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STUDIES ON THE ROLE OF THE COLEOPTERAN SPECIES CALLOSOBROCHUS MACULATUS FAB., SITOPHILUS ZEAMAIS MOTS. AND TRIBOLIUM CASTANEUM HERBST. IN THE DISPERSAL OF FUNGI AMONG STORED GRAINS OF MAIZE (ZEA MAYS L.) AND RICE (ORYZA SATIVA L.) AND SEEDS OF COWPEA (VIGNA UNGUICULATA WALP.) AND BAMBARA GROUNDNUT (VIGNA SUBTERRANEA L.) VERDC]

A Thesis presented by

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in partial fulfillment of the requirements for the M. Phil. Degree of the University of Ghana

The Department of Botany

University of Ghana

Legon.

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DECLARATION

1, the undersigned, KWABENA BADU-YEBOAH, author of this thesis, do hereby declare that the work presented in this thesis,

STUDIES ON THE ROLE OF THE COLEOPTERAN SPECIES CALLOSOBROCHUS MACULATUS FAB., SITOPHILUS ZEAMAIS MOTS. AND TRIBOLIUM CASTANEUM HERBST. IN THE DISPERSAL OF FUNGI AMONG STORED GRAINS OF MAIZE (ZEA MAYS L.) AND RICE (ORYZA SATIVA L.) AND SEEDS OF COWPEA (VIGNA UNGUICULATA WALP.) AND BAMBARA GROUNDNUT (VIGNA SUBTERRANEA L.) VERDC

was done entirely by me in the department of botany, University of Ghana, Legon, from March, 1994 to December, 1995. This work has never been presented either in whole or in part for any other degree of this University or elsewhere.

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ABSTRACT

Seven *Aspergillus* species, *A. clavatus*, *A. flavus*, *A.fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus and A. ustus* have been used to investigate the role of three Coleopteran insect pests, namely, *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* in the persistance and spread of contaminant fungi among grains of maize *(Zea mays)* and rice (*Oryza sativa*) and seeds of bambara groundnut (*Vigna subterranea*) and cowpea *(Vigna unguiculata)*. The *Aspergillus* species were among fungi isolated from the grains and seeds.

Bambara groundnut seeds on sale at Kaneshie, La, Madina, Makola and Mallam Atta markets in Accra district contained species of *Absidia*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Neurospora*, *Paecilomyces*, *Penicillium and Pullularia*. The dominant genera were *Aspergillus* and *Penicillium* represented by five and four species, respectively, and the dominant species were *Aspergillus flavus* and *Aspergillus niger*.

The predominant species of cowpea seeds from the same markets were *Aspergillus flavus* and *Paecilomyces puntonii* among 19 species, and, the dominant genera were *Aspergillus*, *Paecilomyces* and *Penicillium*. The rest of the genera were *Cladosporium*, *Drechslera*, *Epicoccum*, *Fusarium*, *Mucor*, *Neocosmospora*, *Neurospora*, *Phoma*, *Pullularia*, *Rhizopus and Verticillium*.

Aspergillus flavus and *Penicillium citrinum* were the predominant species on maize grains from the five markets among 16 contaminant fungal species belonging to the genera *Aspergillus*, *Fusarium*, *Mucor*, *Neocosmospora*, *Neurospora*, *Penicillium*, *Pullularia* and *Rhizopus*.

Rice grains had the shortest list of genera. The fungi belonged to only four genera, *Aspergillus*, *Cladosporium*, *Mucor* and *Penicillium* and there were 14 species in all. The most frequently occurring species were *Aspergillus oryzae*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Penicillium expansum*.

Although all the seven *Aspergillus* species could grow on insect body leachate agar prepared with leachate of the three insect pests, the conidia of some of them could germinate in the leachate of only some of the insects. Only *A. flavus* and *A. ochraceus* condidia germinated in all the three leachates. Germination of the conidia of all the species occurred, anyway, in leachates containing extracts of various tissues of seeds (axis of the embryo, cotyledon and testa) and extracts of grains. Conidia of all the species germinated in solution of dissolved faecal pellets of *Callosobrochus maculatus*, while conidia of *A. clavatus*, *A. ochraceus* and *A. sulphureus* only germinated in the solution of dissolved faecal pellets of *Sitophilus zeamais* and conidia of also three species, *A. clavatus*, *A. flavus and A. ochraceus* germinated in the solution of faecal pellets of *Tribolium castaneum*.

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Aspergillus flavus conidia adhering to the bodies of *Sitophilus zeamais* and *Tribolium castaneum* were transported through maize grains packed in wide glass tubes. The amount of the conidia detached as the insects moved depended on the size of the spaces among the grains and the frequency of contact between the insects and the grains. *S. zeamais* lost 87.8, 84.7 and 76.7 percent of the original load of conidia as the insects travelled over 100cm through grains measuring 5.3-8.3 x 4.04-7.3mm, 8.1-10.2 x 6.0-8.2mm and 9.5-12.2 x 7.5-9.5mm, respectively. The corresponding figures for conidia on *T. castaneum* were, 88.5, 87.9 and 82.8 per cent respectively.

Dead insect bodies were invaded by many fungi despite the presence of large populations of surface bacteria. The colony-forming-units of bacteria recorded for *C. maculatus, S. zeamais* and *T. castaneum* per ml of suspending medium immediately after death were 27.5×10^4, 281×10^4 and 104.5×10^4 respectively; and six days later they were 37.8×10^4, 5.0×10^4 and zero, respectively. On the sixth day, *Aspergillus flavus* was isolated from the bodies of all the three insect pests. In addition, *Aspergillus niger, Mucor sp., Rhizopus sp.* and *Trichoderma Viride* were isolated from *C. maculatus., Aspergillus niger* and *Curvularia sp.* from *S. zeamais* and *Clasdosporium sp.* from *T. castaneum*.

The mycelium growing in the bodies after death might have arisen from inoculum either on the surface of the body or in the gut. For, the gut of the insects had extensive mycoflora. Fourteen, thirteen and sixteen fungal species were isolated from the gut of *C. maculatus, S. zeamais* and *T. castaneum*, respectively. The predominant genera were *Aspergillus* and *Penicillium* and five species, namely *Aspergillus flavus, Aspergillus fumigatus, Cladosporium herbarum, Penicillium citinum* and *Penicillium purpurogenum* were isolated from the guts of all the three insect pests.

Three *Aspergillus* species fed experimentally to the insects persisted for different lengths of time in the guts. *A. flavus* was isolated 6,8, and 5 days respectively, after feeding the insects, from the guts of *C. maculatus, S. zeamais* and *T. castaneum*. The corresponding survival periods for *A. fumigatus* were 4,6 and 5 days respectively, and for *A. ochraceus*, 6,4 and 3 days respectively.

It was concluded that products and dead bodies of the insects would contribute to the persistence of the contaminant fungi and living insects would be responsible for both persistence and dispersal of the fungi in stored grains and seeds. Persistence and dispersal of the fungi could be reduced by measures which control the insect pest population, by periodic removal of dead insect bodies especially in comparatively smaller stocks kept in the markets and by exposure of the products to light to drive the insects to the dark base and discourage the frequent migration.

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I. INTRODUCTION AND LITERATURE REVIEW

Insects destroy stored produce in tropical and sub-tropical countries and the control of insect infestation is a major part of storage management. They attack both plant and animal products. Insects have become adapted to widely diverse habitats and diets and the breeding rate is so prolific that within a few months, sufficient progeny can develop from a single mating pair to infest several tonnes of produce. Their inter-relationship with other agents of deterioration of products in storage adds another dimension to their economic importance.

Fungi are responsible for more plant diseases and breakdown of dry plant products (eg. grains, paper, seeds, timber, twines, etc.) than any other group of micro-organisms. The great diversity in their morphology, physiology and life cycle makes them a very versatile group of plant pathogens. Their spores, which constitute the principal infection units, are varied in form and are adapted to many different methods of dispersal.

The seemingly limitless profligacy of fungi in the production of spores is impressive. Of much more interest than the ability of fungi to produce spores in abundance is the development of mechanisms or devices that serve to provide maximum distribution of these spores. Survival of given species may in large part be conditioned by dispersal into habitats where food is available. In most species of fungi special mechanisms are lacking, and hence distribution appears to be largely fortuitous. This might appear to be the case in the dispersal of fungi which grow on products in

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storage by storage insect pests. Leach (1935) has expressed the opinion that "insects are not merely disseminators of inoculum, but the insect-fungi relationship is highly organized and has broad biological and evolutionary significance". This was one of the main reasons for carrying out this investigation on fungal contamination of grains of maize (*Zea mays* L.) and rice (*Oryzae sativa* L.) and seeds of bambara groundnut (*Vigna subterranean* (L.) Verdc) and cowpea (*Vigna unguiculata*(L) Walp) in storage.

Bambara groundnut is a widely cultivated tropical leguminous crop of Madagascar origin (Hutchinson, 1964). It has also been found in South Asia, on the banks of the Nile from Khartoum to Gondokora and in Brazil (Candolle, 1959). Kay (1979) also reported that the crop is now grown in Northern Australia and North America.

Bambara groundnut grows at a temperature of 20^0C to 28^0C and a rainfall of 900-1200mm per annum. It grows at elevations of up to 1600 metres. Bunch types normally mature in 90-120 days and the spreading types in 120-150 days (Kay, 1979).

The pods are usually sundried for several days before being stored in the shell in baskets or sealed pots, or shelled and then stored in sacks or plastic bags. The shelled seeds are usually susceptible to insect infestation and are sometimes fumigated with Phosphine, carbon tetrachloride or carbon disulphide. Seeds stored in jute sacks in Burkina Faso have been found to be severely attacked by insects - up to about 39 per cent infestation. Preliminary tests showed that, those stored in plastic bags were relatively free from attack (Kay, 1979).

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Seed yield of Bambara groundnut 30 years ago was between 300 and 800 kg/ha (FAO, 1961). It ranks next to cowpea as the widely grown grain legume in Africa, south of the Sahara (Okigbo, 1977). Stems and leaves are fed to cattle, while the seeds are boiled or made into soup (Bakhareva, 1975).

Total production in Africa has been estimated to be about 330,000 tonnes per annum and in the descending order of production, the important producers in Africa are, Nigeria, Burkina Faso, Niger, Ghana, Togo and Cote Ivoire (Kay, 1979).

Most of the production is consumed domestically and bambara groundnuts do not usually enter international trade, although there have been several unsuccessful attempts to develop exports to Europe for use as animal feed.

Cowpea is also a leguminous plant belonging to the family Papilionaceae. It is an ancient crop whose centre of origin is uncertain having been reported as possibly Asia - Hindestan and from Nigeria and Ethiopia and even South America. It is now widely distributed throughout the tropics and Sub-tropics and is an important food legume crop in Africa, South of the Sahara, particularly, in the West African Savanna Zone. Africa, in fact produces about 95% of the world crop, with Nigeria, Burkina Faso and Uganda as the dominant producing countries in that order. Outside Africa, cowpea is grown in Asia, especially India, in lowland and coastal areas of south and central America (Kay, 1979; Simmonds, 1976).

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Cowpea grows at a temperature of between 20 and 35°C, but can tolerate temperatures as low as 15°C. It grows in regions with rainfall between 600 and 1500mm per annum. In East Africa, the cowpea is normally grown at elevations up to 1500 metres. Depending upon the cultivar and environment, cowpeas may take from 60 to between 210 and 240 days to produce mature seeds (Kay, 1979).

The green pods are edible and are harvested when they are still immature and tender and before the seeds are fully developed. For long storage, pods are harvested when they are fully mature. The seeds range from 5mm to 12 mm in length and can be globular or reniform in shape. The testa may be smooth, rough or wrinkled, and can vary in colour from white, through various shades of buff, green, brown, red and purple to black, sometimes mottled, blotched or speckled patterns. The hilum is white approximately 3mm in length and, in the black-eyed types is surrounded by a dark ring. The seed weight averages from 5g to 30g/100 seeds. After threshing the seeds are thoroughly dried to a moisture content of about 14 percent before being stored.

The world major producing countries in descending order of magnitude of production are Nigeria, Burkina Faso, Uganda and U.S.A. In Africa, although the highest yield in Nigeria may exceed 3,000kg/ha, the average yield of dry seeds for the continent normally averages between 100 and 300kg/ha. World production for 1970-74 averaged 1,100,000 tonnes per annum, (Kay, 1979). With the continual expansion of cultivation, current production must be well beyond this level.

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Botanically maize and rice belong to the family Gramineae and both are annuals. In maize, the grain makes up about 42 percent of the dry weight of the tall (about 2.5 metres) plant. It is, after wheat and rice, the most important cereal grain in the world providing grains for human food, and grains and herbage for animal feed (F.A.O., 1992).

Maize cultivation probably originated in Central America, particularly in Mexico. De Candolle (1880) considered maize to have originated in the New World, in "New Canada", Vavilov (1931) presented criteria for establishing the centres of origin of maize to Mexican-Central American region and flourey maize to Peru, Ecuador and Bolivia.

The chief maize producing regions include the United States of America (U.S.A.) (especially the corn belt region of north central states) which produces almost half of the world's total, south-eastern Europe (especially the former U.S.S.R., Hungary, Romania and Yugoslavia) and Argentina, Brazil, China, India, Indonesia, Italy, Mexico and South Africa. Only about 5 percent of the U.S.A. crop is exported, but that is over half of the world's total exports (Simmonds, 1976).

From the agrobotanical studies of variability as indicated in reviews by Brandolini (1970) and Mangelsdorf (1974), it appears that Mexico and/or lowland central America is the centre of variability for the commercially important dent types. These forms have spread around the tropics since AD 1,500. Derivatives of the Mexican dents apparently spread into the Southern United States of

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America shortly before or after colonization of that region. The Mexican and Lowland Central American dents appear to have been associated with the Mayan civilization, while the conical corns from higher elevations in Central Mexico appear to have been associated with the Aztecs and their predecessors.

Maize grows from latitude 58^{0}N in Canada and the former Union of Soviet Socialist Republics to latitude 40^{0}S at both high altitudes and lowlying regions (F.A.O., 1992).

As in most economies at early stages of development, starchy food-stuffs account for something like 70-90 percent of the calories produced and consumed in tropical Africa. Of the major starchy staple food crops, maize is the most widely grown and has been very closely linked with economic development than any other starchy staple. In fact it is present in every sizeable area where food crops can be raised and in a number of regions - most of Kenya, Malawi and Zimbabwe as well as considerable sections of Angola, Benin, Cameroon, Ghana, Mozambique, Tanzania, Togo and Zambia, it is the leading starchy staple by a large margin.

Maize production in Ghana between 1990 and 1993 fluctuated and the figures in metric tonnes were 553,000, 932,000, 731,000 and 750,000 for 1990, 1991, 1992 and 1993, respectively (F.A.O., 1994).

In recent years, laudable breeding programme carried out by the Crops Research Institute (Council for Scientific and Industrial Research), Kumasi, in collaboration with the Canadian International Development Agency (CIDA) has yielded many high-yielding and high protein varieties. The most important ones being extensively

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cultivated are: Abeelehi, Abrotia, Dobidi, Obaatanpa and Okomasa.

Rice, on the other hand, has been cultivated for such countless ages that its origin must always be a matter for conjecture, Ting (1949) suggested that in view of the number of wild races found in Southern China, rice cultivation probably started in this region and spread northwards. Chang (1975), on the other hand, believed that rice was first domesticated in the area between Northern India and Pacific coast adjoining Vietnam and China.

The genus *Oryzae* comprises 25 species distributed through tropical and sub-tropical regions of Asia, Africa, Central and South America and Australia. There are only two cultivated species, *Oryzae glaberrima* Stead and *Oryzae sativa* Linn. *O. glaberrima* is confined to West Africa where it is an upland crop but is almost now replaced by *O. sativa* (Grist, 1986). The annual rice production in Ghana in 1990, 1991 and 1992 was 81,000, 151,000 and 100,000 metric tonnes respectively (F.A.O. Quarterly Bulletin of Statistics, 1994).

Callosobrochus subinnotatus Pic. is a serious pest of bambara groundnut seeds, with a total life span of 6-7 weeks (Prevett, 1966). Although Bruchus *vicinus* var *subinnotatus* occurs less frequently than *C. subinnotatus* its mode of attack leads to total loss of the invaded seed. For, 3-5 weevils usually burrow into one seed (Zacher, 1921). These two species of weevils occurred at very low freqeuncies in the stored seeds and were therefore, excluded from this investigation.

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Cowpeas are extremely susceptible to insect infestation during storage. Insects covering the main phytophagous taxa attack the cowpea plant in the field and the seeds after harvest. Several species of *Callosobrochus* cause damage to cowpea seeds in storage. The two most important ones are *Callosobrochus maculatus* Fabricius and *Callosobrochus chinensis* Linnaeus.

In Africa, insect pests may be responsible for 100 percent losses of cowpea yields (Raheja, 1976; Singh and Allen, 1980) and if not controlled, may virtually limit yields to less than 300 kg/ha (IITA,1985). Infestation occurs in the field when the pods are nearly mature. Eggs are either laid on the pods or on the seeds by insects which enter the pods through holes made by other pests. On-farm storage for six months is accompanied by about 30% loss in weight with up to 70% of the seeds being infested (Singh and Allen, 1980).

The adult life span is only 5-7 days. The female lays 50-80 eggs which hatch in about 3-5 days. The entire life cycle is completed in about 30 days. The damage is done by the larvae feeding inside the seed.

Other pests of cowpea of lesser importance include aphids, beanfly, leaf hoppers, thrips, podborers, pod sucking bugs, cowpea curculio and the storage bettle (Singh and Allen, 1980).

Investigations in Senegal have shown that cowpeas may be stored satisfactorily for up to one year in plastic sacks using soft capsules of carbon tetrachloride as a fumigant. Seeds treated with palm, groundnut or coconut oils are reported to be protected

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against insect infestation for periods up to six months but they tend to lose viability. The hermetic storage of cowpeas in small granaries, silos and pits has been developed in Nigeria, where a very encouraging development has been the use of plastic liners in traditional dried-earth granaries (Kay, 1979).

Two most important insect pests of maize grains in storage are *Sitophilus zeamais* and *Tribolium castaneum*. *T. castaneum* shows preference for the embryo of grains and it is also a major pest of the milled product.

Up to 400 eggs are laid by the female over a period of about 18 months. The eggs are laid at random in the foodstuff, and hatch in 8-12 days into slender cylindrical 4.8mm long larvae. The larvae pupate in the food in 11-16 days on wheat bran. The pupal stage takes about 4-8 days and the adult may live for as long as 18 months (F.A.O., 1983).

Sitophilus zeamais attacks maize, rice, wheat and sorghum grains causing hollowing out of the grains. Adults may live for up to five months. Under optimum conditions, 100-150 eggs are laid by the female over a period of about five months, but most eggs are laid by the younger adults. Each egg is laid in a minute hole chiselled in the grain by the female and the mouth of the hole is sealed by a secretion. The larva remains in the grain where it feeds and eventually pupates. The adult on emerging eats its way out of the grain. (F.A.O., 1983).

The major pests of rice is the rice weevil, *Sitophilus oryzae*. The adult weevil lives from 4 to 5 months, each female laying

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between 300 and 400 eggs in its life time. The eggs are deposited in small holes dug in the grain by the female. The larva on hatching, remains inside the kernel until it pupates. The young adult that emerges from the pupa bores its way out of the grain. Development from the egg stage to an adult takes about 24 days. (Cotton, 1966).

Six major types of defects caused by fungi growing in stored grains have been identified. (Christensen and Kaufmann, 1969; F.A.O. Tech. Report, 1981). These are: (1) decrease in germinability, (2) discoloration of parts or all of the seed or kernel, (3) heating and mustiness, (4) various biological changes, (5) production of mycotoxins, and (6) loss in weight. The causal fungi fall into two categories, namely, field fungi and storage fungi (Christensen and Kaufmann, 1965; 1969).

Field fungi are those that invade the developing seeds on the plants in the field. They may be pathogens or saprophytes. Common examples are *Alternaria tenuis, Cladosporium herbarum, Curvularia* species, *Epicoccum purpurascens, Fusarium* species, and *Verticillium alboatrum* (Malone and Muskett, 1964). These require high moisture levels and their activities are therefore curtailed in stored grains and seeds whose moisture content is usually kept at low levels.

Storage fungi are those that grow on the stored products. Most of them are able to grow without free water and can withstand high osmotic pressures. Most of the storage flora are xerophilic species of *Aspergillus* and *Penicillium* which are active at relative

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humidities ranging from 70 to 90 percent. In the view of Christensen and Kauffmann (1969), Christensen (1971) and, Warnock and Preeece (1971), a very low frequency of storage fungi of about less than 1.0 percent may be present as dormant mycelium within the tissues of pericarp and seed coats before harvest.

It is well known that most crops are hygroscopic, that is, they have the propensity of exchanging moisture with the storage atmosphere until an equilibrium is reached. The moisture content of crops especially, grains, pulses, oil seeds etc. is required to be reduced to a minimum level known as 'safe level' before such crops can be successfully stored. Therefore, both the moisture content of the produce and the "moisture content" of the storage atmosphere are critical physical factors in crop storage.

Moisture content and temperature are the primary factors determining the development of storage fungi in seeds and grains. Storage fungi grow at moisture contents in equilibrium with relative humidities ranging from 65-70 to 85-90 percent. The minimum air relative humidity for fungal growth varies among different species but growth of most fungi will be prevented if the relative humidity is less than 65 percent (F.A.O., 1985). Relative humidities that permit growth of some prevailing storage fungi are 65% for *Aspergillus halophilicus*; 70-73% for *Aspergillus restrictus* and *Aspergillus repens*; 80% for *Aspergillus candidus* and *Aspergillus ochraceus*, 85% for *Aspergillus flavus* and 85-95% for various species of *Penicillium* (Christensen, 1973).

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There may be considerable variation of the moisture level within a bulk and, therefore, any pockets of high moisture may form a locus for invasion by storage fungi. The problem of unequal distribution of moisture in the stored mass is common in bins where no forced aeration system is available (Neergaard, 1983).

Fungi which have been isolated from stored bambara groundnut seeds are comparatively few. Danquah (1973) isolated *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium sp*; *Macrophomina phaseolina*, *Penicillium sp*; *Rhizopus sp.* and *Tricothecium roseum* from the seeds in Ghana.

The lists of fungi of cowpea seeds and maize and rice grains in storage in Ghana are much longer. Arthur (1994) gave a list of 14 contaminant fungal species and Danquah (1973) isolated 24 species from cowpea seeds. The two lists are compared in Table 1

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TABLE 1: FUNGAL SPECIES ISOLATED FROM COWPEA SEEDS IN STORAGE IN GHANA

LIST OF ARTHUR (1994)	LIST OF DANQUAH (1973)
Aspergillus candidus	*Aspergillus flavus*
Aspergillus flavus	*Aspergillus niger*
Aspergillus fumigatus	*Botryodiplodia theobromae*
Aspergillus niger	*Cercospora sp.*
Aspergillus ochraceus	*Cladosporium oxysporum*
Aspergillus oryzae	*Colletotrichum capsici*
Aspergillus tamarii	*Colletotrichum graminicola*
Cladosporium herbarum	*Colletotrichum lindemuthianum*
Epicoccum nigrum	*Corynespora sp.*
Fusarium moniliforme	*Curvularia lunata*
Neurospora sitophila	*Curvularia pallescens*
Penicillium species	*Curvularia pallexens*
Rhizoctonia solani	*Curvularia trifolii*
Stemphylium lanuginosum	*Fusarium equiseti*
-	*Fusarium moniliforme*
-	*Fusarium poae*
-	*Fusarium semitectum*
-	*Fusarium solani*
-	*Macrophomina phaseolina*
-	*Myrothecium verrucaria*
-	*Nigrospora oryzae*
-	*Pestalotia sp.*
-	*Phoma oryzicola*
-	*Trichothecium roseum*

Simiarly numerous fungal species contaminated maize and rice grains in storage in Ghana. The species isolated from maize grains by Danquah (1973) and Odamtten (1986), and from rice grains by Addison (1971) and Danquah (1973) are shown in Tables 2 and 3, respectively.

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TABLE 2: FUNGAL SPECIES ISOLATED FROM MAIZE GRAINS IN STORAGE IN GHANA

LIST OF DANQUAH (1973)	LIST OF ODAMTTEN (1986)
Aspergillus sp.	*Aspergillus candidus*
Botryodiplodia theobromae	*Aspergillus flavus*
Cephalosporium acremonium	*Aspergillus fumigatus*
Curvularia eragrostides	*Aspergillus niger*
Diplodia macrospora	*Aspergillus ochraceus*
Drechslera maydis	*Aspergillus restrictus*
Fusarium moniliforme	*Aspergillus tamarii*
Macrophomina sp.	*Aspergillus ustus*
Nigrospora sp.	*Aspergillus wentii*
Penicillium species	*Cephalosporium acremonium*
Pestalotia sp.	*Cladosporium herbarum*
-	*Curvularia lunata*
-	*Drechslera maydis*
-	*Fusarium moniliforme*
-	*Neurospora sitophila*
-	*Paecilomyces variotii*
-	*Penicillium expansum*
-	*Penicillium verrucosum*
-	*Phoma glomerata*
-	*Rhizoctonia solani*
-	*Rhizopus oryzae*

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TABLE 3: FUNGAL SPECIES ISOLATED FROM RICE GRAINS IN STORAGE IN GHANA

LIST OF ADDISON (1971)	LIST OF DANQUAH (1973)
Alternaria longissima	*Alternaria longissima*
Cercospora oryzae	*Alternaria padwickii*
Chaetomium spp.	*Colletotrichum graminicola*
Curvularia cymbopogonis	*Curvularia cymbopogonis*
Currularia geniculata	*Curvularia eragrostidis*
Curvularia inaequalis	*Curvularia geniculata*
Curvularia lunata	*Curvularia inaequalis*
Curvularia maculans	*Curvularia intermedia*
Curvularia oryzae	*Curvularia lunata*
Curvularia pallescens	*Curvularia oryzae*
Curvularia trifolii	*Curvularia pallescens*
Drechslera hawaiiensis	*Curvularia trifolii*
Drechslera oryzae	*Drechslera hawaiinensis*
Drechslera rostrata	*Drechslera longirostrata*
Fusarium equiseti	*Drechslera oryzae*
Fusarium moniliforme	*Drechslera rostrata*
Fusarium semitectum	*Drechslera tetramera*
Fusarium solani	*Fusarium avenaceum*
Helminthosporium sativum	*Fusarium dimerum*
Nigrospora oryzae	*Fusarium equiseti*
Pyricularia oryzae	*Fusarium moniliforme*
Trichoconis padwickii	*Fusarium semitectum*
-	*Nigrospora oryzae*
-	*Phaeotrichoconis sp.*
-	*Phoma glomerata*
-	*Pyricularia oryzae*
-	*Stemphylium sp.*
-	*Ulocladictum sp.*
-	*Verticillium sp.*

The cardinal temperature for the growth of most storage fungi are 0-5^0, 30-33^0 and 50-55^{0}C. Some species of *Aspergillus* such as *Aspergillus flavus* and *Aspergillus candidus* have a high optimum temperature ranging from 35 to 40^{0}C. At 12-15^{0}C most storage fungi grow very slowly in cereals with moisture content of 15-16 percent, and at 5-8^{0}C they may cease growing (Christensen and Kauffmann, 1965; Christensen, 1974). At temperatures of 5-10^{0}C sound wheat and maize with a moisture content of 15-16 percent or even higher did

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not deteriorate when stored for a year (Papavizas and Christensen, 1958; Qasem and Christensen, 1958) and maize kept for two years at 5^{0}C still germinated and was entirely free of storage fungi (Qasem and Christensen, 1958). It appears from this that low temperature, in spite of high moisture content can preserve stored grains. It must, however be borne in mind that, if grains are already invaded prior to storage, even moderately, by storage fungi, these can still grow at very low temperatures even down to freezing point or below, provided the relative humidity is high. This holds true for some species of *Penicillium* common in seeds (Christensen, 1973).

