 ± 0.19	7.83 ± 0.23	0.8 ± 0.04	7.8 ± 0.12
20 LOW	3.5	7.21 ± 0.04	6.58 ± 0.18	7.88 ± 0.04	7.9 ± 0.04	7.9 ± 0.09
	4.0	7.13 ± 0.10	6.53 ± 0.27	7.69 ± 0.19	7.8 ± 0.14	8.1 ± 0.10
	0	7.28 ± 0.06	6.64 ± 0.02	7.84 ± 0.22	7.91 ± 0.02	7.95 ± 0.15
20 HIGH	3	7.15 ± 0.02	6.55 ± 0.13	7.74 ± 0.24	7.76 ± 0.12	7.76 ± 0.16
	4.0	7.0 ± 0.07	6.54 ± 0.04	7.8 ± 0.18	7.9 ± 0.05	8.06 ± 0.10
	0	7.02 ± 0.02	6.60 ± 0.02	7.85 ± 0.01	7.89 ± 0.21	8.16 ± 0.04
80 LOW	3.5	7.15 ± 0.02	6.42 ± 0.14	7.85 ± 0.15	7.92 ± 0.16	8.14 ± 0.02
	4.0	7.23 ± 0.09	6.52 ± 0.11	7.6 ± 0.03	7.8 ± 0.33	7.8 ± 0.21
	0	7.24 ± 0.03	6.55 ± 0.03	7 75 ± 0.25	7.8 ± 0.13	8.15 ± 0.05
80 HIGH	3.5	7.25 ± 0.37	6.69 ± 0.05	7.75 ± 0.30	7.8 ± 0.16	7.87 ± 0.10
	4.0	7.29 ± 0.07	6.62 ± 0.04	7.8 ± 0.3	7.9 ± 0.16	7.9 ± 0.11

*Standard Deviation

APPENDIX 24: RECORDS OF FUNGAL INFECTION AFTER COMBINED TREATMENT OF HEAT AND RADIATION AND STORAGE AT 90% R.H.

Temp.	Dose (kGy)	Days after storage 10	14	18	21	24	28
	0	10.9	45	100	100	100	100
	1.5	0	1.8	5.5	7.27	47.3	100
		0	1.9	7.7	9.6	19.2	100
20°C	High						
	0	9.8	100	100	100	0	100
	1.5	0	0	0	1.75	3.5	36.8
	3.5	0	10.3	94.8	100	100	100
60°C	Low						
	0	3.0	0	0	0	0	0
	1.5	0	43.6	100	100	100	100
	3.5	0	0	10	12	22	40
60°C	High						
	0	9.09	3.3	50	1.8	95	95
	1.5	0	0	1.8	0	14.29	16.1
70°C	Low						
	0	3.3	0	0	0	15.1	15.1
	1.5	0	0	0	71.43	0	0
	3.5	0	0	44.6	0	7 43	100
70°C	High						
	0	0	0	0	14.8	0	0
	1.5	0	0	7.4	0	42.6	53.7
	3.5	0	0	0	0	0	0
80°C	Low						
	0	0	0	44.6	71.43	71.43	100
	1.5	0	0	0	0	16.8	16.98
	3.5	0	0	0	0	0	0
80°C	High						
	0	0	0	7.4	14.8	42.6	53.7
	1.5	0	0	0	0	0	0
	3.5	0	0	0	0	0	0

Appendix 25: The effect of combined treatment of heat radiation on the log number of survival of *A. flavus* on cocoa beans

Temp. °C	Dose (kGy)	Before storage: Humidity during Dry	Before storage: Humidity during Moist	After storage 28 days at 75% R.H.: Humidity during Dry	After storage 28 days at 75% R.H.: Humidity during Moist
20	0	6.0	6.4	5.8	6.2
	1.5	5.1	5.2	5.1	5.2
	3.5	3.3	3.5	3.9	3 7
60	0	5.9	5.7	5.8	5.7
	1.5	4.3	3.2	4.3	3.1
	3.5	2.5	2.2	2.5	2.0
70	0	6.4	4.3	5.5	4.2
	1.5	3.7	2.3	3.6	3.5
	3.5	2.5	1.8	2.5	2.2
80	0	5.0	3.2	4.2	3.2
	1.5	2.6	1.6	2.6	1.5
	3.5	1.5	1.2	1.6	1.2