Unfortunately, very low temperatures do not exist in local warehouses, and the existence of high temperatures that encourage high respiration rates of the stored products, rapid population growth of insect pests and fungi contributes to the deterioration of the products. Respiration by the stored products, insect pests and fungi together raises the temperature further, while metabolic water produced by the insects is absorbed by the seeds and grains raising their moisture content. The condition that is congenial to growth of both the fungal and insect population facilitates the association between the two groups of organisms.

Leach (1940) stated that by the specific association with certain species of plants and with particular organs of these plants, insects do not only disperse fungal infection units but also very effectively inoculate the plants. They transport the inoculum to the most favourable place for infection with little loss of material.

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In one group of the larger fungi, the Phallales, structure is obviously related to insect dispersal. In these fungi for example, *Phallus impudicus*, the minute spores embedded in a sugary slime with a strong and an unpleasant smell are displayed at maturity, usually on top of a conspicuous stipe. The spore dispersion depends upon sarcophagid and muscid flies. The penetrating odour is attractive to the flies and in consequence they carry the spore externally and also void them intact in their excreta. *Ithyphallus coralloides* which causes root rot of sugar cane is so attractive to flies that they can be driven away from fructifications only with difficulty. Various ants and beetles are also attracted to this species and no doubt carry the spores underground to situations favourable for germination and development (Wolf and Wolf, 1947). Various flies are also attracted to the saccharine exudate containing conidia of the sphacelial (Conidial) stage of *Claviceps*, especially *Claviceps purpurea* and *Claviceps paspali* (Ainsworth and Sussman, 1966). Similarly the pycniospores of *Puccinia graminis* and *Puccinia helionthi* are transferred by flies and other insects attracted to the sugary exudate of the pycnium of rusts (Craigie, 1931).

Hendree (1933) isolated from the faecal pellets of termites and from the frass and wood enclosing their burrows 33 genera of fungi, among them *Trichoderma* and *Penicillium*. In her opinion these fungi are a common dietary elements of the termites *Reticulitermes hesperus, Zootermopsis angusticollis* and *Kalotermes minor*.

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The only conclusion warranted from the foregoing account, which is representative of a large volume of reports of insects as disseminating agents, is that, many species of insects are involved Furthermore, many fungi, both pathogenic and saprophytic, are insect-borne, it remains to be determined whether virulence in fungi is modified by passage through the alimentary tract. Also nothing is known about the effects of digestive enzymes of insects on germination. In addition to all these, host injuries by insects aid inoculation and infection. Sugarcane injured by the sugarcane borer, *Sphenophorus obscurus* is more subject to attack by *Colletotrichum falcatum*. Moreover, onions infested with thrips are predisposed to infection by *Peronospora destructor* (Wolf and Wolf, 1947). Weevils bore holes in seeds and grains during feeding by both the larvae and adults and fungi enter the seeds and grains by these holes.

Insect dispersal is more highly developed in symbiotic relationships where the insect actively inoculates a vegetable substrate and provides food for itself. Wood wasps have sacs opening to the ovipositor, containing oidia of *Stereum sanguinoleutum*, which are extruded on the eggs as they are laid, thus the fungus is introduced into sound wood during oviposition and the Mycophagous larvae feed on the fungi (Parkin, 1942).

The dispersal of fungi in stored seeds and grains has been studied and the relevant literature contains reports of these studies. Neeta Ponde, Jyoti and Mehrotra (1989), for example found out that some insects transmit fungal spores both externally and

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internally and that the fungi on the surface and within the alimentary canal of insects were the same as isolated from the grain samples in which they were present. They concluded that it might be due more to the movement of spores on the bodies of the insects in the grain lot, rather than dispersal by passage through the alimentary canals.

Their study was conducted on three species of insects, *Sitophilus oryzae, Rhizopertha dominica* and *Sitophilus zeamais*. It was observed that *R. dominica* carried the least percentage of spore load externally as well as internally probably because they are small in size and less active and also not mycophagous. *S. oryzae* usually carried the heaviest spore load possibly ascribable to its feeding habit and body structure. *A. flavus* was the most frequent fungus associated with the three species of insects. They pointed out that earlier authors had found an appreciable amount of spores of *A. flavus* in the alimentary canal of *S. oryzae*. The degree of infestation within the alimentary canal of insects was directly related to the level of infestation of grain samples.

A very important relevant factor which should not be overlooked is the possible contribution of the dead bodies of insect pests and droppings of the insects as substrates for the growth of contaminating fungi and as sources of inoculum for invasion of the products.

Saprophytes growing on dead bodies of insect pests may face some of the problems encountered by fungal parasites of insects at the pre-penetration phase. The destructive effects of fungal

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proteases on cuticles can be attributed, at least in part, to the structural importance and enzymatic accessibility of protein polymers in the cuticle. Chitin fibres also contribute to cuticle structure as a mechanical barrier to penetration and as a stabilizer of the cuticular protein matrix.

Cuticular barriers have yet to be completely characterised for a single insect species, but available data suggest that most, if not all, barriers can be part of a typical response acting in sequence or simultaneously (St. Leger, 1991) (Fig.1). Specifically, establishment by saprophytes may be prevented by low humidity, an inability to utilize available nutrients on the cuticle surface or incidence of antibiosis caused by bacteria which are able to establish on the insect bodies under the normal atmospheric conditions.

This investigation was carried out to extend knowledge on the dispersal of fungi in stored grains and seeds, and the thesis contains results of studies on the possible role of three Coleopteran insects in the persistence and dispersal of the *Aspergillus* species; *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and *A. ustus*, among the contaminating fungi of seeds of bambara groundnut and cowpea and grains of maize and rice. The major phases of the investigation are:

(i) Identity and frequency of fungi in seeds of bambara groundnut and cowpea and grains of maize and rice in storage in Accra.

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(ii) Effects of leachates of the coleopteran species *Callosobrochus maculatus* fab., *Sitophilus zeamais* Linn. and *Tribolium castaneum* Duval. on germination of conidia of *Aspergillus* species among the fungi isolated from seeds and grains: *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and *A. ustus*.

(iii) Effects of leachates of *C. maculatus*, *S. zeamais* and *T. castaneum* on growth of the *Aspergillus* species.

(iv) Bacterial flora of dead bodies of *C. maculatus*, *S. zeamais* and *T. castaneum* that may influence the establishment of fungi on the dead insect bodies.

(v) Transport of hyphae of the *Aspergillus* species through the gut of C. maculatus, S. zeamais and *T. castaneum* and the survival of ingested fungi.

(vi) Dispersal of conidia of the *Aspergillus* species carried externally by the insects.

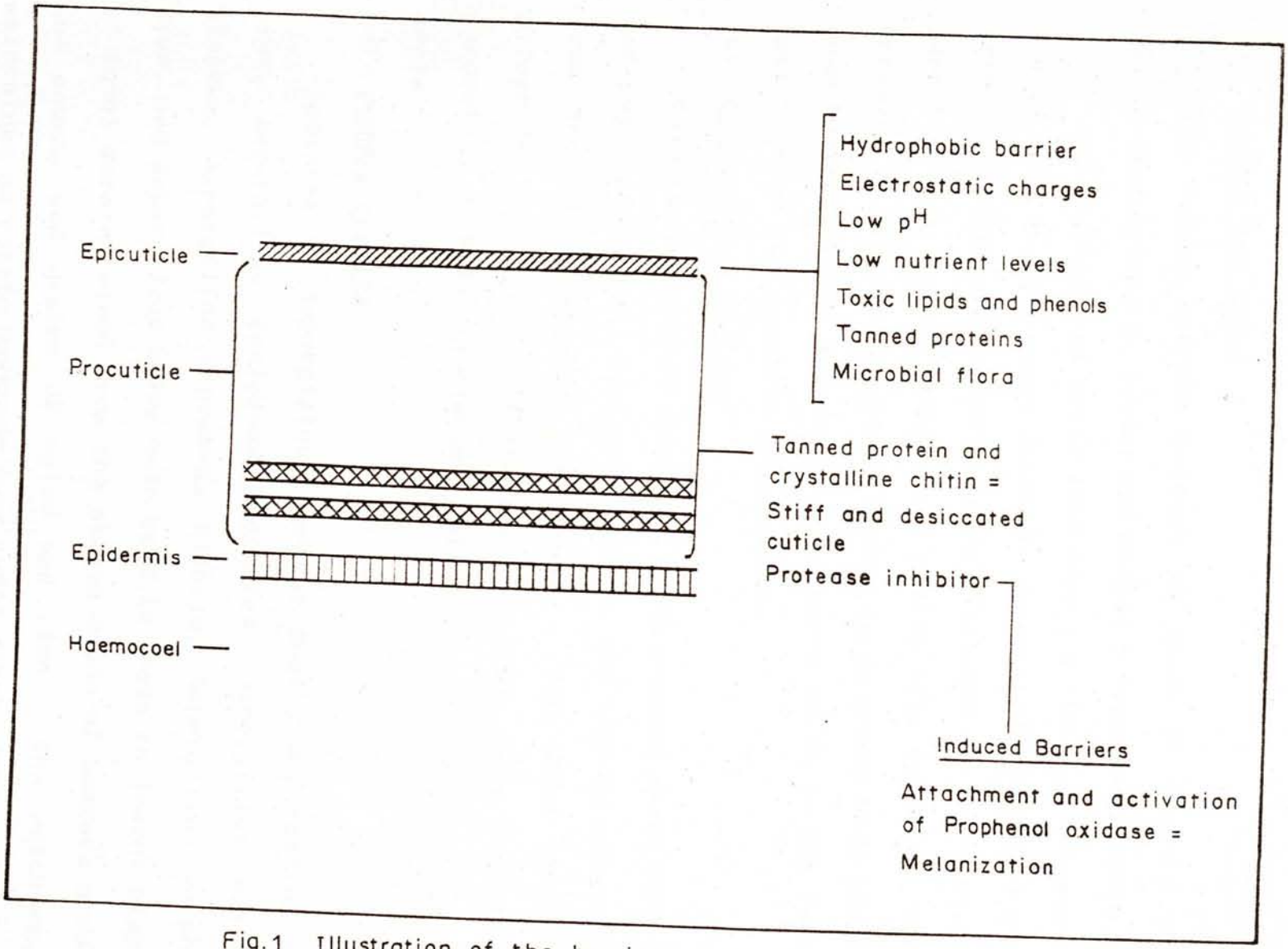

Fig. 1 Illustration of the barriers of insect cuticle against microbial infection (St. Leger, 1991).

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II. MATERIALS AND GENERAL METHODS

1. MATERIALS

(a) GRAINS AND SEEDS

The fungal species present in seeds of bambara groundnut (*Vigna subterranea* L. Verdc) and cowpea (*Vigna unguiculata* L. Walp) and in grains of maize (*Zea mays L.*) and rice (*Oryzae sativum L.*) on sale in Accra were studied. Samples of each were purchased from five different markets widely scattered in the city, namely, Kaneshie, La, Madina, Makola and Mallam Atta Markets. They were conveyed to the Department of Botany in polythene bags and kept at room temperature to keep the insect pests alive in the laboratory until needed for specific experiments.

(b) COLEOPTERAN SPECIES

Coleopteran species which are known storage pests were used in various experiments. For some studies, the insects were collected from the stored grains and seeds. For other experiments, *Sitophilus zeamais* and *Tribolium castaneum* were reared in the laboratory on maize grains and *Callosobrochus maculatus* on cowpea seeds.

(c) FUNGUS SPECIES

Isolates of *Aspergillus clavatus* Desm., *Aspergillus flavus* Link, *Aspergillus fumigatus* Fresenixs, *Aspergillus niger* Van Tieghem, *Aspergillus ochraceus* Wilhelm, *Aspergillus sulphureus* Fres. and *Aspergillus ustus* Bain used in tests on insect dispersal of fungi were obtained from the stored seeds of bambara groundnut and cowpea and grains of maize and rice. The cultures were maintained on Potato Dextrose Agar (PDA) slants in McCartney tubes

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at 3°C. They were sub-cultured every fortnight. For the production of conidia for some of the experiments, cultures were grown on PDA Petri plates.

(d) CULTURE MEDIA

Where an Agar Medium was used, 20ml of the medium was put in a 9cm-diameter Petri dish. The composition of Agar media used were as follows:

Potato Dextrose Agar (PDA) Medium: (Ainsworth and Bisby, 1945).

Potato tuber chips	...	...	200g
Dextrose	...	...	10g
Agar	...	...	15g
Distilled water	...	...	1000ml

Potato chips were boiled in 500ml of distilled water until they started to break up. The extract was strained with muslin and made up to 1000ml with distilled water. To this was added the Dextrose and Agar. The mixture was heated in a water bath to melt the Agar before it was autoclaved.

Bambara groundnut Extract Agar Medium

Bambara groundnut seeds	...	...	64g
Agar	...	...	15g
Distilled water	...	...	1000ml

The bambara groundnut seeds were soaked overnight and ground to a paste and used.

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Cowpea Extract Agar Medium

Prepared as Bambara groundnut Extract Agar but with cowpea seeds.

Maize Meal Agar (Ainsworth and Bisby, 1945)

Maize grain flour	200g
Agar	15g
Distilled water	100ml

Rice Meal/Boiled Rice (Clerk, 1963)

Each Petri dish contained:

Short grain rice	5g
Distilled water	25ml

Callosobrochus Maculatus Leachate Agar Medium
(Prepared with freshly caught live insects)

Insect leachate	200ml
Agar	3g

In all preparations requiring leachate of insects (compounds of insect bodies dissolved in distilled water) the leachate was obtained by placing 1,000 insects in 200ml sterile distilled water at $4^{0}C$ for 24 hours.

Sitophilus Zeamais Leachate Agar Medium
(Prepared with freshly caught live insects)

Insect leachate	200ml
Agar	3g.

Tribolium castaneum Leachate Agar Medium
(Prepared with freshly caught live insects)

Insect leachate	200ml
Agar	3g.

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(e) CHEMICALS

Chemicals used came from a number of manufacturers: Oxoid Limited, London, England; British Drug House (BDH) Chemicals Limited, Poole, England and Accra Chemist Limited, Accra.

2. GENERAL METHODS

A. ISOLATION OF FUNGI FROM GRAINS AND SEEDS

Following the procedure of Lacey, Hill and Edwards, (1980), 10g of either the grain or seed being studied was ground in a sterile grinder (Moulinex; made in France) and 5g of the resulting powder were suspended in 45ml of aqueous 0.1% agar in a 100ml Erlenmeyer flask and shaken for 2 minutes and allowed to stand for 30 minutes. A decimal dilution series was prepared in 0.1% Agar. Aliquots (1ml) of appropriate dilutions were poured into four Petri dishes 2 x 250µl + 1 x 500µl and 20ml of cool Potato Dextrose Agar (PDA) containing 250µg Chloramphenicol ml^{-1} was poured into each Petri dish. The plates were incubated at $30^{0}C$ and colonies which developed were counted after 5 days and the species identified after 7 days using standard text books; An introduction to industrial Mycology, (George Smith, 1960); Illustrated Genera of imperfect fungi, 3rd edition, (Barnett and Hunter, 1972); Manual of *Aspergillus*, (Thom and Raper, 1945); Manual and Atlas of the *penicillia*, (Carlos Ramirez, 1982) and Practical Mycology, Manual for Identification of fungi, (Sigured Funder, 1953), and with the help of my Supervisor. The percentage frequency of each of the species which were identified was calculated.

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B. LEACHATE OF THE INSECTS

One hundred freshly caught insects of each of the three different coleopteran species used in this research (*Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum*) were put in 20ml distilled water in a McCartney tube and kept in a refrigerator (4^0C) for 24 hours. This was regarded as the standard solution with which dilution series of 1/2, 1/4, 1/8, and 1/16 was prepared. These were put in individual McCartney tubes and autoclaved at 1.1kg/cm^2 pressure for 15 minutes at 121^0C.

C. INSECT LEACHATES AS CONIDIUM GERMINATION MEDIUM

The insect leachates were used as conidium germination media. They were either amended with extracts of seeds of bambara groundnut and cowpea and extracts of grains of maize and rice or used without adding any other ingredient.

Ten grams of the grains of either maize or rice were ground in a sterile grinder (Moulinex) and 5g of the resulting powder was suspended in 100ml of distilled water and the mixture strained with muslin cloth. A 1:1 mixture of this extract and insect leachate was prepared and autoclaved in a McCartney tube at 1.1kg/cm^2 pressure for 15 minutes at 121^0C.

In the case of the seeds of bambara groundnut and cowpea, separate extracts were prepared with the testa, cotyledon and embryo axis of each. The 1:1 mixture of extract and insect leachate was then made for each type of extract.

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D. SPORE GERMINATION TESTS: THE SLIDE METHOD

Spore suspension of each of the *Aspergillus* species used in this investigation (*A. clavatus, A flavus, A. fumigatus. A. niger, A. ochraceus, A. sulphureus* and *A. ustus*) was prepared with conidia from 5-day old cultures and dilution series of the insect leachates in McCartney tubes were prepared. The conidia were transferred into the leachates by gently touching the sporulating mycelia with a sterile inoculating loop without touching the supporting Agar medium and stirring the solution with the loop. The spore suspensions were shaken by hand for 10 minutes to give uniform spore dispersion. The number of spores in suspension for every germination test was strictly standardized to 500,000 per millilitre of suspending medium with the aid of a haemacytometer or its equivalent of 30-35 spores per high power (x40 objective) microscope field.

Each of sterile Petri dishes, serving as the germination chamber, contained a sterile slide (7.5 x 2.5cm) supported on a V-shaped short glass rod over a small quantity of sterile water. Using a sterile dropping pipette, three individual drops of spore suspension (about 0.1ml in volume) were placed in a row well separated from each other on each slide. The lid of the dish was replaced and the suspension drops incubated at 30^{0}C for the desired period. Extent of germination was assessed after incubation. All the six drops of suspension (ie. three separate drops on each of two slides for each treatment) were observed for the assessment. At least three random fields were observed from each drop resulting

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in a minimum of eighteen field counts for each treatment.

E. ASSESSMENT OF CONIDIAL GERMINATION AND GERM TUBE GROWTH

At the end of the desired incubation period the incubated spores were examined directly under the microscope and the germinated and ungerminated spores of each microscope field of the drops were counted. If observation could not be made immediately, a drop of 0.1ml formaldehyde was added to each suspension drop to stop further development. The percentage germination was calculated by the following formula.

$$\frac{100X \text{ number of germinated spores}}{\text{Total number of spores (germinated and ungerminated) observed.}}$$

During the count any spore with a discernible germ tube was considered as having germinated.

The lengths of 20 germ tubes were also measured, using an eye-piece graticule, and the mean calculated. Where germ tube branched or where a spore produced more than one germ tube, the length was taken as the sum of the lengths of the main axis and the branches or the sum of the individual germ tubes.

F. MEASUREMENT OF GROWTH OF FUNGAL CULTURES

The *Aspergillus* species were grown on Agar medium plates. After inoculation of the petri plates, the dishes were incubated in an inverted position. Two diameters were drawn at right angles to each other which intersected each other at the centre where the

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plate was inoculated. The diameters of the growing colonies were measured along these two diameters every other day. Each plate in every test was inoculated with a 2mm culture disc removed with a sterile No.2 cork borer from the growing edges of a 5-day old culture raised on PDA.

G. INGESTION OF HYPHAE OF ASPERGILLUS SPECIES BY THE COLEOPTERAN SPECIES AND SURVIVAL OF INGESTED FUNGI

Newly emerged adults of *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* being reared in the laboratory were collected and introduced separately into containers with seeds and grains of cowpea and maize, respectively, previously inoculated with three *Aspergillus species* - *A flavus*, *A. fumigatus* and *A. ochraceus*.

To obtain pure cultures on the grains and seeds, the grains and seeds were first sterilized in an autoclave at 1.1kg/cm^2 pressure for 15 minutes at 121^{0}C, before being inoculated with the selected fungus. An amount of 20ml distilled water was added to 500g of each of the grains and seeds before autoclaving, thereby making them sufficiently moistened before inoculation. Inoculation was done by sprinkling approximately the same amount of conidia of the test fungus on an inoculating loop onto either the grains or seeds. The inoculated grains and seeds were incubated for 7 days at 30^{0}C during which the fungi grew and sporulated heavily.

Although time-consuming and laborious, each grain or seed was picked with a sterile pair of forceps and the crops of sporulating

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hyphae on the surface wiped clean with sterile tissue paper before the insects were introduced. This method was devised when the insects were found to avoid grains and seeds covered with the fur of mycelium. Because conditions of incubation were dry no further external growth occurred. The insects in the course of feeding on the grains and seeds ingested as well the mycelium contained in the tissue.

The insects were left on the fungus-invaded grains and seeds for 7 days. The insects were withdrawn and placed in sterilized glass containers and washed in 10 percent Milton solution and rinsed thoroughly in several changes of sterilized water to remove any conidia adhering to the body. They were then dissected aseptically under dissecting microscope using the dissection kit shown in plate 1. Five alimentary canals of dissected insects of each species were plated on a potato Dextrose Agar and incubated at 30°C for 7 days. Each alimentary canal was first placed in a sterile Petri dish and macerated with a sterile glass rod. An amount of 20ml of the Potato Dextrose Agar and a few drops of streptomycin were then added. The colonies which appeared on the plates were counted after 4 days and identified after 7 days.

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PLATES:1 Photograph showing the instruments for the dissection of the coleopteran insects. FROM LEFT: mounting pin, pair of forceps. Entomological scissors and dissection pins (x 1/2)

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H. MYCOFLORA OF ALIMENTARY CANAL OF INSECTS OF STORED GRAINS AND SEEDS

Live insects were picked from stored maize grains and cowpea seeds and the fungus flora of their guts studied. They were surface - sterilized as was done in the preceding experiment by washing with 10 percent Milton solution and rinsing with sterile distilled water. They were dissected, their guts removed and macerated and plated using Potato Dextrose Agar. The plates were incubated at 30°C, and the colonies which appeared were counted on the 4th day and the fungus species identified after 7 days. The percentage frequencies of the different fungus species were also determined.

I. PREPARATION OF AQUEOUS EXTRACT OF FAECAL PELLETS OF THE INSECT PESTS

Aqueous extract of faecal pellets of each of the three coleopteran insect pests was prepared and used as medium for germination of the conidia of the seven *Aspergillus* species. Each extract of the faecal pellets was prepared as follows:

One thousand live individuals were put in a sterile 400ml beaker. The mouth of the beaker was covered with a nylon netting held in position with a rubber band.

The set up was left on the laboratory bench for 4 days during which masses of tiny whitish faecal pellets were deposited at the bottom of the beaker.

The insects were transferred back into their respective breeding vessels and 10ml of sterile distilled water was added to

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the pellets. The mixture was stirred until the pellets were completely dissolved.

The solution was regarded as the standard solution from which a 1/2 dilution solution was prepared. Conidial germination tests with the different fungi were then carried out, using the slide method and the standard and 1/2 dilution faecal pellet extracts.

J. ESTIMATION OF BACTERIAL FLORA OF THE BODIES OF THE INSECT PESTS

The bacterial flora of the dead bodies of the three coleopteran insect pests which could influence colonisation of the dead bodies by fungi was studied using the viable count method. The bacterial flora was determined from the day of death to the 6th day after death.

One hundred insects were put in 10ml of sterile distilled water in a McCartney tube and shaken for about 10 minutes. The solution served as the standard solution from which dilution series of 1:10, 1:100, 1:1000 and 1:10000 were prepared.

One milliliter each of the various dilutions was put in a sterile Petri dish and 20ml of sterile cool (45^{0}C) Nutrient Agar (Prepared by adding 31g of the powder to 1000ml of distilled water and autoclaved at 1.1kg/cm^{2} pressure for 15 minutes at 121^{0}C) was added. Four replicate plates of each dilution were prepared and incubated at 37^{0}C for 48 hours. Bacterial colonies which appeared on the plates were counted after the incubation period and the mean of the four plates of each dilution calculated.

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K. GRAM STAINING METHOD

The universal gram staining method (eg As stated by Krueger and Johansson (1964) in a book entitled Principles of Microbiology) to determine Gram-positive and Gram-negative cells of bacteria on dead bodies of the insect pests was used.

(a) A film of bacteria was made on a slide and fixed over a flame.

(b) The film was stained with an alcoholic solution of crystal violet for 1 minute.

(c) The stain was briefly rinsed with Lugol's iodine.

(d) The film was next flooded with Lugol's iodine for 1 minute - A dye-iodine complex was then formed inside the cells.

(e) The film or smear was discolorised by running 95% ethanol over the surface of the smear while the slide was held in a tilted position.

(f) The film was rinsed immediately under running tap. At this stage Gram-positive cells stained violet because the ethanol cannot extract the dye-iodine complex, while Gram-negative cells were unstained because the dye-iodine complex has been completely washed out.

(g) The film was then counter-stained with dilute carbol-Fuchsin for 1 minute.

(h) This was briefly rinsed under the tap and blotted dry and observed under the microscope under oil-immersion. Gram-positive cells stained violet and Gram-negative cells stained pink.

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L. DETERMINATION OF FUNGAL FLORA OF DEAD BODIES OF INSECT PESTS

The fungus flora of the dead bodies of the insect pests used in the preceding test was determined after the dead insects had been kept for 6 days. Sample bodies were randomly selected in each case and plated on PDA containing streptomycin which suppressed bacterial growth.

Ten bodies were evenly placed on each Petri medium and the preparation incubated at 30°C for 5 days. The fungus species were identified and the percentage frequency of occurrence of each species was calculated.

M. MEASUREMENT OF pH OF MEDIA

The pH of the media was measured with pH meter HM60s by TOA Electronics Limited, Japan. An extra medium in excess of the number of replicates required for a treatment was used for measuring the pH after autoclaving.

N. STERILIZATION

Media, distilled water, McCartney tubes, dissection kits, beakers and tissue paper were sterilized by autoclaving at 1.1kg/cm^2 pressure for 15 minutes at 121°C. The empty glassware and tissue paper were wrapped in either cellophane or aluminium foil, and the non-absorbent cotton wool plugs of Medicinal flats and flasks were also covered with either aluminium foil or cellophane to prevent the entry of condensed water vapour.

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Pipettes and Petri dishes (9cm diameter) were packed into their respective canisters and sterilized in an oven at 160°C for 12 hours.

Slides and cover slips were thoroughly washed with detergent, rinsed under running tap and in several changes of distilled water and stored in 90% ethanol and flamed-sterilized just before use.

Inoculating needles and loops were flamed to red-heat and cooled immediately before use.

0. MEASUREMENT OF DISTANCES TRAVELLED BY CONIDIA ADHERING TO INSECT BODIES

Experiments were carried out to determine the proportion of conidia attached to the bodies of *Sitophilus zeamais* and *Tribolium castaneum* that were transported to various distances through sets of packed maize grains of different sizes.

Transparent glass tubes measuring 29, 54, 79 and 104cm long and 4cm in diameter were packed with sound and clean maize grains, leaving 2cm of each end free of the grains. The tubes were held in horizontal position with a clamp, and a 2 x 2cm heavily sporulating *A. flavus* agar culture was placed at the floor at one end of the tube. The other end was plugged with a cork.

In each test, 20 active adults of either *S.zeamais* or *T. castaneum* were placed on the *A. flavus* culture while the tube was covered with a brown paper tube. As the brown paper was pulled from the other end, the insects crawled into the shade of the brown paper as they become exposed to the sunlight which was augmented by

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a 100 watt electric bulb. The insects having moved across the fungus culture, naturally picked conidia which became attached to their bodies. They followed the shade as the brown paper tube receeded until they reached the cork plug. The first five insects that arrived at the other end of the tube were collected and all put in a 5ml sterile distilled water in a McCartney tube. A drop of Tween 80 was added and the McCartney tube was vigorously shaken for 10 minutes to dislodge and disperse the spores. The number of spores per milliliter of this suspension was then determined in all the tests, with the aid of a heamacytometer. Counts of 10 suspension drops were made in each treatment.

Before the insects which had crawled over the *A. flavus* culture could all enter the packed grains, five of them were taken and the spore load on their bodies was assessed as has been described above. From the two values, the percentage of spores which was carried over the distance was calculated.

P. PHOTOGRAPHY

Photographs were taken to illustrate some aspects of the investigation with OLYMPUS OM-4Ti camera using KODAK film.

Q. STATISTICAL ANALYSIS

Experimental results were statistically analysed where necessary. Confidence limits at 95% levels have been quoted. Confidence limit was calculated from the formula

Confidence limit = Mean ± 1.96 x standard Error (Kershaw, 1973)

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R. EXPERIMENTAL PRECAUTIONS

1. Except when taking readings, the experiments on culture growth were not disturbed, to avoid inadvertent scattering of conidia over the agar plates.
2. Spores of the same age were used in all experiments involving germination tests.
3. The chamber in which the dissection was done and the dissection microscope were all sterilized by spraying with 5.0 percent dethol.
4. The life histories of the insects were studied, so that it became possible to use young and energetic adult insects for all the experiments.
5. Glassware was kept scrupulously clean. Glassware which had been already cleaned with detergents and tap water was rinsed several times with distilled water and allowed to drain dry before being sterilized.
6. Petri dishes and glass slides which had come in contact with formalin were washed with detergents on three consecutive days to ensure that all traces of the formalin have been completely removed.

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III. EXPERIMENTAL DETAILS

A. ISOLATION OF FUNGI FROM THE SEEDS OF BAMBARA GROUNDNUT AND COWPEA AND GRAINS OF MAIZE AND RICE ON SALE IN VARIOUS MARKETS IN ACCRA

This investigation was carried out for two purposes. First, it was considered desirable to extend knowledge on contaminating fungi of these seeds and grains in Ghana. Secondly, some of the *Aspergillus* species to be encountered would be used in some of the subsequent experiments.

Sufficient quantities of maize and rice grains, and, bambara groundnut and cowpea seeds were purchased in March, 1994 from each of the five markets at different localities in Accra Municipality, namely, Kaneshie, La, Madina, Makola and Mallam Atta. Inoculation material of each sample, prepared as described under 'Materials and General Methods' with surface sterilized grains or seeds was used to inoculate PDA containing streptomycin. There were five replicate plates for each sample. The total number of fungal colonies which occurred after 5 days' incubation at 30°C was recorded. The fungal species were identified and their percentage frequency calculated. The results obtained are shown in Tables 4 to 7 and part of the data illustrated in Fig.2.

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B. GERMINATION OF CONIDIA OF SEVEN ASPERGILLUS SPECIES IN AQUEOUS BODY LEACHATE OF CALLOSOBROCHUS MACULATUS, SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM

The ability of conidia of seven species of *Aspergillus* selected from the isolates of the previous experiment. *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus* *A. sulphureus* and *A. ustus* - to germinate in aqueous body leachate of the three Coleopteran species was next investigated.

Drops of spore suspension prepared with sterile body leachate were placed on sterile glass slides, using the 'slide method' and incubated at 30^{0}C for 24 hours. Each treatment consisted of two slides with six suspension drops. For tests with each fungus, five concentrations of the leachate of each insect species were used. These were, the standard solution and 1/2, 1/4, 1/8 and 1/16 dilutions.

The results indicating percentage germination of the various tests and the mean lengths of the germ tubes are presented in Tables 8, 9 and 10..

C. INDUCING CONIDIAL GERMINATION IN INSECT BODY LEACHATE WITH EXUDATES OF THE GRAINS AND SEEDS

In experiment B the conidia of some of the *Aspergillus* species did not germinate in the insect body leachates. Conidia of *A. fumigatus* did not germinate in the body leachate of *Callosobrochus maculatus* and *Tribolium castaneum*. *A. clavatus* conidia did not germinate in the leachate of *Sitophilus zeamais*. *A. ustus* conidia did not germinate in the leachate of *Callosobrochus moculatus* and

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conidia of *A. niger* and *A. sulphureus* did not germinate in the leachates of both *Sitophilus zeamais* and *Tribolium castaneum*. This could be due to two possible causes. Either the leachate lacked adequate nutrients or it contained inhibitory compounds. In this experiment nutrients were added to leachate to raise the level in case the leachate lacked adequate nutrients or to overcome the effects of the inhibitory compounds. Exudates of the seeds of bambara groundnut and cowpea and of the grains of maize and rice were added individually to the leachates of standard concentration in a series of conidial germination tests. In each test, the percentage germination and mean germ tube length were determined after incubation at 30°C for 12 hours. The data obtained are presented in Tables 12 to 19.

b. GROWTH OF THE SEVEN FUNGUS ASPERGILLUS SPECIES ON INSECT BODY-LEACHATE AGAR MEDIA

Because conidial germination and hyphal growth involve quite different physiological processes, growth of the mycelium of each of the *Aspergillus* species on agar medium prepared with standard concentration of leachate of each of the three insect species was next investigated.

Each test consisted of five replicate Petri plates. Each plate was inoculated with a 3mm-diameter culture disc and incubated at 30°C. The mean colony diameters of the various treatments recorded daily for a total period of 10 days are tabulated in Tables 20 to 26, and displayed graphically in Figs 3.

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E. MYCOFLORA OF GUT OF CALLOSOBROCHUS MACULATUS, SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM

Fungi of stored products also interact with the internal environment of the insect pests. Contaminating fungi ingested with tissues of the grains and seeds may survive for varying lengths of time in the gut of the insects depending on the fungal species. The components of the gut mycoflora would, anyway, depend, on the chance occurrence of the various species of the contaminants on the particular grains attacked by the insect pests.

A general view of the mycoflora of the guts of the three insects pests was obtained by plating the gut contents of the insects on PDA plates containing streptomycin to suppress growth of bacteria. For each test, five individuals of the insect species were dissected and their gut contents suspended in sterile distilled water and used to inoculate the PDA plates as described in 'Materials and General Methods'. The guts of *C. maculatus* picked from cowpea seeds and those of *S. zeamais* and *T.castaneum* from stored maize grains contained the fungal species listed in Tables 27 to 29.

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F. SURVIVAL OF ASPERGILLUS SPECIES IN THE GUT OF CALLOSOBROCHUS MACULATUS, SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM

The results of the previous investigations reflect, first, the fungal species which had been ingested and, secondly, the species which were still viable when the gut contents were extracted. The longivity of fungi in the guts of the three insect species was next investigated. Different batches of cowpea seeds and maize grains invaded with mycelium of either *Aspergillus flavus* or *Aspergillus fumigatus* or *Aspergillus ochraceus* in the manner described under 'Materials and General Methods', were fed to the three insect pests. Inoculated cowpea seeds were fed to *C. maculatus* and inoculated maize grains fed to *S. zeamais* and *T. castaneum*.

Each batch of insects which had fed for seven days on material containing a particular fungus species, was transferred to a dry clean Petri dish. Five were taken each day for 10 consecutive days and dissected and the gut contents of each insect plated on a PDA plate containing streptomycin. The mean number of colonies of that fungus which appeared on the plates after incubation at 30°C for five days was then calculated. The results of all the tests appear in Tables 30 to 32, and values of percentage survival of each fungus in each insect pest are shown graphically in Fig 4.

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G. GERMINATION OF CONIDIA OF ASPERGILLUS SPECIES IN EXTRACTS OF FAECAL PELLETS OF THE INSECTS

The possibility of the faecal pellets serving as medium for germination of the conidia of the different *Aspergillus* species was next investigated. The extract of the faecal pellets of each of the three insects was used in a conidial germination test using the slide method. Two concentrations of standard (faecal pellets of one thousand insects dissolved in 10ml distilled water) and 1/2 dilution of the extract were used. The conidia were incubated at 30°C for 24 hours after which the percentage germinationn was determined and random samples of the germ tubes of the germinated conidia measured and the mean germ tube length calculated. The results are presented in Tables 33 to 35.

H. DISPERSAL OF CONIDIA OF ASPERGILLUS FLAVUS ATTACHED TO THE BODIES OF SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM

Apart from the dispersal of viable fungi in the faecal pellets as was suggested by long survival of the fungi in the gut of the insects in the preceding experiment, dispersal of the fungi by the insects externally is also possible. The success of external dispersal would depend on the ease by which conidia attached to the insect bodies could be dislodged. It was hypothesized that the smaller the spaces among the packed grains or seeds the more frequent the insects would brush against the grains or seeds and the faster the rate of removal of the conidia on the bodies of the insects. This hypothesis was verified in this experiment.

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Maize grains of three different sizes of small, medium and large were packed into 4mm diameter tubes of different lengths of 25, 50, 75 and 100cm. By the habit of these insects to move from light to shade, *S.zeamais* and *T. castaneum* were induced to move from one end of the tubes to the other. Insects reaching the ends of the tubes were submerged in distilled water with Tween 80 to wash off the conidia.

Tables 36 to 41 contain assessments made by spore counts with haemacytometer of proportion of conidia still attached to the insect bodies after moving through the columns of maize grains of different sizes while some aspects of the results are illustrated graphically in Figs 5 and 6.

I. BACTERIAL FLORA OF DEAD BODIES OF THE THREE INSECT SPECIES

The dead insect bodies among the maize grains in the store would most probably be substrates for both fungi and bacteria. They could, therefore, serve as launching pads for fungi that could infect the grains. Perhaps, the metabolites of some of the bacteria which would be established on the insect bodies could depress fungal growth, especially, if the bacterial flora is very large. This investigation was carried out to obtain an idea of the extent of colonization of the insect bodies by bacteria.

Samples of the dead insects, freshly killed with ether and kept at 30°C were taken each day and used to prepare insect body washings as described under 'Materials and General Methods'. Nutrient Agar plates inoculated with the washing were incubated at 30°C and the bacterial colonies which developed after 48 hours were

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counted. There were five replicate plates per each treatment.

Ten colonies of different growth forms, and hence of different species, were selected at random for each treatment and stained using the Gram Staining Method. A record was made of the morphology and the Gram Stain reaction of the cells of each colony. The results obtained are given in Tables 42 to 44 and illustrated in Fig.7.

J. FUNGUS FLORA OF DEAD BODIES OF THE THREE INSECT SPECIES

In the previous investigation, tiny hyphal growths were observed on some of the dead insect bodies on the 4th day after the insects had been killed. Twenty insects of each insect species with visible growth were plated on Potato Dextrose Agar containing streptomycin on the 6th day of the investigation. The plates were incubated at 30^{0}C for 5 days and the fungal species which developed were identified and the percentage frequency calculated. The results are presented in Table 45.

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IV. RESULTS

A. ISOLATION OF FUNGI FROM THE SEEDS OF BAMBARA GROUNDNUT AND COWPEA AND GRAINS OF MAIZE AND RICE ON SALE IN VARIOUS MARKETS IN ACCRA

The samples of seeds of bambara groundnut and cowpea and grains of maize and rice from the various markets in Accra provided many fungal species from which a selection was made for subsequent studies. According to the vendors the different stocks had been in the market for different lengths of time. The fungal populations obtained could not therefore be related to specific period of storage. The species which grew out of the macerated materials plated on Potato Dextrose Agar plates are presented in Tables 4,5,6 and 7.

Considering all the five markets, the largest number of colonies was produced by the maize grains, followed, in descending order, by the colonies from cowpea seeds, bambara groundnut seeds and rice grains. Furthermore, the products at Mallam Atta Market produced the highest number of colonies and products of Madina and Kaneshie Markets produced the smallest number of colonies.

The lists of fungi in the tables of results showed that the number of species on the same crop varied from market to market. The numbers of isolated fungal species from the same grain or seed from the different markets could be summarised as follows:

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Total number of fungal species on

Market	Bambara Groundnut seeds	cowpea seeds	maize grains	rice grains
Kaneshie	9	11	6	5
La	5	7	7	6
Madina	9	9	12	5
Makola	8	14	9	6
Mallam Atta	9	9	8	7

The genera of fungi which were observed on the different products were also not the same. Bambara groundnut seeds contained the genera *Aspergillus, Cladosporium, Fusarium, Neurospora, Paecilomyces, Penicillium, Pullularia and Rhizopus.* Cowpea seeds contained, *Aspergillus, Cladosporium, Drechslera, Epicoccum, Fusarium, Mucor, Neocosmospora, Neurospora, Paecilomyces, Penicillium, Phoma, Rhizopus and Verticillium.* Maize grains contained, *Aspergillus, Fusarium, Mucor, Neocosmospora, Neurospora, Penicillium, Pullularia* and *Rhizopus*, and rice grains were attacked by the genera *Aspergillus, Cladosporium, Fusarium, Mucor* and *Penicillium.* The total number of colonies of the four dominant genera on the agar plates inoculated with the macerated seeds and grains are illustrated in Fig. 2.

The dominant species on all the products put together were *Aspergillus flavus, Aspergillus niger, Paecilomyces puntonii, Penicillium expansum* and *Penicillium citrinum.* The mean percentage occurrence in each product from the five markets were:

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Percentage occurrence of:

Product	*A. flavus*	*A. niger*	*P. puntonii*	*P. expansum*	*P. citrinum*
Bambara groundnut seeds	20	22	14	12	3
Cowpea Seeds	15	6	36	6	0
Maize grains	27	1	0	19	37
Rice grains	18	6	0	19	4

Cladosporium herbarum and *Penicillium chrysogenum* would be considered to form an intermediate group, while the rest, consisting the third group occurred at very low percentage frequencies, or occurred only occasionally even though sometimes, in large numbers, such as, *Aspergillus oryzae* (47 percent: Table 7) *Aspergillus ustus* (14 percent: Table 7) *Dreschslera* species (14 and 43 percent: (Table 5) *Fusarium moniliforme* (11 percent: Table 6) and *Neocosmospora vasinfecta* (14 percent: Table 5).

It was intended from the onset to restrict the subsequent studies on dispersal of the contaminant fungi by insect pests to *Aspergillus* species. As a consequence seven species, namely, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and *A. ustus* were selected for Experiments B to J.

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TABLE 4

Fungus species isolated from Bambara groundnut seed on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Kaneshie	14	*Aspergillus flavus* (2)	15
		Aspergillus fumigatus (2)	15
		Aspergillus niger (1)	7
		Cladosporium herbarum (1)	7
		Neurospora sitophila (2)	14
		Penicillium chrysogenum (1)	7
		Penicillium citrinum (2)	14
		Penicillium expansum (2)	14
		Penicillium sp. (1)	7
La	17	*Aspergillus fumigatus* (1)	6
		Aspergillus niger (1)	6
		Cladosporium herbarum (1)	6
		Paecilomyces Puntonii (9)	53
		Penicillium expansum (5)	29
Madina	37	*Aspergillus flavus* (10)	27
		Aspergillus fumigatus (1)	3
		Aspergillus niger (17)	45
		Aspergillus ustus (1)	3
		Fusarium monilitorme (2)	5
		Neurospora sitophila (1)	3
		Paecilomyces carneus (1)	3
		Paecilomyces puntonii (1)	3
		Penicillium chrysogenum (3)	8

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TABLE 4 CONT'D

Fungus species isolated from Bambara groundnut seed on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Makola	72	*Aspergillus flavus* (18)	25
		Aspergillus niger (30)	41
		Aspergillus ochraceus (6)	8
		Neurospora sitophila (3)	4
		Paecilomyces Puntonii (4)	6
		Penicillium chrysogenum (4	6
		Penicillium sp. (4)	6
		Rhizopus oryzae (3)	4
Mallam Atta	32	*Absidia corymbifera* (2)	6
		Aspergillus flavus (10)	31
		Aspergillus niger (3)	10
		Aspergillus oryzae (2)	6
		Cladosporium herbarum (2)	6
		Paecilomyces puntonii (3)	10
		Penicillium chrysogenum (4)	12
		Penicillium expansum (5)	16
		Pollularia pullulans (1)	3

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TABLE 5

Fungus species isolated from cowpea seeds on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Kaneshie	95	*Aspergillus flavus* (10)	11
		Aspergillus niger (6)	6
		Aspergillus sulphureus (1)	1
		Drechslera species (13)	14
		Fusarium moniliforme (1)	1
		Neocosmospora vesinfecta (13)	14
		Paecilomyces puntonii (33)	35
		Penicillium chrysogenum (5)	5
		Penicillium sp. (2)	14
		Penicillium expansum (2)	2
		Penicillium sp. (1)	1
		Pullularia Pullulans (10)	11
La	102	*Aspergillus flavus* (30)	29
		Aspergillus niger (9)	9
		Mucor species (5)	5
		Paecilomyces Puntonii (42)	41
		Penicillium chrysogenum (3)	3
		Penicillium expansum (10)	10
		Verticillium species (3)	3
Madina	126	*Aspergillus flavus* (13)	10
		Aspergillus niger (3)	2
		Aspergillus ochraceus (5)	4
		Drechslera species (54)	43
		Fusarium moniliforme (9)	7
		Paecilomyces carneus (2)	2
		Paecilomyces Puntonii (37)	29
		Phoma species (2)	2
		Rhizopus stolonifer (1)	1

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TABLE 5 CONT'D

Fungus species isolated from cowpea seeds on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Makola	53		
		Aspergillus flavus (2)	4
		Aspergillus fumigatus (2)	4
		Aspergillus niger (4)	8
		Aspergillus oryzae (1)	2
		Cladosporium herbarum (1)	2
		Drechslera species (5)	9
		Epicoccum nigrum (1)	2
		Mucor species (1)	2
		Neurospora sitophila (1)	2
		Paecilomyces carneus (5)	9
		Paecilomyces puntonii (22)	41
		Penicillium expansum (1)	2
		Penicillium sp. (3)	5
		Rhizopus oryzae (4)	8
Mallam Atta	103	*Aspergillus flavus* (22)	21
		Aspergillus niger (7)	7
		Cladosporium herbarum (3)	3
		Mucor species (2)	2
		Paecilomyces puntonii (34)	33
		Penicillium chrysogenum (14)	14
		Penicillium expansum (19)	18
		Phoma species (1)	1
		Verticillium species (1)	1

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TABLE 6

Fungus species isolated from maize grains on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Kaneshie	378	*Aspergillus flavus* (46)	12
		Aspergillus niger (3)	1
		Fusarium moniliforme (12)	3
		Penicillium citrinum (69)	18
		Penicillium expansum (246)	65
		Penicillium sp. (2)	1
La	1027	*Aspergillus flavus* (96)	9
		Aspergillus niger (17)	1
		Fusarium moniliforme (111)	11
		Mucor species (3)	1
		Penicillium chrysogenum (177)	17
		Penicillium citrinum (622)	60
		Rhizopus oryzae (1)	1
Madina	323	*Aspergillus flavus* (4)	1
		Aspergillus niger (8)	2
		Aspergillus sulphureus (2)	1
		Fusarium moniliforme (10)	3
		Penicillium citrinum (265)	81
		Penicillium expansum (6)	2
		Penicillium funiculosum (5)	2
		Penicillium italicum (3)	1
		Penicillium sp. (14)	4
		Neocosmospora vesinfecta (2)	1
		Neurospora sitophila (2)	1
		Rhizopus oryzae (2)	1

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TABLE 6 CON'T

Fungus species isolated from maize grains on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Makola	657	*Aspergillus flavus* (581)	87
		Aspergillus niger (13)	2
		Aspergillus oryzae (6)	1
		Fusarium moniliforme (8)	1
		Mucor species (2)	1
		Neurospora sitophila (2)	1
		Penicillium citrinum (27)	4
		Penicillium expansum (5)	1
		Penicillium sp. (13)	2
Mallam Atta	1348	*Aspergillus flavus* (366)	27
		Aspergillus ochraceus (2)	1
		Aspergillus oryzae (14)	1
		Penicillium chrysogenum (98)	7
		Penicillum citrinum (308)	23
		Penicillium expansum (400)	29
		Pullularia Pullulans (68)	5
		Penicillium sp. (92)	7

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TABLE 7

Fungus species isolated from rice grains on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Kaneshie	7	*Aspergillus ustus* (1)	14
		Cladosporium herbarum (1)	14
		Penicillium chrysogenum (1)	14
		Penicillium expansum (2)	29
		Penicillium oxalicum (2)	29
La	22	*Aspergillus niger* (4)	19
		Aspergillus ustus (3)	13
		Aspergillus wentii (5)	23
		Cladosporium herbarum (3)	13
		Mucor species (2)	9
		Penicillium expansum (5)	23
Madina	8	*Aspergillus flavus* (1)	13
		Aspergillus flavus-oryzae(1)	13
		Cladosporium herbarum (3)	37
		Penicillium chrysogenum (2)	24
		Penicillium expansum (1)	13
Mokola	9	*Aspergillus flavus* (2)	23
		Aspergillus fumigatus (1)	11
		Aspergillus niger (1)	11
		Cladosporium herbarum (1)	11
		Penicillium citrinum (2)	22
		Penicillium expansum (2)	22

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TABLE 7 CONT'D

Fungus species isolated from rice grains on sale at different markets in Accra

Market	Total No. of Colonies on 5 Plates	Fungus species (Total No. in parenthesis)	% Frequency (to the nearest-whole number)
Mallam Atta	30	*Aspergillus clavatus* (1)	3
		Aspergillus flavus (3)	10
		Aspergillus oryzae (14)	47
		Cladosporium herbarum (4)	13
		Fusaium moniliforme (1)	3
		Penicillium chrysogenum(5)	17
		Penicillium expansum (2)	7

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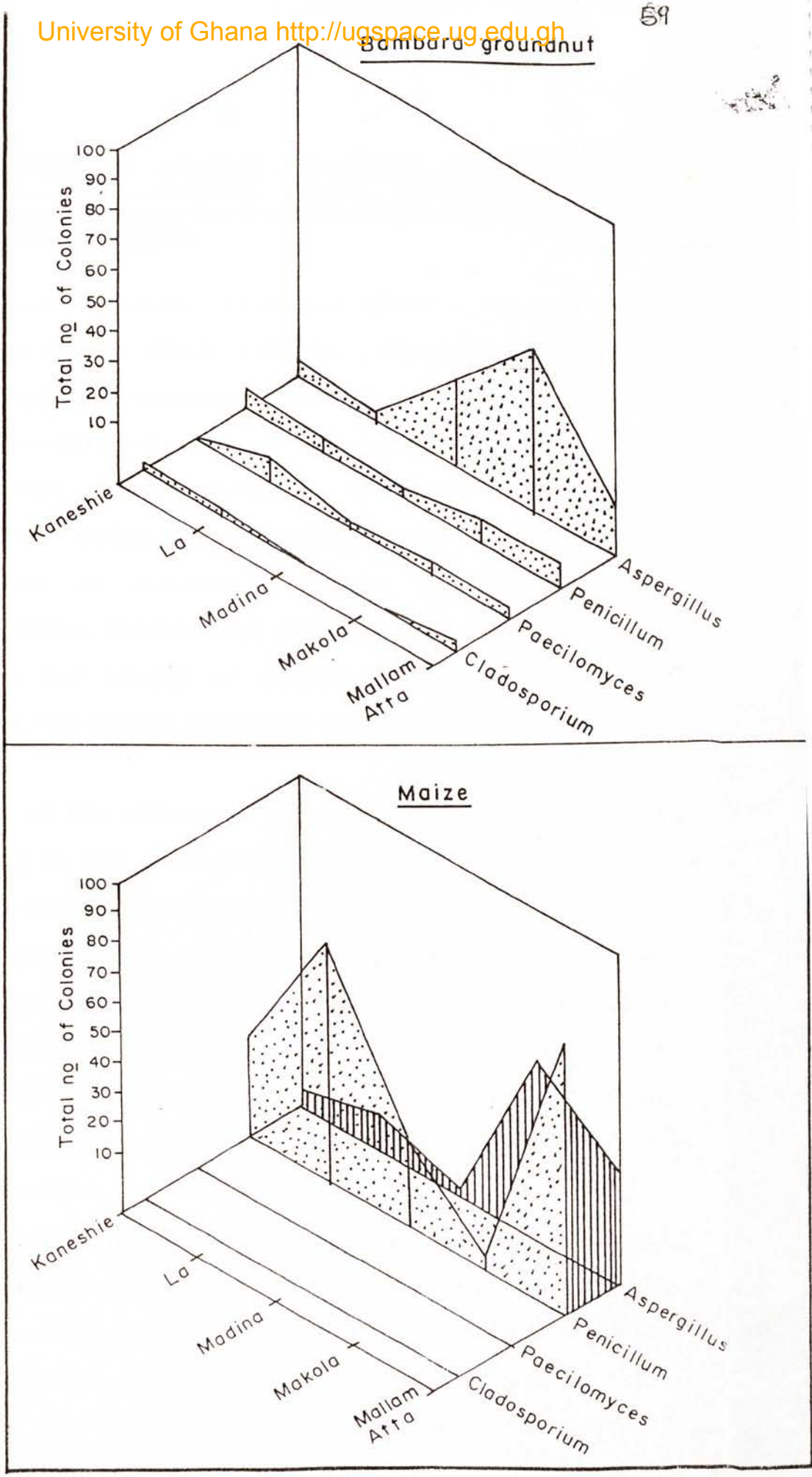

Fig. 2 Graphs of the frequency occurrence of mem
and Penicillum genera in grains of maize a
cowpea obtained from five different marke

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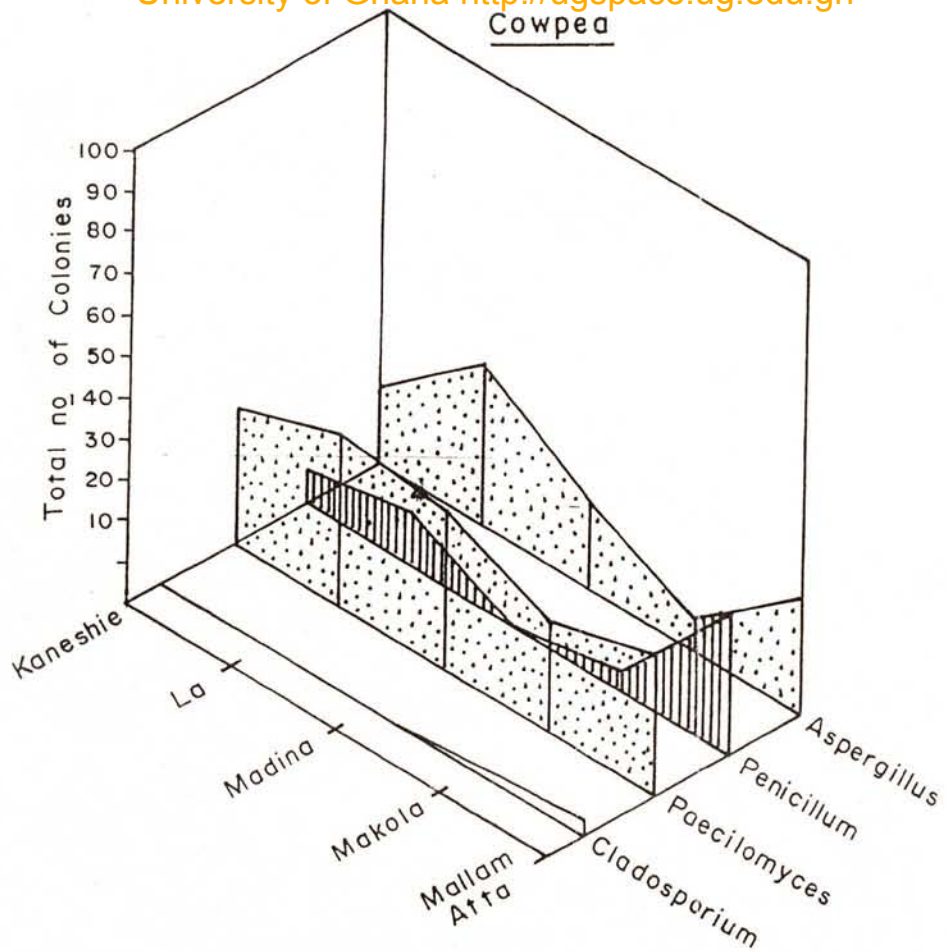

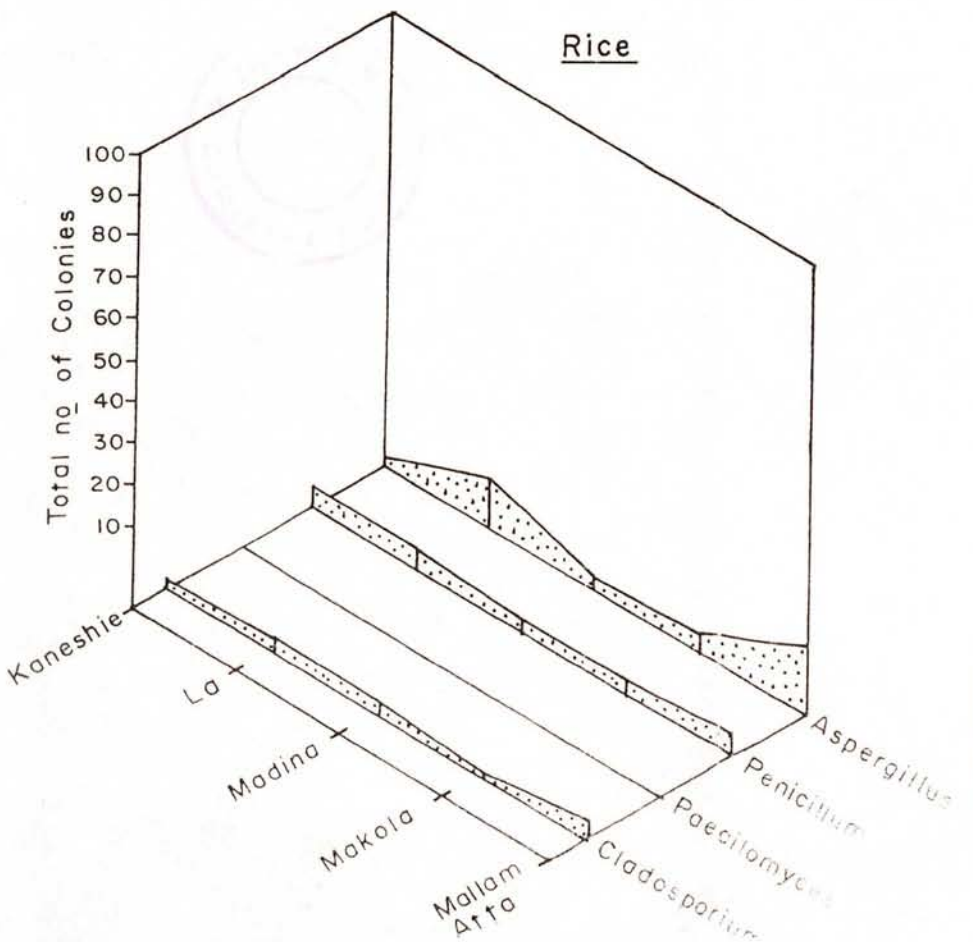

members of Aspergillus, Cladosporium, Paecilomyces
ize and rice and seeds of bambara groundnut and
arkets in the Accra

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B. GERMINATION OF CONIDIA OF SEVEN ASPERGILLUS SPECIES IN AQUEOUS BODY LEACHATE OF CALLOSOBROCHUS MACULATUS, SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM

The surface of the bodies of insect pests in stored grains and seeds would be a most likely site for lodging of the conidia and germination of the conidia. In the first experiment on germination of conidia of the seven *Aspergillus* species, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and *A. ustus*, the conidia were incubated in germination media of aqueous leachate of *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum*. Tables 8,9 and 10 show the levels of germination in the different concentrations of the insect leachates after 24 hours' incubation at 30°C.

The conidia of the different *Aspergillus* species responded quite differently in the germination media. They were affected both by the type and concentration of the leachate. Generally, where there was germination in a particular leachate by conidia of a particular fungal species, the standard concentration of the leachate supported the highest percentage germination, and the percentage germination declined with decreasing leachate concentration. The mean germ tube lengths corresponded to the percentage germination.

The conidia of some species germinated in practically all the concentrations of some of the leachates. This relationship was observed with *A. sulphureus* conidia in *C. maculatus* leachate; conidia of *A. flavus*, *A. fumigatus*, and *A. ochraceus* in *S. zeamais* leachate and conidia of *A. flavus* and *A. ochraceus* in

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leachate of *T. castaneum*. Considering the standard concentration of the leachates alone, *A. flavus* conidia gave the highest germination in *C. maculatus* leachate compared to conidia of the other species, in *S. zeamais* leachate conidia of *A. flavus* and *A. ochraceus* germinated best and in *T. castaneum* leachate, *A. ochraceus* conidia germinated best.

The last trend observed was that although conidia of some species did not germinate at all in certain leachates, there was no species which failed to show germination in all concentrations of all the three leachates. The various trends summarised above have been presented together in Table 11, for clarity, indicating the percentage germination.

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TABLE 8

Germination of conidia of *Aspergillus* species in aqueous solution of faecal pellets of *Callosobrochus maculatus* incubated at 30°C for 24 hours.
(Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Pecentage germination	Mean Germ tube length (μm)
A. clavatus	Undiluted	50.2	109.9
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. flavus	Undiluted	70.4	125.9
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. fumigatus	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. niger	Undiluted	44.0	123.4
	Dilution:		
	1/2	45.5	118.0
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLE 8 CONT'D.

Germination of conidia of *Aspergillus* species in aqueous solution of faecal pellets of *Callosobrochus maculatus* incubated at 30°C for 24 hours.

(Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Percentage germination	Mean Germ tube length (μm)
A. ochraceus	Undiluted	49.6	80.3
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. sulphureus	Undiluted	31.3	149.0
	Dilution:		
	1/2	11.4	116.4
	1/4	6.4	98.7
	1/8	6.4	84.1
	1/16	0.0	-
A. ustus	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLE 9

Germination of conidia of *Aspergillus* species in body leachate of *Sitophilus zeamais* incubated at 30°C for 24 hours (Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Percentage germination	Mean germ tube length (μm)
	Undiluted	0.0	-
A. clavatus	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. flavus	Undiluted	76.2	166.0
	Dilution:		
	1/2	58.6	134.9
	1/4	45.6	122.2
	1/8	19.3	88.9
	1/16	20.9	66.9
A. fumigatus	Undiluted	45.2	91.4
	Dilution:		
	1/2	46.5	53.8
	1/4	36.4	32.8
	1/8	26.8	38.5
	1/16	0.0	-
A. niger	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLE 9 CONT'D

Germination of conidia of *Aspergillus* species in body leachate of *Sitophilus zeamais* incubated at 30°C for 24 hours

(Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Percentage germination	Mean germ tube length (μm)
A. ochraceus	Undiluted	66.3	108.4
	Dilution:		
	1/2	48.1	102.9
	1/4	27.5	99.2
	1/8	29.5	67.9
	1/16	16.4	59.3
A. sulphureus	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. ustus	Undiluted	53.8	56.3
	Dilution:		
	1/2	31.1	31.6
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLE 10

Germination of conidia of *Aspergillus* species in body leachate of *Tribolium castaneum* incubated at 30°C for 24 hours

(Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Percentage germination	Mean germ tube length (μm)
	Undiluted	39.2	121.2
A. clavatus	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. flavus	Undiluted	52.2	131.5
	Dilution:		
	1/2	40.2	99.1
	1/4	37.9	95.1
	1/8	26.2	87.1
	1/16	0.0	-
A. fumigatus	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
A. niger	Undiluted	0.0	-
	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLE 10 CONT'D

Germination of conidia of *Aspergillus* species in body leachate of *Tribolium castaneum* incubated at 30°C for 24 hours

(Percentage germination out of total of 500-600 conidia)

Aspergillus species	Concentration of leachate	Percentage germination	Mean germ tube length (μm)
	Undiluted	69.5	132.4
A. ochraceus	Dilution:		
	1/2	59.3	110.9
	1/4	48.9	88.1
	1/8	37.5	78.1
	1/16	28.1	66.4
	Undiluted	0.0	-
A. Sulphureus	Dilution:		
	1/2	0.0	-
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-
	Undiluted	46.1	119.0
A. ustus	Dilution:		
	1/2	36.7	107.2
	1/4	0.0	-
	1/8	0.0	-
	1/16	0.0	-

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TABLI

Percentage germination of conidia of *Asperg*
Callosobrochus maculatus, *Sitophilus zeamais* a

Body leachate	Concentration of leachate	*A clavatus*	*A flavus*	*A fumig*
	Undiluted	50.2	70.4	-
	Dilution:			
Callosobrochus maculatus	1/2	-	-	-
	1/4	-	-	-
	1/8	-	-	-
	1/16	-	-	-
	Undiluted	-	76.2	4
	Dilution:			
Sitophilus zeamais	1/2	-	58.6	4
	1/4	-	45.6	3
	1/8	-	19.3	2
	1/16	-	20.9	
	Undiluted	39.2	52.2	
	Dilution:			
Tribolium castaneum	1/2	-	40.2	
	1/4	-	37.9	
	1/8	-	26.2	
	1/16	-	-	

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TABLE 11

of *Aspergillus* species in aqueous body leachate of s *zeamais* and *Tribolium castaneum* in 24 hours at 30°C

Aspergillus species					
A *avus*	A *fumigatus*	A *niger*	A *ochraceus*	A *sulphureus*	A *ustus*
70.4	-	44.0	49.6	31.3	-
	-	45.5	-	11.4	-
	-	-	-	6.4	-
	-	-	-	6.4	-
	-	-	-	-	-
6.2	45.2	-	66.3	-	53.8
8.6	46.5	-	48.1	-	31.1
5.6	36.4	-	27.5	-	-
9.3	26.8	-	29.5	-	-
0.9	-	-	16.4	-	-
2.2	-	-	69.5	-	46.1
0.2	-	-	59.3	-	36.7
7.9	-	-	48.9	-	-
5.2	-	-	37.5	-	-
	-	-	28.1	-	-

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C. INDUCING CONIDIAL GERMINATION IN INSECT BODY LEACHATE WITH EXTRACTS OF GRAINS AND SEEDS

Potato Dextrose Broth and extracts of the seeds of bambara groundnut and cowpea and of the grains of maize and rice were able to induce germination of conidia of *Aspergillus* species which failed to germinate in the insect body leachates in the previous experiment. The results of the experiment are shown in Tables 12 to 19. In almost all cases, conidial germination was observed first after 9 hours' incubation. The extent of germination depended on the type of leachate-amendment combination.

Only Potato Dextrose Broth, and extracts of cotyledons of bambara groundnut and cowpea encouraged very high percentage germination of 81.5 - 84.9 percent in 12 hours of conidia of *A. fumigatus* in *Callosobrochus maculatus* leachate (Table 12).

The highly effective amendments for the other *Aspergillus* species, stimulating more than 75 percent germinations in 12 hours were:

A. ustus: Extracts of cotyledons and embryo axis of bambara groundnut in *C. maculatus* leachate (77.2 and 83.6 percent) (Table 12).

A. clavatus: Potato Dextose Broth, extracts of embryo axis of bambara groundnut, cowpea cotyledon, cowpea testa and maize grains in *S. zeamais* leachate (76.5 - 90.6 percent) (Table 14).

A. niger: Potato Dextrose Broth in *S. zeamais* leachate (78.1 percent) (Table 15) and in *T. castaneum* leachate (78.7 percent) (Table 18).

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A. fumigatus in *T. castaneum* leachate (Table 17) and *A. suphureus* in leachates of *S. zeamais* (Table 16) and *T. castaneum* (Table 19) were not so highly stimutated as in the other tests.

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TAB

Germination of conidia of Aspergillus fumigatus at 30°C in body leachate of C and cowpea and grains of maize and rice.

Medium	% Germination after indicated h			
	3	6	9	12
Standard C. maculatus leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	0.0	53.7	82.7
Standard C. maculatus leachate + Extract of axis of bambara groundnut seed	0.0	0.0	9.8	39.3
Standard C. maculatus leachate + Extract of cotyledon of bambara groundnut	0.0	0.0	64.3	81.5
Standard C. maculatus leachate + Extract of testa of bambara groundnut	0.0	0.0	5.7	25.0
Standard C. maculatus leachate + Extract of axis of cowpea	0.0	0.0	29.1	51.7
Standard C. maculatus leachate + Extract of cotyledon of cowpea	0.0	0.0	57.1	84.9
Standard C. maculatus leachate + Extract of testa of cowpea	0.0	0.0	0.0	13.3
Standard C. maculatus leachate Extract of Maize grain	0.0	0.0	7.2	37.8
Standard C. maculatus leachate + Extract of rice grain	0.0	0.0	4.9	24.9

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TABLE 12

eachate of Callosobrochus maculatus amended with extracts of seeds of bambara groundnut

indicated hours	*Mean Germ Tube Length after indicated hours (µM)*			
12	*3*	*6*	*9*	*12*
0.0	-	-	-	-
82.7	-	-	*19.9*	*53.3*
39.3	-	-	*13.7*	*42.3*
81.5	-	-	*44.8*	*76.1*
25.0	-	-	*16.9*	*35.9*
51.7	-	-	*28.9*	*57.9*
84.9	-	-	*33.6*	*62.6*
13.3	-	-	-	*13.2*
37.8	-	-	*22.3*	*48.8*
24.9	-	-	*13.6*	*30.9*

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TAB

Germination of conidia of Aspergillus ustus at 30°C in body leachate of Cali and cowpea and grains of maize and rice.

Medium	% Germination after indicated ho			
	3	6	9	12
Standard *C. maculatus* leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	0.0	33.3	63.0
Standard *C. maculatus* leachate + Extract of axis of bambara groundnut seed	0.0	0.0	18.9	41.6
Standard *C. maculatus* leachate + Extract of cotyledon of bambara groundnut	0.0	0.0	68.9	83.6
Standard *C. maculatus* leachate + Extract of testa of bambara groundnut	0.0	0.0	9.9	36.9
Standard *C. maculatus* leachate + Extract of axis of cowpea	0.0	0.0	57.5	77.2
Standard *C. maculatus* leachate + Extract of cotyledon of cowpea	0.0	0.0	38.9	64.8
Standard *C. maculatus* leachate + Extract of testa of cowpea	0.0	0.0	0.0	6.1
Standard *C. maculatus* leachate + Extract of maize grains	0.0	0.0	40.5	69.9
Standard *C. maculatus* leachate + Extract of rice grain	0.0	0.0	6.0	35.0

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TABLE 13

: of Callosobrochus maculatus amended with extracts of seeds of bambara groundnut

cated hours	Mean Germ Tube Length after indicated hours (µm)			
12	3	6	9	12
0.0	-	-	-	-
63.0	-	-	12.7	50.1
41.6	-	-	17.1	38.8
83.6	-	-	29.8	68.3
36.9	-	-	12.9	29.8
77.2	-	-	25.6	44.5
64.8	-	-	17.5	56.3
6.1	-	-	-	12.9
69.9	-	-	25.3	60.8
35.0	-	-	19.3	49.3

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TAB

Germination of conidia of Aspergillus clavatus at 30°C in body leachate of S cowpea and grains of maize and rice.

Medium	% Germination after indicated ho			
	3	6	9	12
Standard *S. zeamais* leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	65.8	84.3	85.9
Standard *S. zeamais* leachate + Extract of axis of bambara groundnut seed	0.0	45.9	65.8	78.9
Standard *S. zeamais* leachate + Extract of cotyledon of bambara groundnut	51.2	57.2	68.9	70.5
Standard *S. zeamais* leachate + Extract of testa of bambara groundnut	0.0	16.4	23.4	29.6
Standard *S. zeamais* leachate + Extract of axis of cowpea	0.0	74.4	80.5	82.9
Standard *S. zeamais* leachate + Extract of cotyledon of cowpea	0.0	76.7	81.0	81.4
Standard *S. zeamais* leachate + Extract of testa of cowpea	0.0	81.5	85.6	90.6
Standard *S. zeamais* leachate + Extract of maize grain	51.1	64.3	70.1	76.5
Standard *S. zeamais* leachate + Extract of rice grain	31.3	43.7	58.7	67.8

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TABLE 14

chate of Sitophilus zeamais amended with extracts of seeds of bambara groundnut and

dicated hours	Mean Germ Tube Length after indicated hours (µm)			
12	3	6	9	12
0.0	-	-	-	-
85.9	-	26.3	56.9	81.6
78.9	-	18.8	31.3	57.6
70.5	17.8	36.9	57.1	82.8
29.6	-	12.7	30.9	41.3
82.9	-	16.3	49.1	56.8
81.4	-	16.8	40.6	63.9
90.6	-	29.8	54.3	77.9
76.5	24.5	34.1	58.3	88.1
67.8	14.9	40.8	89.7	94.1

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TABL

Germination of conidia of Aspergillus niger at 30°C in body leachate of Sitophil and grains of maize and rice.

Medium	% Germination after indicated hou			
	3	6	9	12
Standard S. zeamais leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	62.0	71.0	78.1
Standard S. zeamais leachate + Extract of axis of bambara groundnut seed	0.0	0.0	7.01	15.6
Standard S. zeamais leachate + Extract of cotyledon of bambara groundnut	0.0	14.3	30.5	44.4
Standard S. zeamais leachate + Extract of testa of bambara groundnut	0.0	0.0	6.6	19.7
Standard S. zeamais leachate + Extract of axis of cowpea	0.0	0.0	17.7	22.9
Standard S. zeamais leachate + Extract of cotyledon of cowpea	0.0	22.7	34.4	57.4
Standard S. zeamais leachate + Extract of testa of cowpea	0.0	0.0	61.3	67.8
Standard S. zeamais leachate + Extract of maize grain	0.0	9.1	28.2	55.3
Standard S. zeamais leachate + Extract of rice grain	0.0	4.5	32.4	57.7

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TABLE 15

of Sitophilus zeamais amended with extracts of seeds of bambara groundnut and cowpea

dicated hours	Mean Germ Tube Length after indicated hours (μm)			
12	3	6	9	12
0.0	-	-	-	-
78.1	-	21.1	58.9	84.9
15.6	-	-	10.3	31.8
44.4	-	40.8	57.4	75.3
19.7	-	-	9.92	27.1
22.9	-	-	16.2	32.6
57.4	-	19.1	45.5	73.9
67.8	-	-	29.5	52.9
55.3	-	19.8	29.2	38.9
57.7	-	14.5	20.9	31.3

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TAB

Germination of conidia of Aspergillus sulphureus at 30°C in body leachate of cowpea and grains of maize and rice.

Medium	% Germination after indicated h			
	3	6	9	12
Standard S. zeamais leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	31.6	40.5	50.7
Standard S. zeamais leachate + Extract of axis of bambara groundnut seed	0.0	0.0	0.0	8.9
Standard S. zeamais leachate + Extract of cotyledon of bambara groundnut	0.0	6.3	16.9	38.6
Standard S. zeamais leachate + Extract of testa of bambara groundnut	0.0	0.0	0.0	7.1
Standard S. zeamais leachate + Extract of axis of cowpea	0.0	0.0	5.8	20.0
Standard S. zeamais leachate + Extract of cotyledon of cowpea	0.0	8.2	15.2	30.2
Standard S. zeamais leachate + Extract of testa of cowpea	0.0	0.0	19.9	32.1
Standard S. zeamais leachate + Extract of maize grain	0.0	0.0	7.9	17.6
Standard S. zeamais leachate + Extract of rice grain	0.0	0.0	0.0	16.6

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TABLE 16

eachate of Sitophilus zeamais amended with extracts of seeds of bambara groundnut and

dicated hours	Mean Germ Tube Length after indicated hours (µm)			
12	3	6	9	12
0.0	-	-	-	-
50.7	-	14.8	38.6	65.9
8.9	-	-	-	15.8
38.6	-	25.3	48.8	67.6
7.1	-	-	-	12.8
20.0	-	-	11.8	25.6
30.2	-	12.7	34.8	56.3
32.1	-	-	20.9	38.6
17.6	-	-	15.2	29.6
16.6	-	-	-	16.5

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TAE

Germination of conidia of Aspergillus fumigatus at 30°C in body leachate o and cowpea and grains of maize and rice.

Medium	% Germination after indicated h			
	3	6	9	12
Standard T.castaneum leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	18.7	31.5	42.4
Standard T.castaneum leachate + Extract of axis of bambara groundnut seed	0.0	0.0	13.3	34.6
Standard T.castaneum leachate + Extract of cotyledon of bambara groundnut	0.0	0.0	5.6	14.4
Standard T.castaneum leachate + Extract of testa of bambara groundnut	0.0	0.0	0.0	5.2
Standard T.castaneum leachate + Extract of axis of cowpea	0.0	0.0	15.9	33.6
Standard T.castaneum leachate + Extract of cotyledon of cowpea	0.0	0.0	5.6	13.7
Standard T.castaneum leachate + Extract of testa of cowpea	0.0	0.0	0.0	7.1
Standard T. castaneum leachate + Extract of maize grain	0.0	0.0	0.0	18.1
Standard T. castaneum leachate + Extract of rice grain	0.0	0.0	0.0	10.5

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TABLE 17

leachate of Tribolium castaneum amended with extracts of seeds of bambara groundnut

ndicated hours	Mean Germ Tube Length after indicated hours (µm)			
12	3	6	9	12
0.0	-	-	-	-
42.4	-	27.6	38.6	57.8
34.6	-	-	17.3	43.6
14.4	-	-	13.8	34.8
5.2	-	-	-	12.2
33.6	-	-	24.1	40.6
13.7	-	-	14.5	30.9
7.1	-	-	-	12.2
18.1	-	-	-	40.1
10.5	-	-	-	34.5

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TAB

Germination of conidia of Aspergillus niger at 30°C in body leachate of Tri cowpea and grains of maize and rice.

Medium	% Germination after indicated h 3	6	9	12
Standard T.castaneum leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	69.1	93.2	98.7
Standard T.castaneum leachate + Extract of axis of bambara groundnut seed	0.0	0.0	32.1	64.4
Standard T.castaneum leachate + Extract of cotyledon of bambara groundnut	0.0	0.0	9.8	26.6
Standard T.castaneum leachate + Extract of testa of bambara groundnut	0.0	0.0	0.0	7.5
Standard T.castaneum leachate + Extract of axis of cowpea	0.0	36.8	53.1	66.9
Standard T.castaneum leachate + Extract of cotyledon of cowpea	0.0	6.72	27.1	46.9
Standard T.castaneum leachate + Extract of testa of cowpea	0.0	0.0	9.4	32.0
Standard T. castaneum leachate + Extract of maize grain	0.0	0.0	16.1	40.6
Standard T. castaneum leachate + Extract of rice grain	0.0	0.0	0.0	12.9

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TABLE 18

of Tribolium castaneum amended with extracts of seeds of bambara groundnut and

ted hours	Mean Germ Tube Length after indicated hours (μm)			
12	3	6	9	12
0.0	-	-	-	-
3.7	-	18.6	68.8	96.7
4.4	-	-	12.5	42.5
6.6	-	-	16.9	32.5
7.5	-	-	-	11.8
5.9	-	20.5	36.5	54.8
5.9	-	30.1	41.1	39.5
2.0	-	-	10.9	30.0
0.6	-	-	29.5	84.1
2.9	-	-	-	56.6

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TABL

Germination of conidia of Aspergillus sulphureus at 30°C in body leachate of and cowpea and grains of maize and rice.

Medium	% Germination after indicated hou			
	3	6	9	12
Standard T.castaneum leachate	0.0	0.0	0.0	0.0
Potato Dextrose Broth	0.0	0.0	16.7	32.7
Standard T.castaneum leachate + Extract of axis of bambara groundnut seed	0.0	0.0	5.1	9.4
Standard T.castaneum leachate + Extract of cotyledon of bambara groundnut	0.0	0.0	0.0	12.1
Standard T.castaneum leachate + Extract of testa of bambara groundnut	0.0	0.0	6.7	8.2
Standard T.castaneum leachate + Extract of axis of cowpea	0.0	0.0	5.4	22.8
Standard T.castaneum leachate + Extract of cotyledon of cowpea	0.0	0.0	0.0	25.1
Standard T.castaneum leachate + Extract of testa of cowpea	0.0	0.0	0.0	7.0
Extract of maize grain	0.0	0.0	8.9	34.5
Standard T. castaneum leachate + Extract of rice grain	0.0	0.0	0.0	5.6

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TABLE 19

achate of Tribolium castaneum amended with extracts of seeds of bambara groundnut

cated hours	Mean Germ Tube Length after indicated hours (μm)			
12	3	6	9	12
0.0	-	-	-	-
32.7	-	-	18.4	46.1
9.4	-	-	-	13.3
12.1	-	-	12.3	22.1
8.2	-	-	-	16.2
22.8	-	-	17.8	42.6
25.1	-	-	15.8	31.3
7.0	-	-	-	16.2
34.5	-	-	19.6	46.1
5.6	-	-	-	13.9

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D. GROWTH OF SEVEN ASPERGILLUS SPECIES ON INSECT BODY LEACHATE AGAR MEDIA

The results in Tables 20 to 26 showed that the leachates of *Callosobrochus maculatus, sitophilus zeamais and Tribolium castaneum* supported mycelial growth of all the seven *Aspergillus* species. By their response the *Aspergillus* species could be separated into three categories.

i. *A. ustus* alone grew to the same extent on all the three media.

ii. *A. fumigatus, A. niger and A. ochraceus* grew better and to almost the same extent on the *Sitophilus zemais* and *Tribolium castaneum* media than on the *Callosobrochus maculatus* medium. (Tables 22,23,24 respectively)

iii. *A. clavatus, A. flavus and A. sulphureus* grew better on the *callosobrochurs maculatus* and *sitophilus zeamais* and to practically the same extent than on the Tribolium castaneum medium. (Tables 20,21,25 respectively)

Apart from A. ustus which grew very well and to the same extent on all the media (Table 26) growth of the others was generally better on the other media (Bambara groundnut Extract Agar, Cowpea Extract Agar. Maize meal Agar, Potato Dextrose Agar, and rice Meal) than on the insect leachate Agar Media. Plates 2 to 5 show some of the colonies on the various media on the 7th day of incubation. Maize meal Agar consistently supported very good growth and the mean colony diameters of all the species on this medium and on the insect Leachate Agar Media have been compared in the histograms in Fig. 3.

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TAB

Growth of Aspergillus clavatus on insect leachate Agar media and other na

Medium	Mean diameter of colony (mm) 2	4
Callosobrochus maculatus		
leachate Agar	8.4 ± 0.2	21.6 ± 0.7
Sitophilus zeamais		
leachate Agar	10.6 ± 0.5	24.9 ± 1.0
Tribolium castaneum		
leachate Agar	11.3 ± 0.3	21.3 ± 0.4
Bambara groundnut Extract Agar	13.1 ± 0.1	24.8 ± 0.2
Cowpea Extract Agar	9.4 ± 0.2	24.0 ± 0.3
Maize Meal Agar	12.8 ± 0.5	37.3 ± 1.1
Potato Dextrose Agar	9.6 ± 0.3	20.3 ± 0.6
Rice meal	11.3 ± 0.4	33.3 ± 1.4

Means with the same letter are not significantly differen. Confidence limits

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TABLE 20

and other natural media at 30°C

colony (mm) after indicated incubation days			
4	6	8	10
.6 ± 0.7	37.7 ± 0.8	53.0 ± 1.0	68.0 ± 0.6 D
9 ± 1.0	38.8 ± 0.4	51.5 ± 0.8	64.2 ± 1.2 C
3 ± 0.4	32.6 ± 0.5	41.6 ± 0.7	53.8 ± 2.5 B
8 ± 0.2	37.6 ± 0.5	50.3 ± 0.4	65.1 ± 0.3 C
0 ± 0.3	38.2 ± 0.7	53.3 ± 0.6	67.3 ± 1.2CD
3 ± 1.1	54.0 ± 0.9	76.5 ± 2.2	89.5 ± 0.5 F
3 ± 0.6	30.0 ± 0.8	40.3 ± 1.0	50.9 ± 0.9 A
3 ± 1.4	49.5 ± 0.4	68.0 ± 0.7	82.2 ± 1.0 E

fidence limits calculated at 95%.

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TAE

Growth of Aspergillus flavus on insect leachate Agar media and other nat

Medium	Mean diameter of colony (mm) 2	4
Callosobrochus maculatus		
leachate Agar	15.0 ± 0.3	29.9 ± 0.2
Sitophilus zeamais		
leachate Agar	14.8 ± 0.3	33.4 ± 0.4
Tribolium castaneum		
leachate Agar	16.8 ± 0.3	28.9 ± 0.5
Bambara groundnut Extract Agar	20.1 ± 0.1	39.9 ± 0.3
Cowpea Extract Agar	10.8 ± 0.2	32.1 ± 0.2
Maize Meal Agar	11.4 ± 0.6	44.2 ± 1.6
Potato Dextrose Agar	13.3 ± 0.3	29.4 ± 0.3
Rice meal	12.9 ± 0.6	35.9 ± 1.9

Means with the same letter are not significantly different. Confidence limit

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TABLE 21

other natural media at 30°C

y (mm) after indicated incubation days			
4	*6*	*8*	*10*
0.2	*44.1 ± 0.5*	*54.3 ± 1.6*	*68.8 ± 1.3 B*
0.4	*50.2 ± 0.4*	*66.0 ± 0.5*	*76.5 ± 0.6 C*
0.5	*42.3 ± 0.6*	*52.9 ± 1.0*	*66.5 ± 0.8 AB*
0.3	*61.6 ± 0.7*	*80.0 ± 0.4*	*90.0 ± 0.0 F*
0.2	*50.5 ± 0.5*	*71.0 ± 0.4*	*80.3 ± 0.3 E*
1.6	*61.3 ± 2.4*	*82.5 ± 1.0*	*90.0 ± 0.0 F*
0.3	*41.6 ± 0.5*	*54.1 ± 0.4*	*65.1 ± 0.8 A*
1.9	*50.3 ± 1.0*	*64.7 ± 0.3*	*78.8 ± 0.7 D*

ence limits calculated at 95%.

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TAB

Growth of Aspergillus fumigatus on insect leachate Agar media and other n

Medium	Mean diameter of colony (mm) 2	4
Callosobrochus maculatus		
leachate Agar	8.6 ± 0.7	28.0 ± 0.6
Sitophilus zeamais		
leachate Agar	9.8 ± 0.2	31.8 ± 0.5
Tribolium castaneum		
leachate Agar	10.4 ± 0.2	24.8 ± 0.7
Bambara groundnut Extract Agar	13.6 ± 0.8	31.8 ± 0.7
Cowpea Extract Agar	4.0 ± 0.0	24.3 ± 0.2
Maize Meal Agar	6.9 ± 0.3	40.9 ± 0.7
Potato Dextrose Agar	5.9 ± 0.4	29.0 ± 0.4
Rice meal	7.3 ± 0.4	27.5 ± 0.8

Means with the same letter are not significantly different. Confidence limit

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TABLE 22

ther natural media at 30°C

m)	*after indicated incubation days*	
6	*8*	*10*
41.8 ± 0.7	*59.8 ± 0.4*	*71.8 ± 0.8AB*
52.8 ± 0.9	*74.5 ± 0.9*	*86.8 ± 0.8E*
44.4 ± 0.6	*62.5 ± 0.6*	*81.4 ±0.8CD*
53.9 ± 0.7	*77.4 ± 0.8*	*90.0 ± 0.8 F*
45.8 ± 0.6	*69.3 ± 0.5*	*79.8 ± 0.3 C*
64.8 ± 2.8	*87.8 ± 1.3*	*90.0 ± 0.0 F*
45.8 ± 0.4	*58.8 ± 0.9*	*70.0 ± 0.9 A*
41.5 ± 0.4	*66.3 ± 2.7*	*82.2 ± 1.1 D*

limits calculated at 95%.

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TABL

Growth of Aspergillus niger on insect leachate Agar media and other natura

Medium	Mean diameter of colony (mm) 2	4	a
Callosobrochus maculatus			
leachate Agar	7.5 ± 0.3	21.3 ± 0.4	3
Sitophilus zeamais			
leachate Agar	14.0 ± 2.1	37.7 ± 0.8	5
Tribolium castaneum			
leachate Agar	5.3 ± 0.2	26.1 ± 0.4	4
Bambara groundnut Extract Agar	16.4 ± 0.4	31.5 ± 0.7	4
Cowpea Extract Agar	10.8 ± 0.3	31.1 ± 0.2	5
Maize Meal Agar	8.8 ± 0.4	46.5 ± 1.3	7
Potato Dextrose Agar	15.8 ± 0.3	33.3 ± 0.4	4
Rice meal	8.5 ± 0.7	31.9 ± 1.1	4

Means with the same letter are not significantly different. Confidence limits

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TABLE 23

d other natural media at 30°C

olony (mm) after indicated incubation days			
4	*6*	*8*	*10*
3 ± 0.4	*36.5 ± 0.5*	*50.8 ± 0.9*	*68.3 ± 0.4A*
7 ± 0.8	*53.2 ± 2.4*	*59.3 ± 0.8*	*83.2 ± 0.8CD*
1 ± 0.4	*45.3 ± 0.3*	*66.8 ± 0.4*	*81.5 ± 0.5C*
5 ± 0.7	*46.4 ± 0.3*	*66.8 ± 0.4*	*82.8 ± 0.3CD*
' ± 0.2	*52.0 ± 0.4*	*77.8 ± 0.3*	*83.3 ± 0.3D*
; ± 1.3	*76.3 ± 1.5*	*86.7 ± 1.1*	*90.0 ± 0.0 E*
' ± 0.4	*44.0 ± 1.5*	*60.3 ± 0.3*	*69.0 ± 0.7AB*
' ± 1.1	*49.5 ± 1.1*	*72.3 ± 1.7*	*84.8 ± 0.8CD*

fidence limits calculated at 95%.

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TA

Growth of Aspergillus ochraceus on insect leachate Agar media and other

Medium	Mean diameter of colony (mm) 2	4
Callosobrochus maculatus		
leachate Agar	7.5 ± 0.2	14.6 ± 0.3
Sitophilus zeamais		
leachate Agar	11.0 ± 0.4	19.5 ± 0.8
Tribolium castaneum		
leachate Agar	7.6 ± 0.3	16.0 ± 0.4
Bambara groundnut extract Agar	10.4 ± 0.2	25.5 ± 0.3
Cowpea Extract Agar	6.6 ± 0.4	20.6 ± 0.4
Maize Meal Agar	10.5 ± 0.4	25.0 ± 0.9
Potato Dextrose Agar	7.9 ± 0.4	24.1 ± 0.4
Rice Meal	5.3 ± 0.3	17.0 ± 0.8

Means with the same letter are not significantly different. Confidence limi

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TABLE 24

lia and other natural media at 30°C

olony (mm) after indicated incubation days			
4	6	8	10
.6 ± 0.3	21.4 ± 0.5	27.6 ± 0.8	31.8 ± 1.5A
.5 ± 0.8	28.0 ± 0.7	35.5 ± 0.7	41.2 ± 0.7B
.0 ± 0.4	25.1 ± 0.3	33.3 ± 0.5	41.5 ± 1.0B
.5 ± 0.3	41.9 ± 0.2	52.5 ± 0.3	65.9 ± 0.3G
.6 ± 0.4	34.2 ± 0.4	45.2 ± 0.1	57.8 ± 0.3D
0 ± 0.9	38.5 ± 0.6	52.8 ± 0.5	60.0 ± 0.8 E
1 ± 0.4	35.5 ± 0.3	43.8 ± 0.6	50.9 ± 0.5C
0 ± 0.8	33.2 ± 0.6	47.8 ± 1.0	63.7 ± 0.4F

nfidence limits calculated at 95%.

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TAB

Growth of Aspergillus sulphureus on insect leachate Agar media and other

Medium	Mean diameter of colony (mm) 2	4
Callosobrochus maculatus		
leachate Agar	7.0 ± 0.2	15.3 ± 0.3
Sitophilus zeamais		
leachate Agar	9.8 ± 0.3	18.4 ± 0.6
Tribolium castaneum		
leachate Agar	7.6 ± 0.5	15.6 ± 1.1
Bambara groundnut Extract Agar	11.1 ± 0.4	24.8 ± 0.5
Cowpea Extract Agar	7.3 ± 0.3	23.5 ± 0.5
Maize Meal Agar	10.0 ± 0.4	41.8 ± 0.8
Potato Dextrose Agar	10.5 ± 0.2	24.4 ± 0.4
Rice meal	5.5 ± 0.3	18.4 ± 0.6

Means with the same letter are not significantly differe. Confidence limit

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TABLE 25

dia and other natural media at 30°C

olony (mm) after indicated incubation days			
4	6	8	10
.3 ± 0.3	24.2 ± 0.6	30.2 ± 0.8	38.7 ± 1.2B
.4 ± 0.6	24.0 ± 0.8	33.1 ± 1.2	43.7 ± 0.6C
.6 ± 1.1	19.9 ± 0.3	22.0 ± 0.7	29.4 ± 0.3A
.8 ± 0.5	40.4 ± 0.8	59.8 ± 0.4	74.5 ± 0.3F
.5 ± 0.5	39.6 ± 0.4	55.5 ± 0.3	59.0 ± 0.4E
.8 ± 0.8	56.8 ± 0.8	68.8 ± 0.8	84.8 ± 0.5G
.4 ± 0.4	36.9 ± 0.9	54.5 ± 0.3	69.6 ± 0.3E
.4 ± 0.6	34.1 ± 1.3	45.0 ± 1.3	64.0 ± 1.3D

nfidence limits calculated at 95%.

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TAB

Growth of Aspergillus ustus on insect leachate Agar media and other natur

Medium	Mean diameter of colony (mm) after ind 2	4	6
Callosobrochus maculatus			
leachate Agar	14.1 ± 0.2	33.5 ± 0.8	61.8 ±
Sitophilus zeamais			
leachate Agar	12.9 ± 0.4	37.1 ± 0.7	62.3 ±
Tribolium castaneum			
leachate Agar	14.6 ± 0.3	32.1 ± 0.4	59.3 ±
Bambara groundnut Extract Agar	15.9 ± 0.4	32.3 ± 0.5	54.9 ±
Cowpea Extract Agar	5.0 ± 0.2	26.4 ± 0.2	50.3 ±
Maize Meal Agar	7.4 ± 0.3	40.0 ± 1.8	60.8 ±
Potato Dextrose Agar	11.1 ± 0.3	34.6 ± 0.3	60.3 ±
Rice meal	7.4 ± 0.8	28.0 ± 0.9	40.7 ±

Means with the same letter are not significantly different. Confidence limit

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TABLE 26

ther natural media at 30°C

after indicated incubation days		
6	*8*	*10*
61.8 ± 1.2	*78.8 ± 1.2*	*89.0 ± 1.0B*
62.3 ± 1.0	*85.3 ± 0.3*	*90.0 ± 0.0B*
59.3 ± 0.5	*73.4 ± 0.5*	*90.0 ± 0.0B*
54.9 ± 0.4	*80.0 ± 0.0*	*90.0 ± 0.0B*
50.3 ± 0.3	*74.8 ± 0.3*	*90.0 ± 0.0B*
60.8 ± 0.5	*79.3 ± 0.9*	*90.0 ± 0.0B*
60.3 ± 0.3	*81.8 ± 0.6*	*90.0 ± 0.0B*
40.7 ± 0.7	*61.3 ± 0.7*	*79.0 ± 0.7A*

ence limits calculated at 95%.

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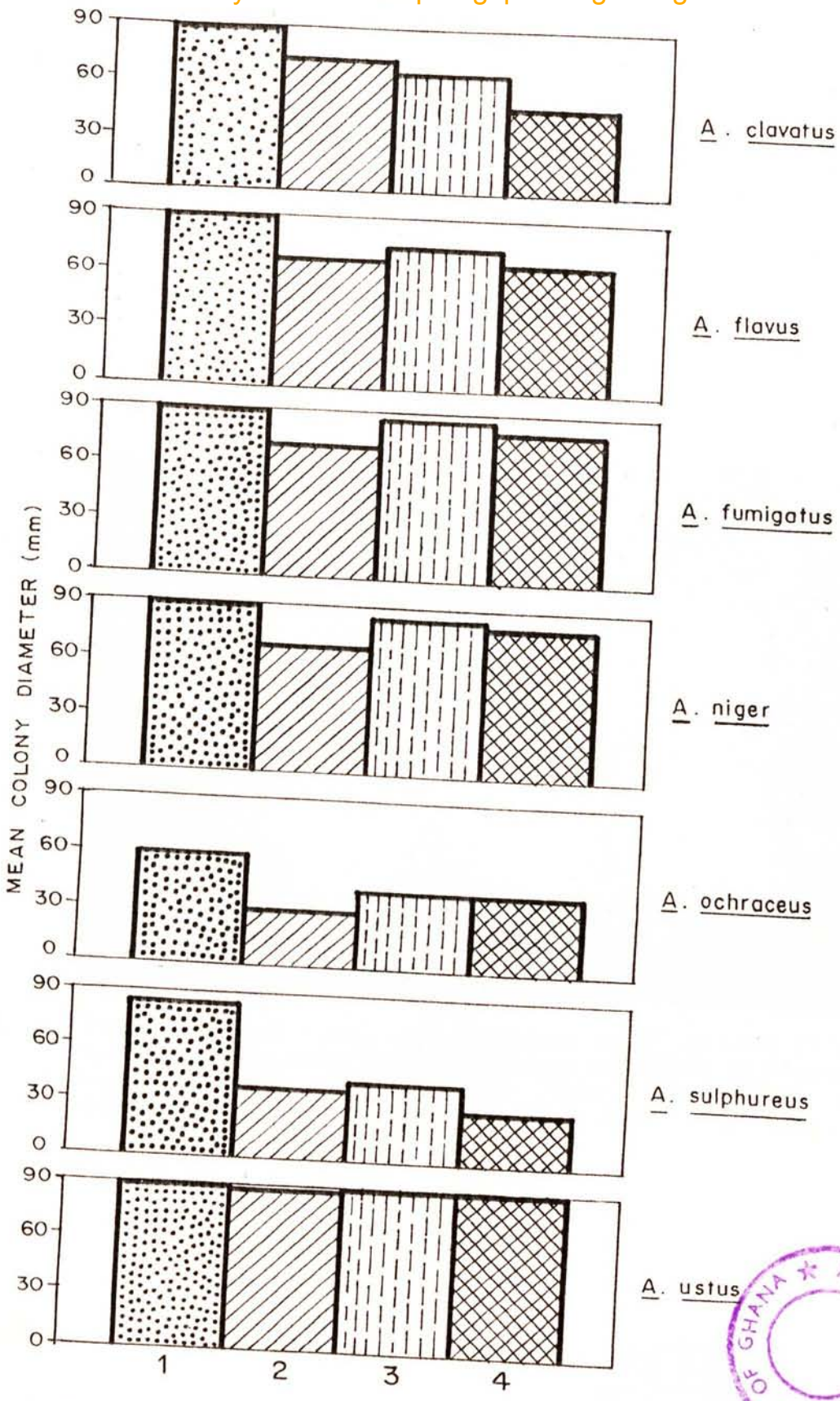

Fig. 3 Mean colony diameters of A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. sulphureus, A. ustus on Maize Meal Agar (1) Leachate Agar Media prepared with Callosobrochus maculatus (2), Sitophilus zeamais (3), Tribolium castaneum (4) on the 10th day of incubation at 30°C.

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PLATES 2: Photograph of 7-day old cultures of *A. flavus* (TOP) and *A. sulphureus* (BOTTOM) grown on three different media at 30°c. FROM LEFT: Maize Meal Agar, *C. maculatus* Leachate Agar and Rice Meal. (x 1/3).

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PLATE 3: Photograph of 7-day old cultures of A. ochraceus (TOP) and *A. niger* (BOTTOM) grown on three different media at 30°C
FROM LEFT: Maize Meal Agar, *C. maculatus* leachate Agar and Rice Meal (x 1/3)

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PLATES 4: Photograph of 7-day old cultures of *A. flavus*(TOP) and *A. clavatus* (BOTTOM) grown on three different media at 30°c. FROM LEFT: Maize Meal Agar, *S. zeamais* Leachate Agar and Cowpea Extract Agar. (x 1/3).

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PLATE 5: Photograph of 7-day old cultures of A. ochraceus (TOP) and A. sulphureus (BOTTOM) grown on three different media at 30°C
FROM LEFT: Potato Dextrose Agar, *T. castaneum* Agar and cowpea Extract Agar (x 1/3)

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E. MYCOFLORA OF GUT OF CALLOSOBROCHUS MACOLATUS, SITOPHILUS ZEAMAIS AND TRIBOLIUM COSTANEUM

The data in Tables 27 to 29 show that samples of the three insect pests *C. maculatus*, *S. zeamais* and *T. costaneum* taken randomly from the stored cowpea seeds and maize grains had live propagules of many fungi in their guts. The flora which grew on Potato Dextrose Agar plated with the gut contents of *C. maculatus* from the stored cowpea seeds consisted of 14 species. The most important genera were *Aspergillus* and *Penicillium whose members made 34.4 and 39.2 percent, respectively, of the total number of colonies (Table 27).*

Aspergillus and *Penicillium were also the dominant genera in the guts of Sitophilus zeamais and T. cdstaneum.* The respective percentage occurrence of members of *Aspergillus* and *Penicillium* was 45.2 and 40.8 percent in *Sitophilus zeamais* (Table 28) and 22.6 and 50.9 percent in *Tribolium castaneum* (Table 29). With all three insects *Aspergillus flavus* was dominant *Aspergillus* species and *Penicillium citrinum* was the predominant Penicillium species. Plates 6 to 8 are photographs of fungi which grew on samples of the Potato Dextrose Agar plates inoculated with the gut contents of *C. maculatus, S. zeamais* and *T. castaneum* respectively.

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TABLE 27

Fungi isolated from the gut of *Callosobrochus maculatus* collected from naturally infested stored maize grains (Colonies on inoculated PDA after 7 days' incubation at 30°C)

Fungi isolated	No of colonies	Percentage occurrence
Absidia species	4	5.8
Aspergillus flavus	11	15.9
Aspergillus fumigatus	4	5.8
Aspergillus niger	2	2.9
Aspergillus ochraceus	4	6.8
Aspergillus terreus	2	2.9
Cephalosporium species	1	1.4
Cladosporium herbarum	4	5.8
Penicillium citrinum	19	27.6
Penicillium expansum	2	2.9
Penicillium purpurogenum	6	8.7
Pullularia pullulans	2	2.9
Rhizopus species	1	1.4
Syncephalastrum racemosum	3	4.3
Sterilia mycelia	4	5.8

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TABLE 28

Fungi isolated from the gut of *Sitophilus zeamais* collected from naturally infested stored maize grains (Colonies on inoculated PDA after 7 days' incubation at 30°C

Fungi isolated	No of colonies	Percentage occurrence
Aspergillus clavatus	1	2.4
Aspergillus flavus	11	26.2
Aspergillus fumigatus	4	9.4
Aspergillus sulphureus	1	2.4
Aspergillus wentii	2	4.8
Cladosporium herbarum	2	4.8
Curvularia species	1	2.4
Fusarium moniliforme	2	4.8
Penicillium citrinum	12	28.6
Penicillium chrysogenum	3	7.4
Penicillium purpurogenum	1	2.4
Penicillium spinulosum	1	2.4
Rhizopus species	1	2.4

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TABLE 29

Fungi isolated from the gut of *Tribolium castaneum* collected from naturally infested stored maize grains (Colonies on inoculated PDA after 7 days' incubation at 30°C

Fungi isolated	No of colonies	Percentage occurrence
Aspergillus clavatus	1	0.6
Aspergillus effusus	3	1.9
Aspergillus flavus	18	11.2
Aspergillus fumigatus	5	3.2
Aspergillus niger	3	1.9
Aspergillus ochraceus	5	3.2
Aspergillus tamarii	1	0.6
Botrytis species	2	1.3
Cephalosporium species	26	16.4
Cladosporium herbarum	4	2.5
Fusarium moniliforme	1	0.6
Mucor plumbens	1	0.6
Penicillium citrinum	68	42.7
Penicillium chrysogenum	5	3.2
Penicillium purpurogenum	8	5.0
Pullularia pullulans	2	1.3
Sterilia mycelia	6	3.8

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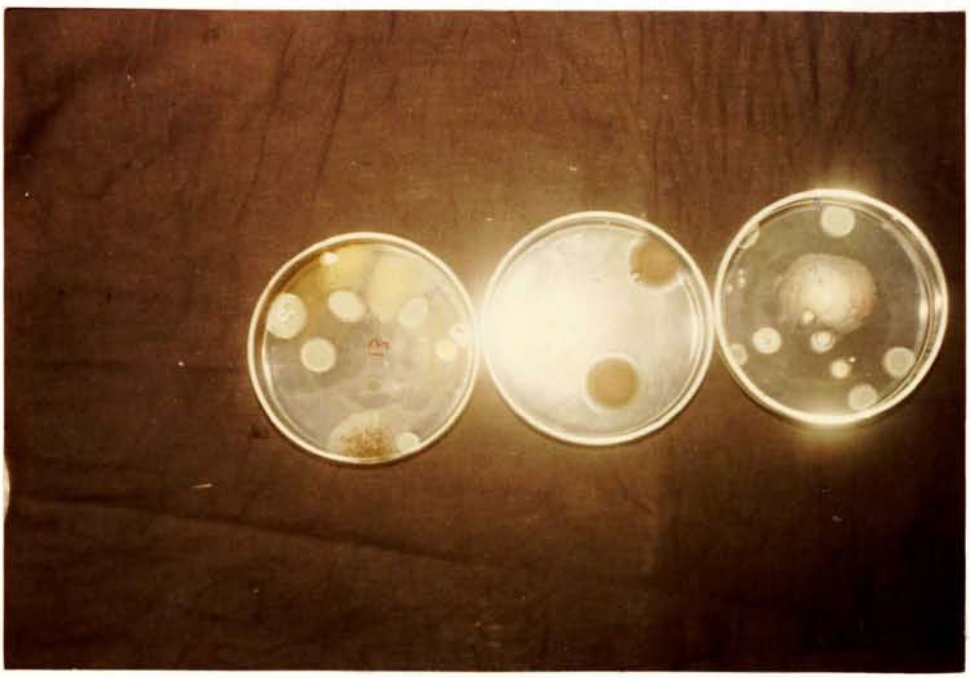

PLATE 6: Photograph of PDA Plates inoculated with gut contents of *Callosobrochus maculatus* showing fungal colonies after incubation at 30°C for 7-days (x 1/3)

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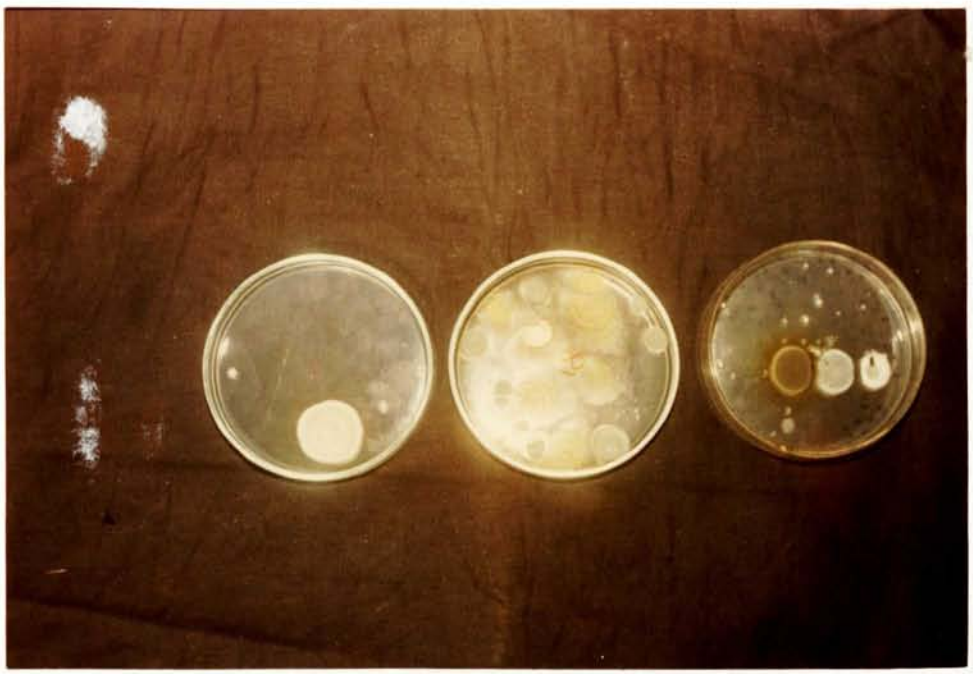

PLATES 7: Photograph of PDA plates inoculated with gut contents of *Sitophilus zeamais* showing developing fungal colonies after incubation at 30°C for 7 days (x 1/3)

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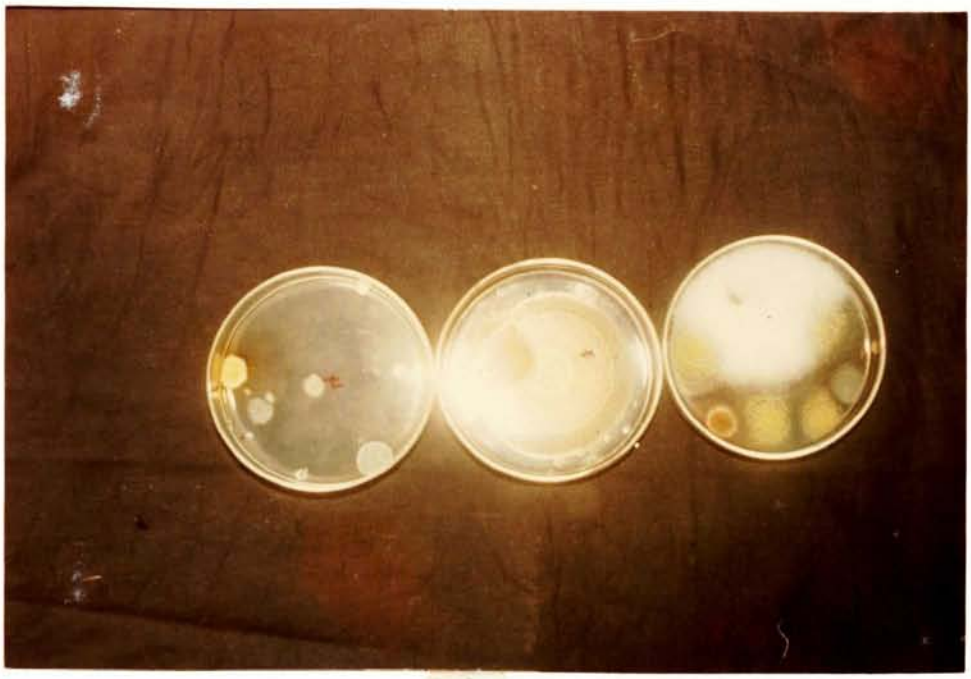

PLATES 8: Photograph of PDA plates inoculated with gut contents of *Tribolium castaneum* showing developing fungal colonies after incubation at 30°C for 7 days (x 1/3)

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F. SURVIVAL OF *ASPERGILLUS* SPECIES IN THE GUTS OF *COLLOSOBROCHUS MACULATUS*, *SITOPHILUS ZEAMAIS* AND *TRIBOLIUM CASTANEUM*

The results of the experiment contain important information on how long ingested *A. flavus*, *A. fumigatus* and *A ochraceus* would remain viable in the guts of *C. maculatus*, *S. zeamais* and *T. castaneum*. Plates 9 to 11 are photographs of 7 day old pure cultures which developed on PDA plates inoculated with gut contents taken two days after feeding of the insects.

The results in Tables 30 to 32 showed two notable aspects of survival. First, *A. flavus* consistently survived longest in the guts of the three insect species. Secondly, with each fungus species, longevity in the guts of the three insect pests varied. The latter has been illustrated in Fig 4 which was constructed with percentage viability calculated from the data in Tables 30 to 32.

A. flavus survived longest (8 days) (Table 31) in the gut of *S. zeamais* and shortest (5 days) (Table 32) in the gut of *T. castaneum*.

A. fumigatus survived longest (6 days) (Table 31) in the gut of *S. zeamais* and shortest (4 days) (Table 30) in the gut of *C. maculatus*.

A. ochraceus survived longest (6 days) (Table 30) in the gut of *C. maculatus* and shortest (3 days) (Table 32) in the gut of *T. castaneum*.

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TABLE 30

Survival of *Aspergillus* species in the gut of *Collosobrochus maculatus* fed on inoculated cowpea seeds with the species (Contents of gut plated on PDA and incubated at 30°C for 7 days)

Time after feeding (Days)	Mean no. of colonies Per Petri Plate of		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	29	32	87
2	21	37	28
3	23	3	14
4	15	1	13
5	14	0	7
6	4	-	2
7	0	-	0
8	-*	-	-
9	-	-	-

* Readings dicontinued

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TABLE 31

Survival of *Aspergillus* species in the gut of *Sitophilus zeamais* fed on inoculated maize grains with the species (Contents of gut plated on PDA and incubated at 30°C for 7 days)

Time after feeding (Days)	Mean no. of colonies Per Petri Plate of		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	38	41	5
2	98	42	33
3	20	16	5
4	6	25	2
5	7	12	0
6	14	2	-*
7	1	0	-
8	1	-	-
9	0	-	-

*

Reading discontiued.

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TABLE 32

Survival of *Aspergillus* species in the gut of *Tribolium casaneum* fed on inoculated maize grains with the species (Contents of gut plated on PDA and incubated at 30°C for 7 days)

Time after feeding (Days)	Mean no. of colonies Per Petri Plate of		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	22	33	87
2	15	18	1
3	6	9	7
4	13	3	0
5	3	4	-
6	0	0	-
7	-*	-	-
8	-	-	-
9	-	-	-

* Reading discontinued.

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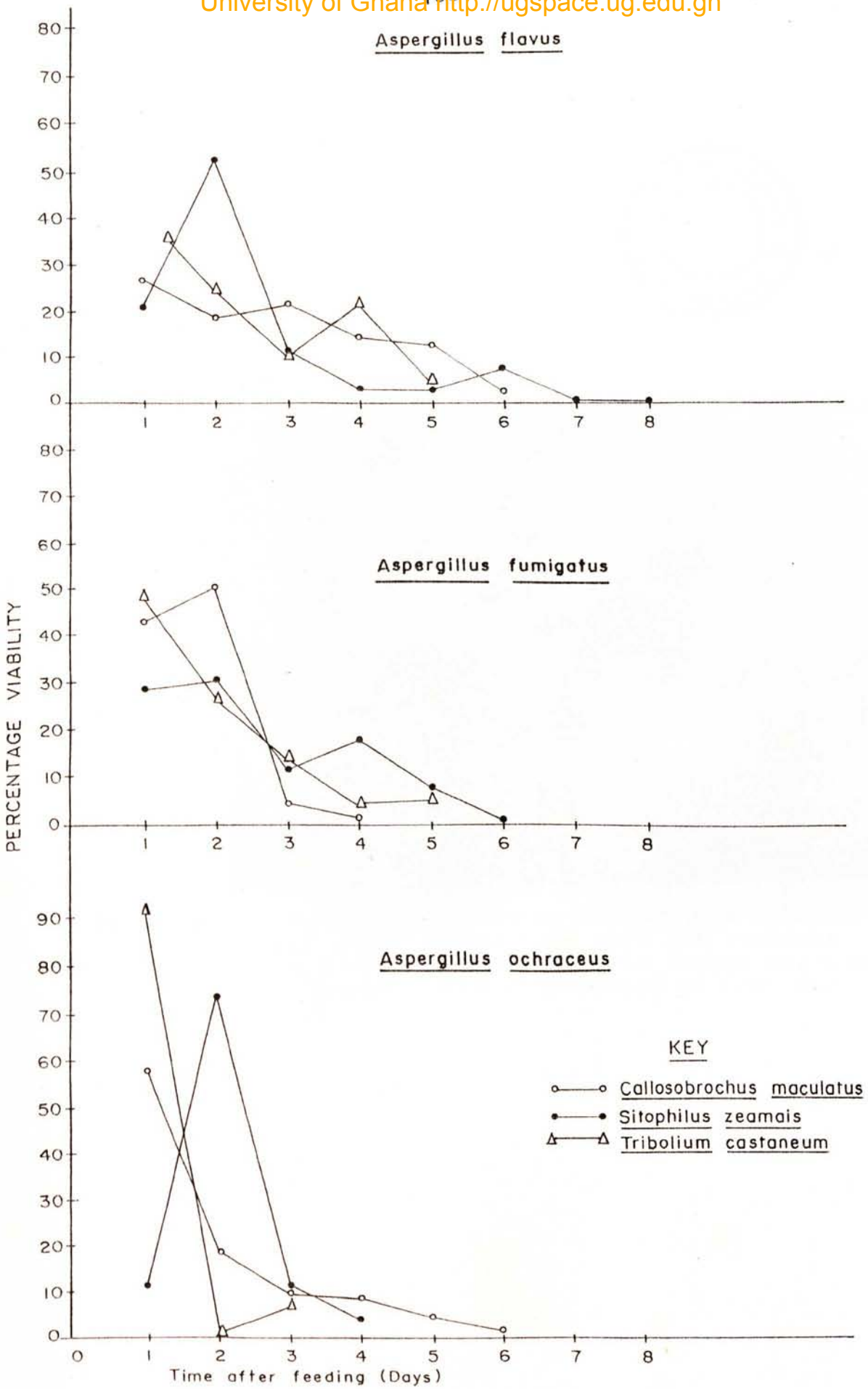

Fig. 4 Percentage frequency of Aspergillus flavus, Aspergillus fumigatus and Aspergillus ochraceus isolated from the guts of C. maculatus, S. zeamais and T. castaneum on consecutive days following feeding.

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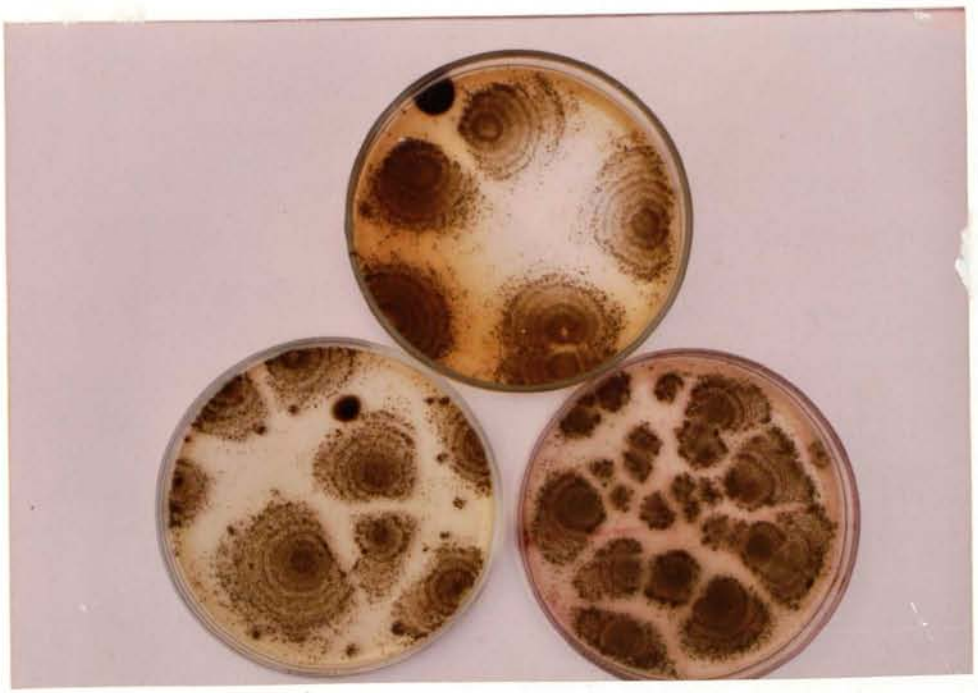

PLATES 9: Photograph of PDA Plates showing pure cultures of A. flavus after inoculation with gut contents of *S. zeamais* taken 2 days after the insect had been fed with fungus. Plates incubated at 30°c. for 7 days (x 1/2).

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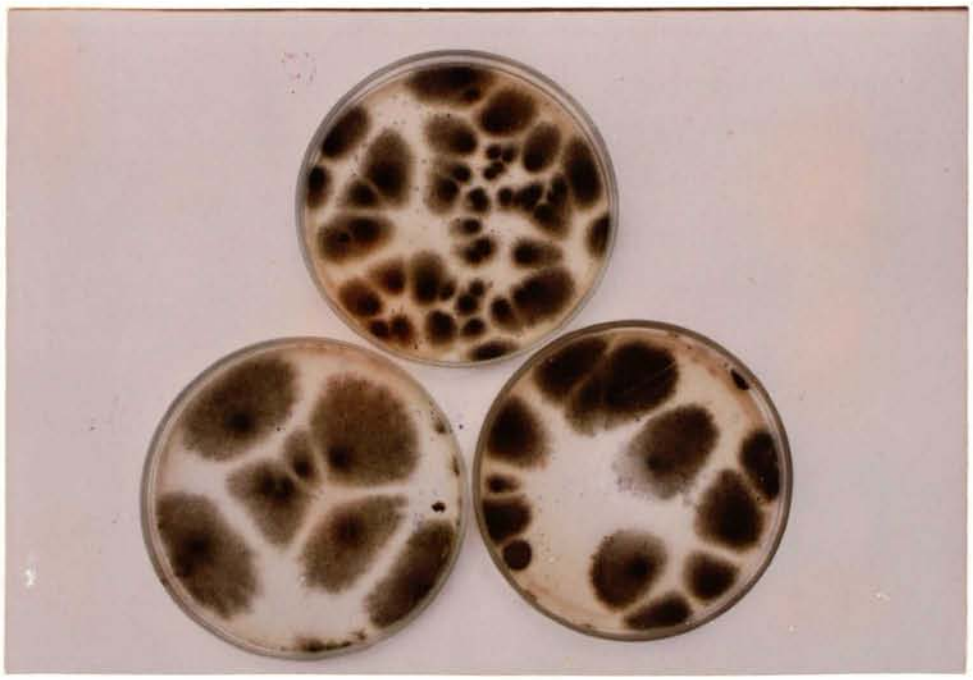

PLATES 10: Photograph of PDA Plates showing pure cultures of *A. fumigatus* after inoculation with gut contents of *C. maculatus* taken 2 days after the insect had been fed with the fungus. Plates incubated at 30°c. for 7 days (x 1/2).

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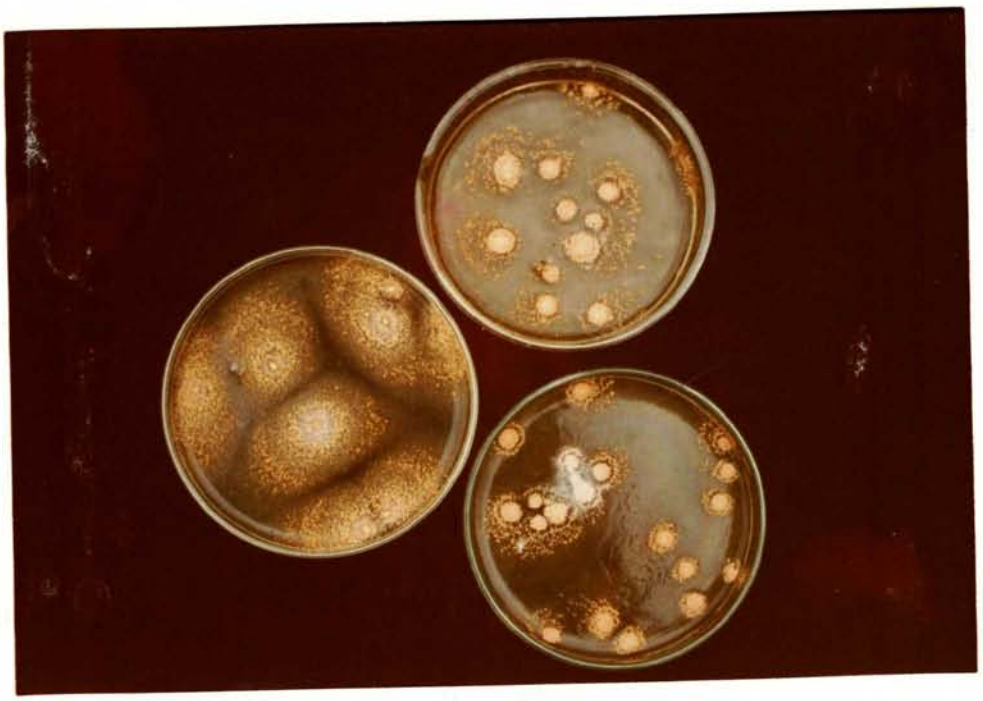

PLATES 11: Photograph of PDA Plates showing pure cultures of *A. ochraceus* after inoculation with gut contents of *T. castaneum* taken 2 days after the insect had been fed with the fungus. Plates incubated at 30°c. for 7 days (x 1/2).

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G. GERMINATION OF CONIDIA OF *ASPERGILLUS* SPECIES IN EXTRACTS OF FAECAL PELLETS OF THE INSECTS

The results of this experiment tabulated in Tables 33 to 35 showed that the faecal pellets of the insect pests could serve as a medium for germination of the conidia of some of the *Aspergillus species*. The extracts of the faecal pellets of *C. maculatus* supported germination of conidia of all the seven *Aspergillus* species to varying degrees at the standard concentration (faecal pellets of 1,000 individuals dissolved in 10 ml of sterile distilled water) used (Table 33).

Extract of the faecal pellets of *S. zeamais* at the standard concentration supported very low percentage germination of conidia of *A. clavatus, A. ochraceus and A. sulphureus* only (Table 34) while the only species which showed germination in standard extract of the faecal pellets of *T. castaneum* were *A. clavatus, A. flavus* and *A. ochraceus* (Table 35).

The result also showed that some of the conidia became swollen, and thus initiated the germination processes, but were unable to produce germ tubes during the incubation period of 24 hours.

There was neither conidial swelling nor germ tube development when the extracts were diluted to half the concentration of the standard.

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TABLE 33

Germination of conidia of *Aspergillus* species in aqueous solution of faecal pellets of *Callosobrochus maculatus* incubated at 30°C for 24 hours.

(Each value of Percentage Germination based on a total of 500-600 observed conidia)

Aspergillus species	Concentration of aqueous faecal Pellet solution	Percentage of conidia swollen	Percentage germination	Mean Germ tube length (µm)
A. clavatus	Standard	22.8	6.9	96.9
	1/2 Dilution	0.0	0.0	-
A. flavus	Standard	29.7	6.9	104.6
	1/2 Dulution	0.0	0.0	-
A. fumigatus	Standard	19.1	8.6	115.2
	1/2 Dilution	0.0	0.0	-
A. niger	Standard	12.54	10.0	108.6
	1/2 Dilution	0.0	0.0	-
A. ochraceus	Standard	18.2	7.1	85.9
	1/2 Dilution	0.0	0.0	-
A. sulphureus	Standard	16.2	8.3	84.6
	1/2 Dilution	0.0	0.0	0.0
A. ustus	Standard	12.7	6.0	71.6
	1/2 Dilution	0.0	0.0	-

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TABLE 34

Germination of conidia of *Aspergillus* species in aqueous solution of faecal pellets of *Sitophilus zeamais* incubated at 30°C for 24 hours.
(Each value of Percentage Germination based on a total of 500-600 observed conidia)

Aspergillus species	Concentration of aqueous faecal Pellet solution	Percentage of conidia swollen	Percentage germination	Mean Germ tube length (µm)
A. clavatus	Standard	34.8	1.0	93.2
	1/2 Dilution	0.0	0.0	-
A. flavus	Standard	29.7	0.0	-
	1/2 Dulution	0.0	0.0	-
A. fumigatus	Standard	38.8	0.0	-
	1/2 Dilution	0.0	0.0	-
A. niger	Standard	17.8	0.0	-
	1/2 Dilution	0.0	0.0	-
A. ochraceus	Standard	25.5	0.7	183.14
	1/2 Dilution	0.0	0.0	-
A. sulphureus	Standard	13.9	0.7	111.6
	1/2 Dilution	0.0	0.0	-
A. ustus	Standard	6.5	0.0	-
	1/2 Dilution	0.0	0.0	-

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TABLE 35

Germination of conidia of *Aspergillus* species in aqueous solution of faecal pellets of *Tribolium castaneum* incubated at 30°C for 24 hours.

(Each value of Percentage Germination based on a total of 500-600 observed conidia)

Aspergillus species	Concentration of aqueous faecal Pellet solution	Percentage of conidia swollen	Percentage germination	Mean Germ tube length (μM)
A. clavatus	Standard	15.1	21.5	110.6
	1/2 Dilution	0.0	0.0	-
A. flavus	Standard	12.0	13.7	112.2
	1/2 Dulution	0.0	0.0	-
A. fumigatus	Standard	14.3	0.0	-
	1/2 Dilution	0.0	0.0	-
A. niger	Standard	11.7	0.0	-
	1/2 Dilution	0.0	0.0	-
A. ochraceus	Standard	11.1	5.4	75.9
	1/2 Dilution	0.0	0.0	-
A. sulphureus	Standard	0.0	0.0	-
	1/2 Dilution	0.0	0.0	-
A. ustus	Standard	0.0	0.0	-
	1/2 Dilution	0.0	0.0	-

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H. DISPERSAL OF CONIDIA OF *ASPERGILLUS FLAVUS* ATTACHED TO THE BODIES OF *SITOPHILUS ZEAMAIS AND TRIBOLIUM CASTANEUM*

The conidia of the sporulating contaminant fungi would definitely adhere to the body of insects which would brush against the conidial apparatus. The results of this experiment have shown how much of the initial load of conidia would be lost as the insects migrated among the heaps of grains and seeds. The results of this investigation in which the insects were made to pick up conidia and then move along a column of maize grains in glass tubes are presented in Tables 36 to 41 and in Figs. 5 and 6, Plate 12 is a photograph of the set up.

The results could be summarized as follows:

(a) The percentage of conidia removed depended on the pore size among the maize grains irrespective of the insect species. For the two insect pests, the respective percentages of conidia still adhering to insects which had travelled over 100cm through the small-sized, medium-sized and large-sized maize grains were:

Sitophilus zeamais: 12.2, 15.3 and 23.3 percent (Tables 36-38, Fig. 5).

Tribolium castaneum: 11.5, 12.1 and 17.2 percent (Tables 39-41, Fig. 6).

(b) The conidia were generally more easily removed from the bodies of *Tribolium castaneum* than those of *Sitophilus zeamais*. The values from the experiment carried out with the large-sized

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maize grains showed marked differences. The percentage of conidia remaining on the bodies of *Sitophilus zeamais* after travelling over 25, 50, 75 and 100cm was 27.5, 19.6, 18.0 and 23.3 percent, respectively, (Table 38). The corresponding figures for *Tribolium castaneum* were 19.5, 17.2, 13.8 and 17.2 percent respectively (Table 41).

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TABLE 36

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Sitophilus zeamais* after travelling through small-sized maize grains (5.3-8.5 x 4.04-7.30 mm) over different distances

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of $76x100ml^{-1}$
25	24.8C	32.8C
50	10.4A	13.8A
75	12.4B	16.4B
100	9.2A	12.2A

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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TABLE 37

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Sitophilus zeamais* after travelling through Medium-sized maize grains (8.1-10.2 x 6.00-8.21 mm) over different distances

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of $76x100ml^{-1}$
25	15.6C	20.6C
50	12.8BC	16.9BC
75	9.6A	12.7A
100	11.6B	15.3B

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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TABLE 38

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Sitophilus zeamais* after travelling through Large-sized maize grains (size range of 9.5-12.2 x 7.5-9.5mm) over different distances.

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of 76x100ml^{-1}
25	20.8D	27.5D
50	14.8AB	19.6AB
75	13.6A	18.0A
100	17.6C	23.6C

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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TABLE 39

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Tribolium castaneum* after travelling through small-sized maize grains (5.3-8.5 x 4.04-7.30 mm) over different distances

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of $70x100ml^{-1}$
25	12.4B	17.8B
50	8.4A	12.1A
75	9.2AB	13.2AB
100	8.0A	11.5A

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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TABLE 40

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Tribolium castaneum* after travelling through Medium-sized maize grains (8.1-10.2 x 6.00-8.21 mm) over different distances

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of $70x100ml^{-1}$
25	15.6C	22.4C
50	10.0B	14.4B
75	9.6AB	13.8AB
100	8.4A	12.1A

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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TABLE 41

Proportion of *Aspergillus flavus* conidia still adhering to the body of *Tribolium castaneum* after travelling through Large-sized maize grains (size range of 9.5-12.2 x 7.5-9.5mm) over different distances.

Length of column of packed grains (cm)	Mean No. of conidia per insect ml^{-1} (x100)	Percentage of original load of $70x100ml^{-1}$
25	13.6B	19.5B
50	12.0B	17.2B
75	9.6A	13.8A
100	12.0B	17.2 B

Means with the same letter are not significantly different. Confidence limits calculated at 95%.

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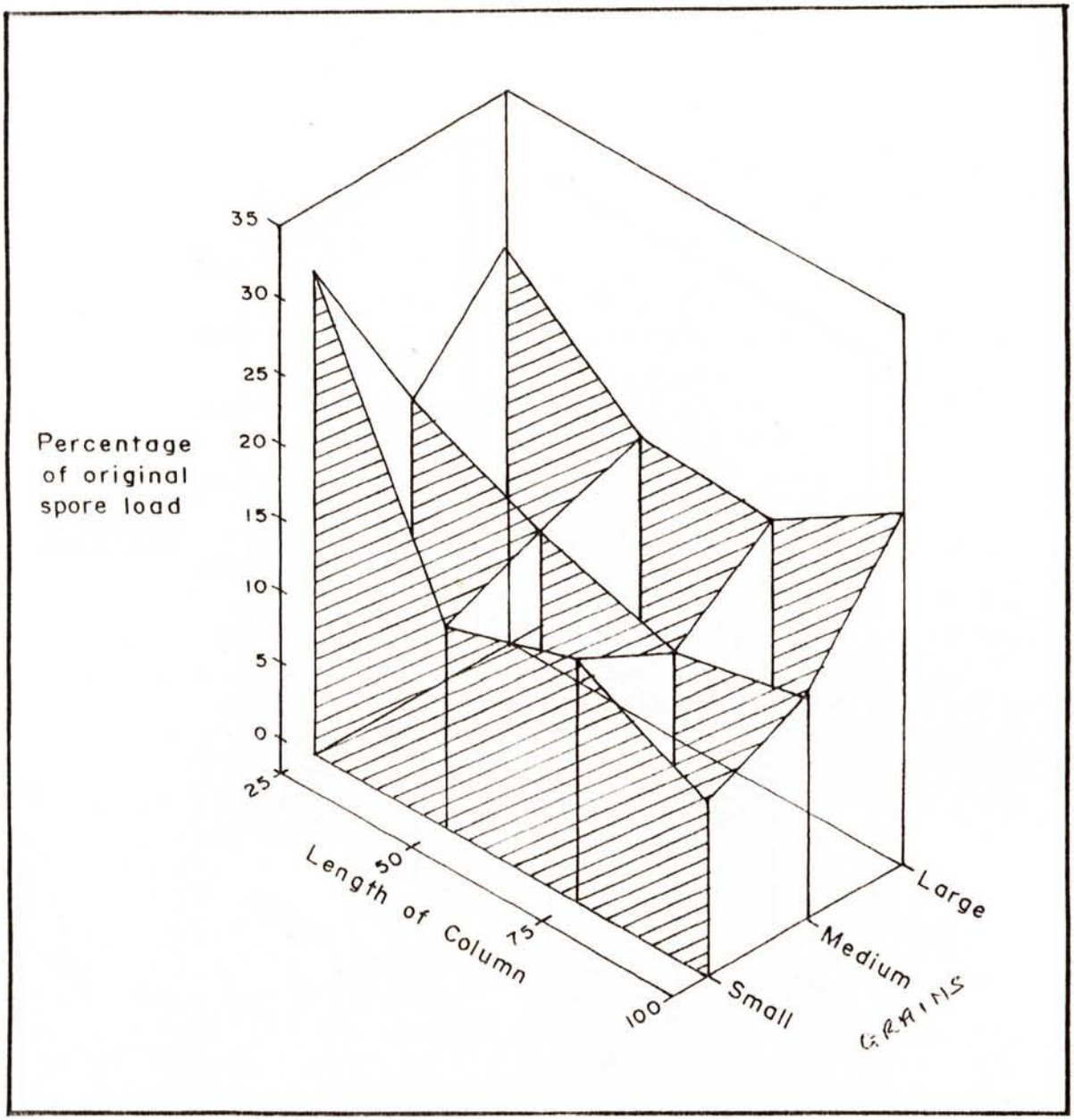

Fig.5 Three-dimensional graph showing percentage of the original load of conidia still adhering to the bodies of S. zeamais after travelling through small-, medium- and large-sized maize grains over a total distance of 100 cm.

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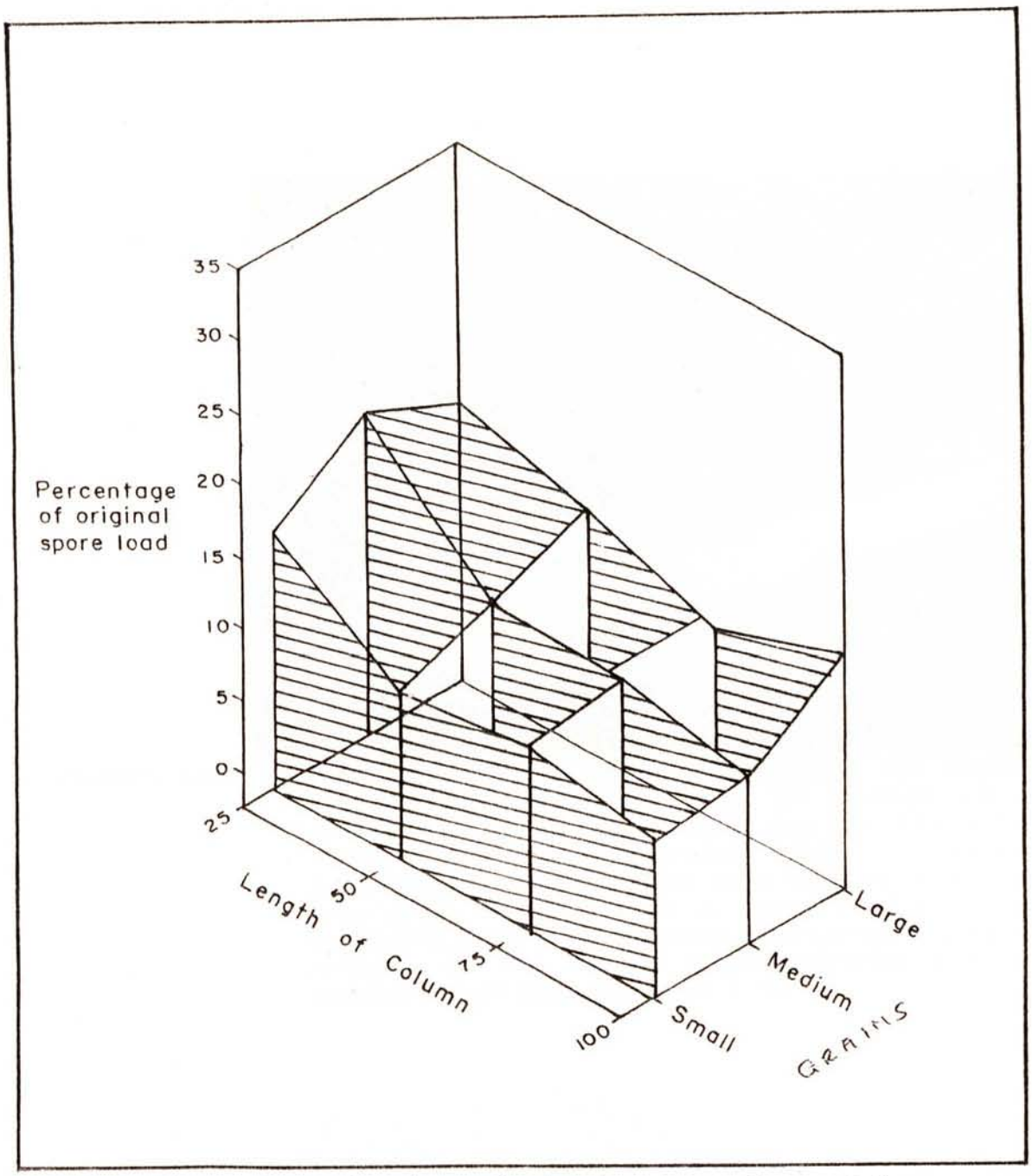

Fig. 6 Three-dimensional graph showing percentage of the original load of conidia still adhering to the bodies of T. castaneum after travelling through small, medium and large-sized maize grains over a total distance of 100 cm.

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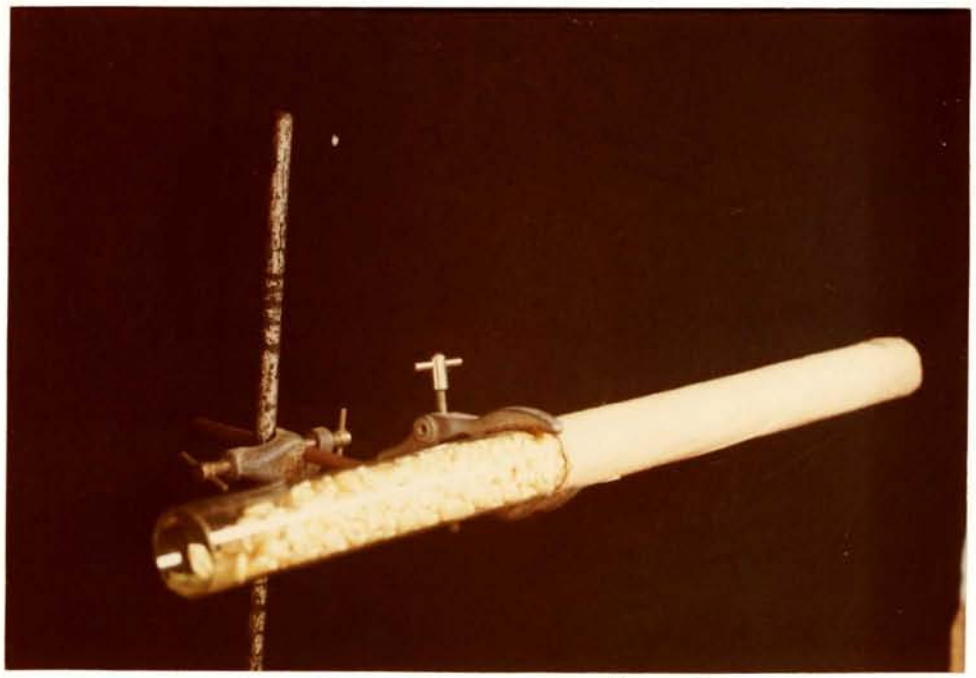

PLATES 12: Photograph of apparatus designed to measure loss of conidia adhering to bodies of *S. zeamais* and *T. castaneum* moving through a column of maize grains (M). Insects introduced through the opening at the left (arrowed) crawled over a culture disc at the entrance and were induced to travel along the column as the brown paper shield (S) was gradually pulled back (x 1.5).

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I. BACTERIAL FLORA OF DEAD BODIES OF THE THREE INSECT SPECIES

The results of this investigation are presented in Tables 42 to 44 and Fig 7. Insects which had been bred for this experiment as shown in plates 13 a and b and then killed with ether had even at the time of killing, a bacterial flora which was not affected by the ether. Plate 14 shows 48 hours-old bacterial colonies on Nutrient Agar inoculated with different dilutions of *Tribolium castaneum* body washings prepared immediately after etherizing the insects. The initial bacterial load varied with the insect species. *Sitophilus zeamais* had the highest colony forming units of 281×10^4 per ml of suspension (Table 42) and *C. maculatus* had the least of only 27×10^4 per ml of suspension (Table 42).

As can be clearly observed in the graphs of Fig 7 the population fluctuation on the three insect species then followed different patterns. The population on *T. castaneum* fell sharply within 24 hours to 12.8×10^4 per ml and disappeared totally by the 4th day (Table 44).

There were, however, bacteria on the bodies of the other two species on the 6th day. There was a low population of 5.0×10^4 per ml of suspension on *S. zeamais* on the 6th day (Table 43) while the *C. maculatus* insects had a population of 37.8×10^4 per ml of suspension on that day (Table 42). The bacterial population on *C. maculatus* showed a significant increase to a peak of 201.3×10^4 per ml of suspension on the second day after death before declining gradually to 37.8×10^4 per ml of suspension on the 6th day. More varied bacterial forms occurred on the bodies of *S. zeamais* (Table

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43) than on those of the other two species. Generally, the majority of the bacterial species were bacilli and cocci, and they were a mixture of Gram-positive and Gram-negative as shown in the tables of results. There were more Gram-positive than Gram-negative colonies.

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TABLE

Viable count of bacterial flora on dead bodies of

Time after insects were etherized (Days)	Mean No. of colony forming units per ml of suspension ($\times 10^4$)	Morphology of cells of 10 randomly selected different colony types (species)			
		Bacilli	Cocci	Vibro	Fila to
0	27.5	10	0	0	C
1	108.8	3	7	0	C
2	201.3	7	3	0	C
3	141.8	3	7	0	C
4	76.3	6	4	0	C
5	72.3	5	5	0	C
6	37.8	6	4	0	C

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ABLE 42

of *Callosobrochus maculatus* kept at 30°C for 6 days

f 10 erent es)	Reaction of cells of the 10 colonies to Gram staining					
	Reaction of bacilli			Reaction of cocci		
Filamen-tous	No.of Colonies	No. of Colonies		No. of Colonies	No. of Colonies	
		+ve	-ve		+ve	-ve
0	10	8	2	0	0	0
0	4	2	2	6	4	2
0	7	6	1	3	2	1
0	3	2	1	7	4	3
0	6	6	0	4	1	3
0	5	3	2	5	2	3
0	6	4	2	4	2	2

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TABL

Viable count of bacterial flora on dead bodies of

Time after insects were etherized (Days)	Mean No. of colony forming units per ml of suspension (x 10^4)	Morphology of cells of 1 randomly selected differe colony types (species)			
		Bacilli	Cocci	Vibro	Fil tc
0	281.0	3	5	2	(
1	4.3	5	5	0	(
2	12.0	4	6	0	(
3	13.3	6	4	0	(
4	33.0	3	5	0	2
5	25.0	3	4	3	0
6	5.0	8	2	0	0

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ABLE 43
of *Sitophilus zeamais* kept at 30°C for 6 days

f 10 erent es)	Reaction of cells of the 10 colonies to Gram staining					
	Reaction of bacilli			Reaction of cocci		
Filamen-tous	No.of Colonies	No. of Colonies		No. of Colonies	No. of Colonies	
		+ve	-ve		+ve	-ve
0	2	0	2	6	4	2
0	5	5	0	5	5	0
0	4	3	1	6	4	2
0	6	5	1	4	3	1
2	3	3	0	5	3	2
0	3	3	0	4	0	4
0	8	4	4	2	0	2

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TABLE

Viable count of bacterial flora on dead bodies of

Time after insects were etherized (Days)	Mean No. of colony forming units per ml of suspension ($\times 10^4$)	Morphology of cells of 10 randomly selected differen colony types (species)			
		Bacilli	Cocci	Vibro	Fila to
0	104.5	8	2	0	0
1	12.8	10	0	0	0
2	2.5	5	5	0	0
3	4.3	0	10	0	0
4	0.0	-	-	-	-
5	0.0	-	-	-	-
6	0.0	-	-	-	-

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TABLE 44

es of *Tribolium castaneum* kept at 30°C for 6 days

of 10 fferent ies)	Reaction of cells of the 10 colonies to Gram staining					
	Reaction of bacilli			Reaction of cocci		
Filamentous	No.of Colonies	No. of Colonies		No. of Colonies	No. of Colonies	
		+ve	-ve		+ve	-ve
0	8	6	2	2	2	0
0	10	3	7	0	0	0
0	0	0	0	10	10	0
0	10	10	0	0	0	0
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-

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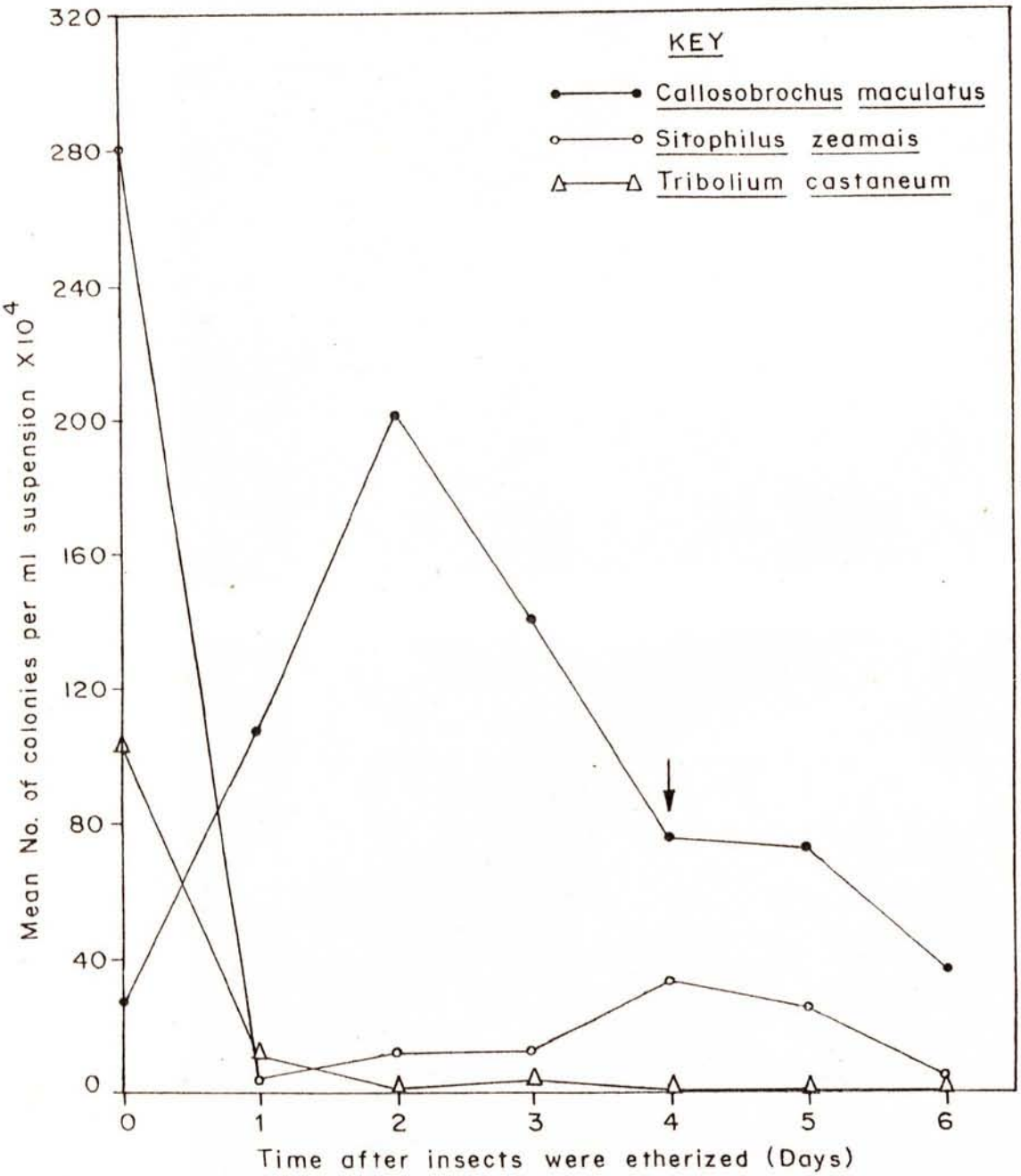

Fig.7 Number of bacterial colonies on Nutrient Agar plates inoculated with washings of dead bodies of three Coleopteran species and incubated at 37°C for 48 hours.

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PLATES 13: Photograph of insect-breeding chambers (x 1/3
- a: closed chambers
- b: open chambers showing *S. zeamais* (left) and *T. castaneum* (right)

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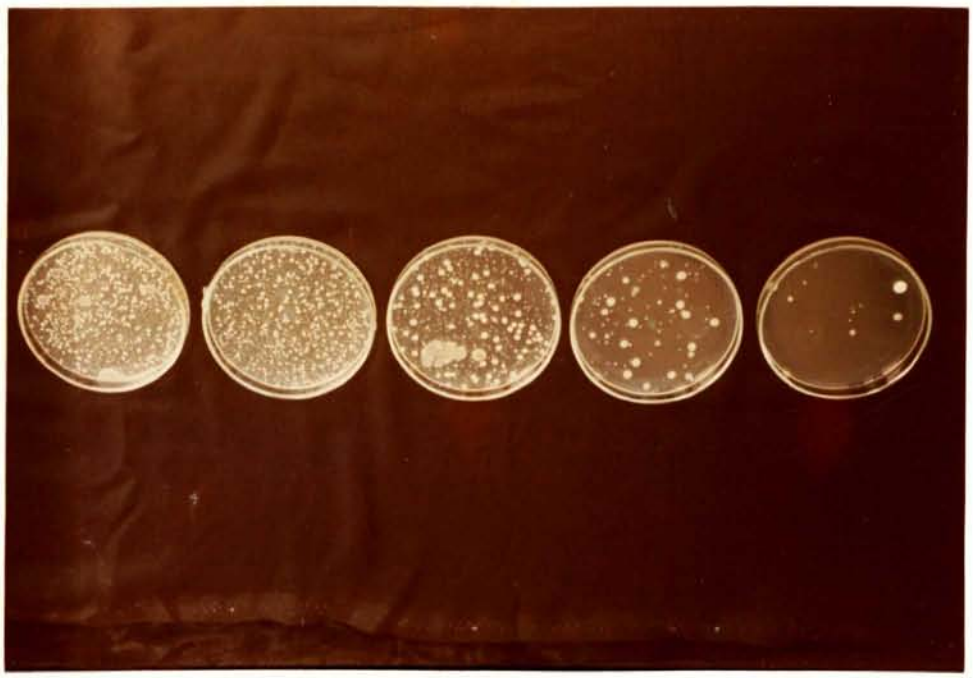

PLATE 14: Photograph showing 48 hour-old bacterial colonies on Nutrient Agar inoculated with different dilutions of *T. castauem* body washings prepared immediately after etherizing the insects.
FROM LEFT: standard solution and 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions (x 1/4)

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J. FUNGUS FLORA OF DEAD BODIES OF THE THREE INSECT SPECIES

Despite the bacterial growth on the etherized insect bodies, fungi could also colonize the dead bodies. The results in Table 45 showed that all the 20 bodies of either *C. maculatus* or *S. zeamais* randomly selected on the 6th day were colonized. There was a lower level of invasion of *T. castaneum* bodies (70 per cent).

T. castaneum bodies had the smallest number of fungal species of two, *S. zeamais* had three fungal species and five fungal species were isolated from bodies of *C. maculatus*. *Aspergillus flavus* was by far the predominant species present being 90, 70 and 65 per cent on the bodies of *S. zeamais*, *C. maculatus* and *T. castaneum*, respectively.

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TABLE 45

Fungi isolated from dead bodies of the three Coleopteran insect species, six days after they had been killed with ether and kept at 30°C.

Insect species	Percentage of Bodies showing fungal growth (out of total of 20)	Fungal species and Percentage of bodies contaminated in parenthesis
Callosobrochus maculatus	100	*Aspergillus flavus* (70)
		Aspergillus niger (10)
		Mucor sp. (10)
		Rhizopus sp. (5)
		Trichoderma viride (5)
Sitophilus zeamais	100	*Aspergillus flavus* (90)
		Aspergillus niger (5)
		Curvularia sp. (5)
Tribolium castaneum	70	*Aspergillus flavus* (65)
		Cladosporium sp. (5)

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V. DISCUSSION

The role of pests of stored grains and seeds in the persistence and spread of contaminant fungi has been the major subject of the investigation reported in this thesis. The dispersal of fungi has been of interest and subject of many investigators from many different points of view. The insects as a group are prominent agents of dispersal.

One aspect of general biological interest is the cultivation of fungi by ants, beetles and termites. Approximately 100 species of tropical and sub-tropical Myrmicine ants have the remarkable habit of cultivating fungi on which they feed. These ants cut out bits of leaves and carry them into their underground nests which are used in building spongy masses that serve as a fungal culture medium. The medium is then inoculated with spores and mycelium of a particular species with which the ant associates. The inoculum is usually carried by the queen in her infrabuccal pouch (Wolf and Wolf 1947). Specific fungi are therefore found in the gardens of particular ants. For example, *Cladosporium myrmecophilum* is cultivated by *Lasius fuliginosus*, *Hormiscium pithyophilum* is cultivated by *Lasius umbratus* and *Lentinus alticolus* is cultivated by *Atta cephalotes* (Weber 1938).

Similarly termites raise fungi in their termitaria essentially for feeding the nymphs. Among the species of these fungal gardens are *Agaricus termitigina*, *Pluteus termitus*, *Tricholoma Subgambosum* and *Xylaria nigripes* (Wolf and Wolf, 1947). Members of the genus *Termitomyces* are of particular interest. They proceed to produce basidiocarps when the termitarium is abandoned by the termites, and they are freed from

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grazing. The fruiting bodies are long-stiped large edible basidiocarps which emerge above the surface of the mound. Some of the cherished species in the tropical regions are *Termitomyces fuliginosus* and *Termitomyces le Testui*. In the Western Region in Ghana, farmers encourage termites to extend their fungus gardens and plant more *Termitomyces Le Testui* by piling leaves of oil palm (*Elaeis guineensis* Jacq.) in the immediate vicinity of termitaria and, thereby, place abundant vegetable matter at the door of the termites for their use (Clerk, G.C., personal communication).

Another story of dispersal of fungi which are food for the insects is the associations between the stinkhorns (Phallus species) and flies and between rusts and members of Hymenomycetes and mites. The stinkhorns produce a gelatinized glebal mass which is attractive to carrion flies because of its putrid odour. The basidiospores embedded in the glebal mass are ingested with the slime and are later voided after passing unharmed through the alimentary canal.

Oidia of members of the *Hymenomycetes* and pycniospores of rusts are embedded in a sugary mucilage. Mites attracted by the mucilage carry oidia and pycniospores which have adhered to their bodies to the genetically complementary monokaryotic hyphae of the Hymenomycetes and the receptive hyphae of the rusts, respectively, to bring about diploidization which initiates the formation of the dikaryotic secondary phase of these fungi (Alexopoulos and Mims, 1979; Webster, 1970).

Many other fungi are dispersed by insects not because part of the thallus is food for the insects, but because of the

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association between the fungi and food of the insects. Palm wine, the common tropical drink made of the fluid from the phloem of oil palm (*Elaeis guineensis*) and raffia palm (*Raphia* sp.) is inoculated naturally with yeast introduced by bees and fruitflies seeking the sugary palm wine. The insects had picked up the yeast cells from a previous feeding source.

Crowe (1963) reported that the uredospores of the rust *Puccinia graminis* parasitizing maize and rice are transported to remote plantations by hymenopherous parasites (*Leptacis* sp. and *Synopeas* sp.) which burrow into the uredori in search of mycophagous cecidiomyid larvae.

This association is similar to that between some pollinating insects and some smut fungi. The dispersal of the chlamydospores of the smut is accomplished quite fortuitously. The insects visit flowers in search of nectar and pollen. Because the chlamydospores of smut are formed in the ovaries of the flowers, chlamydospores from ruptured ovaries are placed directly in the path of the foraging insects to be carried away on the insect bodies. The smut fungus *ustilago violacea* of campions (*Melandrium* sp.) is dispersed in this way (Ingold, 1965).

Naturally if tissues of plants which are food source to insects are diseased, the pests would ingest both the plant and the fungus it is harbouring. If the fungus is unharmed in the insect gut, viable and effective, inoculum will be voided and deposited on another host plant. *Septoria lycopersici*, the cause of leaf spot of tomato, is among the many fungi dispersed this way by insects.

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Finally, an insect which happens to be the host of a fungus parasite will have the chance of dispersing the fungus before it succumbs. Among the better-known species of the parasitic *Entomophthoras* may be mentioned *Entomophthora muscae* on houseflies, *Entomophthora grylii* on crickets and *Entomophthora sphaerosperma* on caterpillars of cabbage butterflies. In each of these cases, the diseased insect dies far away from the source of infection.

What occurs in the 'Seventeen-year locusts', *Tibicina septendecem*, when it is infected by *Massospora cicadina* is more spectacular and remarkable than events in hosts of *Entomophthora* species. The fungus grows within the insect's body and causes the posterior segments to drop off while the insect is still alive. The conidia are then dispersed as the insect crawls or flies (Speare, 1921).

The fungal contaminants of the maize and rice grains and of bambara groundnut and cowpea seeds studied during this work were so many that the three coleopteran insect pests, *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* among the stored products might find suitable associate fungi for any form of dispersal they are capable of. The four products were all infected while on sale at five different markets at Kaneshie, La, Madina, Makola and Mallam Atta in the Accra district.

Thirty-one species were isolated from the four products representing 15 genera - *Absidia*, *Aspergillus*, *Cladosporium*, *Drechslera*, *Epicoccum*, *Fusarium*, *Mucor*, *Neocosmospora*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Pullularia*, *Rhizopus* and *Verticillium*. All except three were represented by

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one species only. Of the three exceptions, there were two *Paecilomyces* species, *P. carneus* and *P. puntonii*, six fully identified *Penicillium* species, *P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. funiculosum*, *P. italicum* and *P. oxalicum* and one species which proved difficult to be fully identified, and as many as ten *Aspergillus species*, *A. clavatus*, *A. flavus*, *A. flavus-oryzae*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. oryzae*, *A. sulphureus*, *A. ustus* and *A. wentii* (see Tables 4-7).

Based on their number (see Fig.2) the *Aspergillus* species would, first, attract the greatest attention as they would together produce abundant dispersal units and abundant inoculum. Secondly, they would cause the greatest deterioration of the grains and seeds, and thirdly, the type of species which were identified warned of the danger of mycotoxicosis if large quantities of the products were consumed.

The mycotoxin producers among the contaminant *Aspergillus* species were *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus* and *A. ustus*. *A. clavatus* produces Ascladiol and cytochalasin E which affects cell division resulting in the formation of multinucleate or anucleate cells. The most widely studied mycotoxins are Aflatoxins produced by *A. flavus*. The most potent among them is *Aflatoxim* B_1 which is hepato-carcinogenic. *A. niger*, *A. ochraceus* and *A. ustus* produce Malformin A^1, Ochratoxin A and Austdiol respectively. Malformin A, is toxic to Mammals, Ochratoxin A causes nephrotrophy and Austdiol is a gastro-intestinal toxin (Moss, 1977). *A. fumigatus* produces fumigatin and fumigacin both of which have been found to be toxic to experimental animals (Thom and Raper, 1945).

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A. flavus is most important as it constituted 17.6 percent of the entire population, in comparison *A. niger* constituted 7.9 percent and *A. fumigatus* constituted a mere 2.0 percent. Maize grains were most susceptible to *A. flavus* attack and rice grains were the least susceptible. The percentage frequency of *A. flavus* on maize grains, rice grains, bambara groundnut seed and cowpea seeds were 27.2, 9.2, 19.6 and 15.0 percent, respectively.

It is, however, reassuring that *A. flavus* had not been consistently the dominant contaminant species of grains and seeds according to reports of earlier workers such as, Addison (1971), Danquah (1973) and Odamtten (1986). Danquah and Odamtten isolated 11 and 21 contaminants, respectively, from maize. Odamtten did not identify any particular genus, including *Aspergillus*, as dominating the mycoflora. Also Addison (1971) and Danquah (1973) listed 22 and 29 contaminant fungi from rice grains with *Curvularia* emerging as the dominant genus. Be as it may, the importance of the *Aspergillus* species should not be ignored as their presence in these reports was noteworthy if not overly spectacular.

Furthermore, the quality and quantity of the contaminants might differ depending on the crop variety, region of cultivation, extent of drying of the products, length of storage period, the region of storage with its peculiar airspora and climatic conditions, and, the nature of chemical changes brought about by the first colonizers. Each product in storage, therefore, needs to be studied to determine its level of wholesomeness.

Notwithstanding, specific prodcuts would be associated with

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a certain physiological group of fungi. Thus, oil seeds such as groundnut and castor oil seed would be attacked generally by fungi with the capacity to produce adequate levels of lipases. Seeds with high levels of protein particularly legume seeds would be prone to attack by fungi able to synthesize proteolytic enzymes while fungi able to produce copious amounts of amylases are more associated with the grains (Cochrane, 1958; Hawker, 1950). But within each group differences may exist in the quality and quantity of the constituent compounds. That might explain the richer growth of fungi in cowpea seeds than bambara groundnut seeds (see Tables 4 and 5). The higher contamination of maize than rice grains in this work (see Tables 6 and 7) could be explained. The proteinaceous aleurone layer of the maize grains was intact making them a richer substrate than the polished rice grains which had lost the aleurone layer in the process. Both Addison (1971) and Danquah (1973) studied contamination of unpolished rice grains in their husks. The comparatively higher values of 22 and 29 species, respectively, they recorded were, therefore, in accord with the expected higher nutrient levels of unpolished grains.

The most obvious and first symptoms of fungal contamination are the discoloration and wrinkling of the seeds and grains. It is when the moisture content of the product rises beyond a certain threshold that a visible fungal growth on the surface becomes obvious. Insect pests would, therefore, primarily disperse contaminant fungi internally after they have consumed infected grains and seeds.

The results in Tables 27 to 29 showed that the lists of

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fungi of the mycoflora of the guts of *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* are fairly long. The respective list contained 14, 13 and 16 fungal species. The mycoflora composition reflected the quality of the flora of the grains and seeds, showing a domination of *Aspergillus* and *Penicillium* species.

The members of the gut mycoflora would apparently remain viable for reasonable lengths of time, thereby allowing the pests to release viable inoculum of an ingested fungus over a number of days. The data in Tables 30 to 32 indeed, demonstrated that after a single feeding, viable *A. flavus* was present in the gut of *C. maculatus*, *S. zeamais* and *T. castaneum* for 6,8 and 5 days, respectively. The corresponding days for *A. fumigatus* were 4,6 and 5 days, respectively, and 6,3 and 3 days, respectively, for *A. ochraceus* (see Fig.4).

But feeding is a continuous event, and it is, therefore, reasonable to suggest that throughout the lives of these insect pests, they would carry viable fungi in their guts and disperse them with their faecal pellets. Later investigations should find out whether the mycoflora of the gut has any effect on the life span and activities, both physiological and reproductive, of the insects.

The acquision of viable fungi during feeding is quite a common phenomenon. The results of the present studies have shown that there was an indiscriminate acquision of the fungi by *C. maculatus*, *S. zeamais* and *T. castaneum*. The idea that some animals are selective is very interesting, and the external mycoflora and the gut mycoflora could be quite different

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qualitatively. Pitts and Cowley (1973) observed that the percentage frequency of the yeast, *Rhodotorula mucilaginosa*, in the surface slime and in the burrow was 65.0 and 20.0 percent respectively while a higher percentage frequency of 94.0 occurred in the midgut of the fiddler crabs *Uca pugilator*. With the exception of the yeast *Rhodotorula mucilaginosa*, there appeared to be no evidence of selection of specific fungi in the feeding of the fiddler crab.

When the moisture content of the grains and seeds rise, surface fungal growth would appear and internal dispersal would be accompanied by external dispersal by the insect pests. This could be important if abundant spores are produced by the fungi. In an experiment using only *Sitophilus zeamais* and *Tribolium castaneum* which are not active fliers, a lot of the conidia adhering to the bodies of these insects was easily removed as the insects brushed against the maize grains among which they were travelling. The insects were induced to migrate over a distance of 100cm. Over a distance of only 25cm, 67.2 percent of the conidia attached to the body of *S. zeamais* travelling through small-sized grains was removed; 79.4 percent of the conidia on *S. zeamais* travelling through medium-sized grains was removed, and in tests using large-sized grains 72.5 percent of the conidia was removed. Over a distance of 100cm the percentages of conidia removed were 87.8, 84.7 and 76.7, respectively (see Tables 36 to 38). A similar trend was observed with experiments with *T. castaneum* (see Tables 39 to 41). There was, thus, a direct relationship between the size of the pores among the grains, and, therefore, the frequency of collision of the insects with the

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grains.

Whether the spores were dispersed internally or externally the faecal pellets could become a substrate for the germination of spores and growth of mycelia. Germination in condensed water droplets on the insect bodies would, however, concern conidia and hyphal frangments carried on the insect body only. The body leachates, equivalent to condensed water droplets on the bodies, of *C. maculatus, S. zeamais* and *T. castaneum* had different effects on the conidia (see Tables 8-11). Considering the undiluted leachates only, leachate of *C. maculatus* supported germination of conidia of *A. clavatus* (50.2 percent) *A. flavus* (70.4 percent). *A niger* (44.0 percent), *A. ochraceus* (49.6 percent), and *A. sulphureus* (31.3 percent). The leachate of *S. zeamais* supported germination of conidia of *A. flavus* (76.2 percent), *A fumigatus* (45.2 percent), *A. ochraceus* (66.3 percent) and *A. ustus* (53.8 percent) (see Table 9) and the leachate of *T. castaneum* supported germination of conidia of *A. clavatus* (39.2 percent), *A. flavus* (52.2 percent) *A. ochraceus* (69.5 percent) and *A. ustus* (46.1 percent) (see Table 10). It is worrying that *A. flavus* and *A. ochraceus* which produce such potent mycotoxins could germinate so well in all the leachates, while the conidia of the rest were induced to germinate by adding extracts of the axes of the embryo, cotyledons and testa of the seeds of bambara groundnut and cowpea and extracts of maize and rice grains to the leachates (see Tables 12 to 19). That is likely to occur in nature when condensed water droplets on the insect body mixes with washings of surfaces of wounds on the products.

Because conidia of *Aspergillus* species did not germinate in

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water, it was difficult to ascertain whether inability to germinate in the leachates was due to lack of nutrients or presence of inhibitory factors. Inhibitory factors could be overcome by reasonable high concentrations of nutrients and the extracts of the tissues of the grains and seeds could have played that role. It is a common practice to offset the inhibitory effects of soil fungistasis by adding nutrients (Clerk, 1969).

If the atmospheric humidity of the storage rooms were kept low, it would play many roles. It will restrict the activity of the fungi in the grains, it would prevent surface mycelial growth, condensed water droplets would not form and the faecal pellets would remain dry. Avoiding the formation of condensed water droplets would remove the danger of conidial germination of some species and the growth of hyphal fragments of all the species used in the test. For, the leachates of *C. maculatus*, *S. zeamais* and *T. castaneum* individually supported growth of the seven *Aspergillus* species (see Tables 20 to 26).

Dry faecal pellets are unsuitable substratum for conidial germination and hyphal growth. However, once they are moistened some of the seven *Aspergillus* species would find a congenial medium for conidial germination. Conidia of all the seven *Aspergillus* species germinated in aqueous solution of faecal pellets of *Callosobrochus maculatus*, even if the percentage germination was low in each case, ranging from 6.0 to 10.0 percent (see Table 33). Conidia of only three species, *A. clavatus* (21.5 percent), *A. flavus* (13.7 percent) and *A. ochraceus* (5.4 percent) could germinate in solution of faecal pellets of *Tribolium castaneum*, (see Table 35) and again conidia

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of only three species namely *A. clavatus, A. ochraceus* and *A. sulphureus* germinated feebly (0.7-1.0 percent) in the solution of faecal pellets of *A. sulphureus*. The pellets were externally tiny and would easily pass through jute sacks. It is likely that because of the low germination level they supported, their contribution to the persistence and dispersal of the seven *Aspergillus* species in stored grains and seeds would be insignificant.

In contrast, the bodies of the insects were large and on death would contribute a substantial amount of substrate for fungal growth irrespective of the ambient relative humidity because there exists already fluids in the gut and haemocoel. All dead bodies of *Callosobrochus maculatus* and *Sitophilus zeamais* picked randomly from maize grains kept at median atmospheric humidities were invaded by fungi. *A. flavus, A. niger, mucor sp., Rhizopus sp. and Trichoderma viride* were isolated from the bodies of *C. maculatus*. The presence of *Mucor sp. and Rhizopus* sp. supported the suggestion that there was adequate water content in the bodies of *C. maculatus*, *S. zeamais* was invaded by *A. flavus, A. niger* and *Curvularia sp.* and *T. castaneum* dead bodies had *A. flavus* and *Cladosporium* sp. which were isolated from 70 percent of the bodies plated (see Table 45). Wolf and Wolf (1947) noted that the genera *Alternaria, Aspergillus, Cladosporium* and *Penicillium* appear on insect bodies only after death, and not on the living insects. This, at least, is no more applicable to *Aspergillus* species, and the living and dead insect bodies may both support *Aspergillus* growth and serve as sources of inoculum. A wide range of insects have been shown

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in the last three decades to be susceptible to infections by *Aspergillus* species. Locusts are prey to these species: for example, *Schistocerca gregaria* in Turkey (Madelin, 1966) and Pakistan (Abas, Hasan, Haq and Hashir, 1959) to *A. flavus*, and *Locustana pardalina* in South Africa to *A. parasiticus* (Prinsloo, 1960). New hosts found to be susceptible to *A. flavus* in the laboratory include the bedbug, *Cimex lectularius* (Cockbain and Hastie, 1961), and the termite, *Reticulitermes virginicus* (Beal and Kais, 1962), while insect parasitism has been demonstrated for the first time in *Aspergillus ustus* which was found attacking larvae of *Heliozela staneela* (Prota, 1962). It is possible that *A. flavus*, which is such a nuisance in stored grains and seeds may do a good turn by attacking the pests.

Furthermore, some *Aspergillus* species have been shown by Kodaira (1961) to produce toxins active against Silkworms. *Aspergillus flavus*, *A. japonicus*, *A. ochraceus* and *A. oryzae* on various culture media produced materials which were toxic when ingested by silkworms. The toxins formed by *A. ochraceus* were two dipeptides,-L-prolyl-L-leucine anhydride and L-prolyl-L-valine anhydride. It remains to be found out whether the two common species *A. flavus* and *A. ochraceus* will find the grains and seeds suitable substrates for the production of these toxic materials for a welcome biological control.

It was noteworthy that the large bacterial populatins on the dead insect bodies did not inhibit the growth of the fungal species. However, the lists for each was short, and the low population could be due to antibiosis against some fungal species by the bacteria or to the absence of essential compounds

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required by some fungal species.

The hypothesis of presence of antibiosis is reasonable, because of the levels of the bacterial populations which were initially high on the bodies of *S. zeamais* (281.0×10^4 per ml of suspension) (see Table 43) and *T. castaneum* (104.5×10^4 per ml of suspension) (see Table 44 and Plate 14). Even though the level was quite low initially on *C. maculatus* bodies it increased to 108.8 and 201.3×10^4 per ml of suspension, respectively, on the second and third days, respectively (see Table 42). The bacteria included many Gram - positive bacilli which are noted for the production of antibiotics. As decomposition progressed the bacterial population naturally declined in the face of diminishing levels of nutrients (see Fig.7).

There are many records of bacterial flora of insects. Cocoons of newly emerged pine sawflies, Diprions sinilis, each contain a resin filled sac-like stomodaen attached to the cast skin of the prepupal moult. Investigations by Phillipsen and Coppel (1975) revealed that the stomodaeal sacs harboured several species of bacteria . Both Gram-positive and Gram-negative forms were observed among the isolates. Representatives of the families Enterobacteraceae, Micro coccaceae, Pseudomonadaceae and Lactobacillaceae were recovered. Numbers of Pseudomonadaceae and Enterobactereaceae were encountered most frequently. The present studies emphasised that fungal flora and the bacterial isolates were not identified by name. This could be a major subject of future investigations. Hardly are all insect pests removed from products used in food preparation. There could be serious consequences if members of the Enterobacteraceae are common and

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are consumed along with the products, unless they are destroyed by cooking during the preparation of the food.

It is clear that the three insect pests, *C.maculatus*, *S. zeamais* and *T.castaneum* would contribute to the persistence and dispersal of contaminant fungi of stored grains and seeds in different sorts of ways. By controlling the insect pests, dispersal of the fungi would be considerably curtailed. Small heaps of stored grains and seeds could also be exposed to continuous light so that the pests, which are negatively phototactic, would drift to the bottom and would be confined there and discouraged from migration. Lastly, the products could be sieved occasionally with sieves with appropriate pore size to get rid of the dead bodies of pests.

The present observations were based on xenospores which are readily dispersed because they are minute and usually germinate readiy in suitable conditions. The menospores were not considered. They are large, often very durable and thick-walled. Above all they need a resting period or only germinate after applying some specific stimulus, shock or nutrient or after removing an inhibitor (Gregory, 1966). If menospores are formed in sufficient quantities the full picture of fungal persistence and dispersal in stored grains and seeds could only emerge when investigations are extended to them also.

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SUMMARY

1(a) Bambara groundnut seeds from the Kaneshie, La, Madina, Makola and Mallam Atta markets contained 9,5,,9,8 and 9 fungal species, respectively.

(b) The fungal species isolated from the bambara groundnut seeds from the Kaneshie, La, Madina, Makola and Mallam Atta markets belonged to 4,4,5,5 and 6 genera respectively.

(c) The predominant genera were *Aspergillus* and *Penicillium* which occurred in products from each market.

(d) *Aspergillus niger* was present in all the five seed samples followed by *Aspergillus flavus*, *Paecilomyces puntonii*, and *Penicillium chrysogenum* isolated from four of the five samples.

(e) The total number of colonies which appeared on five agar plates inoculated with material from Kaneshie, La, Madina, Mokola and Mallam Atta was 14,17,37,72 and 32, respectively.

2(a) Cowpea seeds from the Kaneshie, La, Madina, Makola and Mallam Atta Markets contained 11,7,9,14 and 9 fungal species, respectively.

(b) The fungal species isolated from the cowpea seeds from the Kaneshie, La, Madina, Makola and Mallam Atta markets belonged to 7,5,6,9, and 7 genera, respectively.

(c) The predominant genera were *Aspergillus* and *Paecilomyces* which occurred in products from all the five markets.

(d) *Aspergillus flavus*, *Aspergillus niger* and *Paecilomyces puntonii* were present in all the five seed samples, followed

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by *Penicillium expansum* isolated from four of the five samples.

(e) The total number of colonies which appeared on five agar plates inoculated with material from Kaneshie, La, Madina, Makola and Mallam Atta markets was 95, 102,126,53 and 103, respectively.

3(a) Maize grains from the Kaneshie, La, Madina, Makola and Mallam Atta markets contained 6,7,12,9 and 8 fungal species, respectively.

(b) The fungal species isolated from the maize grains from the Kaneshie, La, Madina, Makola and Mallam Atta markets belonged to 3,5,6,5 and 3 genera, respectively.

(c) The predominant genera were *Aspergillus* and *Penicillium* which occurred in products from all the five markets.

(d) *Aspergillus flavus* was present in all the five grain samples followed by *Fusarium moniliforme* and *Penicillium expansum* isolated from four of the five samples.

(e) The total number of colonies which appeared on five agar plates inoculated with material from Kaneshie, La, Madina, Makola and Mallam Atta markets was 378,1027,323,657 and 1348, respectively.

4(a) Rice grains from the Kaneshie, La, Madina, Makola and Mallam Atta markets contained 5,6,5,6 and 7 fungal species, respectively.

(b) The fungal species isolated from rice grains from Kaneshie, La, Madina, Makola and Mallam Atta markets belonged to

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3,4,3,3, and 4 genera, respectively.

(c) The predominant genera were *Aspergillus*, *Cladosporium* and *Penicillium* which occurred in products from all the five markets.

(d) *Cladosporium herbarum* and *Penicillium expansum* were present in all the five samples, followed by *Aspergillus flavus* and *Penicillium chrysogenum* isolated from three of the five samples.

(e) The total number of colonies which appeared on five agar plates inoculated with material from Kaneshie, La, Madina, Makola and Mallam Atta markets was 7,22,8,9 and 30/ respectively.

5 The highest concentration of insect body leachate (100 bodies in 20ml distilled water) of three insect pests, *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* supported the following germination of conidia of seven Aspergillus species after 24 hours.

(a) Percentage germination of 50.2, 70.4, 0.0, 44.0,49.6, 31.3 and 0.0 percent of conidia of *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and *A. ustus*, respectively, in *C. maculatus* body leachate.

(b) Percentage germination of 0.0, 76.2, 45.2, 0.0, 66.3, 0.0 and 53.8 percent of conidia of *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus and A. ustus*, respectively, in *S. zeamais*, body leachate.

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(c) Percentage germination of 39.2, 52.2, 0.0, 0.0, 69.5, 0.0 and 46.1 percent of conidia of *A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. sulphureus* and A. ustus, respectively, in *T. castaneum* body leachate.

6. Extracts of the seeds of bambara groundnut and cowpea and grains of maize and rice were able to induce germination of conidia of *Aspergillus* species which failed to germinate in the insect body leachates.

7. After 12 hours' incubation, *Callosobrochus maculatus* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of *Aspergillus fumigatus*

(a) axis of bambara groundnut seed:	39.3 percent
(b) cotyledon of bambara groundnut seed:	81.5 percent
(c) testa of bambara groundnut seed:	25.0 percent
(d) axis of cowpea seed:	51.7 percent
(e) cotyledon of cowpea seed:	84.9 percent
(f) testa of cowpea seed:	13.3 percent
(g) maize grain:	37.8 percent
(h) rice grain :	24.9 percent

8. After 12 hours' of incubation, *Callosobrochus maculatus* body leachate with extracts of different tissues indicated below supported the following percentage germination of conidia of *Aspergillus ustus*:

(a) axis of bambara groundnut seed: 41.6 percent

(b) cotyledon of bambara groundnut seed: 83.6 percent

(c) testa of bambara groundnut seed: 36.9 percent

(d) axis of cowpea seed 77.2 percent

(e) cotyledon of cowpea seed 64.8 percent

(f) testa of cowpea seed 6.1 percent

(g) maize grain 69.9 percent

(h) rice grain 35.0 percent

9. After 12 hours' incubation, *Sitophilus zeamais* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of Aspergillus clavatus:

(a) axis of bambara groundnut seed 78.9 percent

(b) cotyledon of bambara groundnuts seed 70.5 percent

(c) testa of bambara groundnut seed 29.6 percent

(d) axis of cowpea seed 82.9 percent

(e) cotyledon of cowpea seed 81.4 percent

(f) testa of cowpea seed 90.6 percent

(g) maize grain 76.5 percent

(h) rice grain 67.8 percent

10. After 12 hours'incubation, *Sitophilus zeamais* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of *Aspergillus niger*:

(a) axis of bambara groundnut seed	15.6 per cent
(b) cotyledon of bambara groundnut seed	44.4 per cent
(c) testa of bambara groundnut seed	19.2 per cent
(d) axis of cowpea seed	22.9 per cent
(e) cotyledon of cowpea seed	57.4 per cent
(f) testa of cowpea seed	67.8 per cent
(g) maize grain	55.3 per cent
(h) rice grain	57.7 per cent

11. After 12 hours' incubation, *Sitophilus zeamais* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of *Aspergillus sulphureus*:

(a) axis of bambara groundnut seed	5.9 per cent
(b) cotyledon of bambara groundnut seed	38.6 per cent
(c) testa of bambara groundnut seed	7.1 per cent
(d) axis of cowpea seed	20.0 per cent
(e) cotyledon of cowpea seed	30.2 per cent
(f) testa of cowpea seed	32.1 per cent
(g) Maize grain	17.6 per cent
(h) Rice grain	16.6 per cent

12. After 12 hours' incubation, *Tribolium castaneum* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of *Aspergillus fumigatus*:

(a) axis of bambara groundnut seed 34.6 per cent

(b) cotyledon of bambara groundnut seed 14.4 per cent

(c) testa of bambara groundnut seed 5.2 per cent

(d) axis of cowpea seed 33.6 per cent

(e) cotyledon of cowpea seed 13.7 per cent

(f) testa of cowpea seed 7.1 per cent

(g) maize grain 18.1 per cent

(h) rice grain 10.5 per cent

13. After 12 hours' incubation, *Tribolium castaneum* body leachate with extracts of the different tissues indicated below supported the following percentage germintion of conidia of *Aspergillus niger*:

(a) axis of bambara groundnut seed 64.4 per cent

(b) cotyledon of bambara groundnut seed 26.6 per cent

(c) testa of bambara groundnut seed 7.5 per cent

(d) axis of cowpea seed 66.9 per cent

(e) cotyledon of cowpea seed 46.9 per cent

(f) testa of cowpea seed 32.0 per cent

(g) maize grain 40.6 per cent

(h) rice grain 12.9 per cent

14. After 12 hours' incubation *Tribolium castaneum* body leachate with extracts of the different tissues indicated below supported the following percentage germination of conidia of *Aspergillus sulphureus*:

(a) axis of bambara groundnut seed 9.4 per cent

(b) cotyledon of bambara groundnut seed	12.1 per cent
(c) testa of bambara groundnut seed	8.2 per cent
(d) axis of cowpea seed	22.8 per cent
(e) cotyledon of cowpea seed	25.1 per cent
(f) testa of cowpea seed	7.6 per cent
(g) maize grain	34.5 per cent
(h) rice grain	5.6 per cent

15. Growth rates of each of the *Aspergillus* species on insect body leachate-agar prepared with leachate of the three insects were quite close.

(a) Mean culture diameters of *Aspergillus clavatus* by the 10th day were 53.8, 64.2 and 68.0mm, with the best growth on the *Callosobrochus maculatus* leachate agar.

(b) Mean culture diameters of *Aspergillus flavus* by the 10th day were 66.5, 68.8 and 76.5mm, with the best growth on the *Sitophilus zeamais* leachate agar.

(c) Mean culture diameters of *Aspergillus fumigatus* by the 10th day were 71.8, 81.4 and 86.8mm with the best growth on the *Sitophilus zeamais* leachate agar.

(d) Mean culture diameters of *Aspergillus niger* by the 10th day were 68.3, 81.5 and 83.2mm with the best growth on the *Sitophilus zeamais* leachate agar.

(e) Mean culture diameters of *Aspergillus ochraceus* by the 10th day were 31.8, 41.2 and 41.5mm with practically the same growth (41.2 and 41.3mm) on the *Sitophilus zeamais* and

Triboium castaneum leachate agar.

(f) Mean culture diameters of *Aspergillus sulphureus* by the 10th day were 29.4, 38.7 and 43.7 with the best growth on the *Sitophilus zeamais* leachate agar.

(g) Mean culture diameters of *Aspergillus ustus* by the 10th day were 89.0, 90.0 and 90.0mm. The growth rates were virtually identical.

16. The gut of *Callosobrochus maculatus* had a mycoflora made up of 14 species. The dominant species and their percentage occurrence were *Penicillium citrinum* (27.6 per cent). *Aspergillus flavus* (15.9 per cent) and *Penicillium purpurogenum* (8.7 per cent).

17. The gut of *Sitophilus zeamais* had 13 fungal species. The dominant species and their percentage occurrence were *Penicillium citrinum* (28.6 per cent), *Aspergillus flavus* (26.2 percent) and *Aspergillus fumigatus* (9.4 percent).

18. The gut of *Tribolium castaneum* had 16 fungal species. The dominant species and their percentage occurrence were *Penicillium citrinum* (42.7 percent), *Cephalosporium* sp. (16.4 per cent) and *Aspergillus flavus* (11.2 percent).

19. The genus *Aspergillus* was represented by the largest number of species in each mycoflora, followed by the genus *Penicillium*.

20. *Aspergillus flavus, A. fumigatus* and *A. ochraceus* experimentally fed to the insects survived for different lengths of periods in the guts of the insects:

(a) *A. flavus* was recovered after 5,6, and 8 days from the guts of

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Tribolium castaneum, *Callosobrochus maculatus* and *Sitophilus zeamais*, respectively.

(b) *A. fumigatus* was recovered after 4,5 and 6 days from the guts of *Callosobrochus maculatus*, *Tribolium castaneum* and *Sitophilus zeamais*, respectively.

(c) A. ochraceus was recovered after 3,4 and 6 days from he guts of *Tribolium castaneum*, *Sitophilus zeamais* and *Callosobrochus maculatus*, respectively.

21. Germination of the conidia of the *Aspergillus* species in extracts of faecal pellets of the insects was rather low.

(a) In the standard extracts of the faecal pellets of *Callosobrochus maculatus*, 6.9, 6.9, 8.6, 10.0, 7.1, 8.3 and 6.0 percent of the conidia of *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sulphureus* and A. ustus, respectively germinated in 24 hours.

(b) In the standard extract of the faecal pellets of *Sitophilus zeamais*, there was poor germination of 1.0, 0.7 and 0.7 percent by conidia of *A. clavatus*, *A. ochraceus* and *A. sulphureus*, respecively. The conidia of *A. flavus*, *A. fumigatus*, *A. niger* and *A. ustus* did not germinate.

(c) In the extract of faecal pellets of *Tribolium castaneum* 21.5, 13.7 and 5.4 percent of *A. clavatus*, *A. fumigatus* and *A. ochraceus*, respectively, germinated. Conidia of the four remaining species did not germinate.

22. Conidia adhering to the bodies of the insect pests were

detached as they brushed against maize grains among which they were crawling. The rate of loss of the spore load depended on the size of the maize grains and, therefore, the size of spaces among the grains. The conidia were rubbed off fastest among maize grains measuring 5.3-8.3 x 4.04-7.3mm, and slowest among maize grains measuring 9.5-12.2 x 7.5-9.5mm. The medium sized grains giving an intermediate rate measured 8.1-10.2 x 6.0-8.2mm.

23. The respective percentages of conidia still adhering to insects which had travelled over 100 cm through the small-sized, medium-sized and large-sized maize grains were: *Sitophilus zeamais*, 12.2, 15.3 and 23.3 percent *Tribolium castaneum* 11.5, 12.1 and 17.2 percent.

24. The three insect pests had surface bacterial flora. The density of colony forming units of *Callosobrochus maculatus*, *Sitophilus zeamais* and *Tribolium castaneum* was 27.5 x 10^4, 281.0 x 10^4 and 104 x 10^4 per ml of suspending medium, respectively.

25. The population of bacteria on *T. castaneum* decreased quickly after death of the insect and no bacterium was isolated from the bodies on the 4th day after death. Bacteria were still present on the bodies of *C. maculatus* and *S. zeamais* after six days. The respective population densities recorded were 37.8 x 10^4 and 5.0 x 10^4 per ml of suspending medium.

26. The bacteria on *C. maculatus* consisted of bacilli and cocci; those on *S. zeamais* were bacilli, cocci, vibro and filamentous

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forms and the flora of *T. castaneum* consisted of bacilli and cocci.

27. Both Gram-positive and Gram-negative bacteria were present on all the three insect pests.

28. Despite the presence of the large bacterial flora, fungi could also grow on the dead insect bodies. The fungi were isolated from insects which had been killed with ether and kept for 6 days.

(a) Species isolated from *Callosobrochus maculatus* in decending order of percentage frequency were, *Aspergillus flavus, Aspergillus niger, Mucor sp., Rhizopus sp. and Trichoderma vivide.*

(b) Species isolated from *Sitophilus zeamais* in descending order of percentage frequency were *Aspergillus flavus, Aspergillus niger and Curvularia* sp.

(c) Only two species were isolated from *Tribolium castaneum,* namely, *Aspergillus* and *Cladosporium* sp.

29. *Aspergillus flavus* consistently occurred in large numbers giving percentage frequency of 70,90 and 65 percent on *C. maculatus, S. zeamais and T. castaneum,* respectively.

30. All the bodies of *C. maculatus* and *S. zeamais* randomly selected for the tests contained fungi while fungi were isolated from only 70 percent of the *T. castaneum* bodies.

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APPENDIX A

Survival of *Aspergillus* species from the gut of *Callosobrochus maculatus* fed on cowpea seeds inoculated with the species (Based on data in Table 30).

Time after Feeding (Days)	Percentage of total Population of indicated species occurring in the gut on different days		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	27.4	43.8	57.6
2	19.8	50.7	18.5
3	21.7	4.1	9.4
4	14.1	1.4	8.6
5	13.2	0	4.6
6	3.8	-	1.3
7	0	-	0
8	-	-	0
9	-	-	-

APPENDIX B

Survival of *Aspergillus* species from the gut of *Sitophilus zeamais* fed on maize grains inoculated with the species (Based on data in Table 31).

Time after Feeding (Days)	Percentage of total Population of indicated species occurring in the gut on different days		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	20.6	29.7	11.1
2	53.0	30.4	73.4
3	10.8	11.6	11.1
4	3.2	18.26	4.4
5	3.8	8.7	0
6	7.6	1.4	-
7	0.5	0	-
8	0.5	-	-
9	0	-	-

APPENDIX C

Survival of *Aspergillus* species from the gut of *Tribolium castaneum* fed on maize grains inoculated with the species (Based on data in Table 32).

Time after Feeding (Days)	Percentage of total Population of indicated species occurring in the gut on different days		
	A. flavus	*A. fumigatus*	*A. ochraceus*
1	37.3	49.3	91.5
2	25.4	26.9	1.1
3	10.2	13.4	7.4
4	22.0	4.5	0
5	5.1	5.9	-
6	0	0	-
7	-	-	-
8	-	-	-
9	-	-	-