MYCOFLORA AND AFLATOXIN LEVELS IN MILLET SAMPLES FROM TEN (10) MARKETS IN ACCRA.

A TECHNICAL REPORT PRESENTED TO CSIR-FRI

BY

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SUMMARY

The mycoflora of millet (*Pennisetum glaucum* (L) R. Br.) grains from ten (10) markets in the Greater Accra Region have been examined with the view to identifying the resident mycoflora and potential mycotoxin-producing fungi especially aflatoxin-producing species.

The mycoflora profile of the resident species varied from one market to another. Millet grains from La market did not harbour any fungi. Eight different fungal species belonging to seven genera (*Aspergillus*, *Alternaria*, *Cladosporium*, *Helminthosporium* (=*Bipolaris*), *Paecilomyces*, *Rhizopus*, and *Fusarium*) were encountered. *A. flavus* was detected in grains from 80% (8/10) of the markets; *F. moniliforme* 80% (8/10) of the markets. *A. flavus* constituted 3.3-13.3% of the total mycoflora isolated on Potato Dextrose Agar from the different markets. The moisture content of the millet flour from the ten (10) markets varied from 8.6-12.35 and the germination capacity was variably affected commensurate with the intrinsic mycoflora contamination.

Aflatoxin G_1 and G_2 were not produced by all the strains of *A. flavus* isolated from the millet grains from all the markets except Nima, which was contaminated by 5.7 μg/kg of aflatoxin G_1. Aflatoxin B_1 and B_2 were detected in all grains except samples from Mallam Atta, Agbogbloshie and La markets. The highest level of total aflatoxin (79.8 μg/kg) was obtained from Makola market samples with the least (1.3 μg/kg) detected in the millet from Kanashie market.

Boiling koko for 10 minutes reduced aflatoxin B_1 and B_2 levels by 42.2% and 83.3% respectively. The same treatment reduced aflatoxin B_1 and B_2 levels in Tuo zaafi. The practical implications of these findings are discussed and future research work suggested.

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INTRODUCTION AND LITERATURE REVIEW

It is quite obvious that the past century has seen great advances in the method of cultivation of wheat, rice, and maize. This has been largely due to immense research bias to these crops. Consequently, humanity may have a buffer stock of grains and cereals to counteract the threat of over population especially in Africa. However, in the present century, where there is the real likelihood of the human population doubling, man cannot afford to build on the expectation of redoubling the production of these three crops (wheat, rice, and maize). International research efforts have made maize more productive than millet, government incentives have given maize added financial advantage, and easier processing has made maize more convenient to use (National Research Council, 1996).

Sorghum and millet are the fifth and sixth most important cereals in the world (National Research Council,1996). Generally, they are crops of the poorest countries, which means that their improvement could directly bring considerable benefit to the people in greatest need and as a result contribute a lot to the present global commitment to poverty reduction and ultimately elimination of this undesirable social menace.

Several reasons can be attributed to the concern shown for seed-borne fungi. One of the commonest effects is the influence of infection on the viability of the seed and the loss of planting material. Decrease in germination capacity could be due to destruction of the meristematic regions or the storage tissues that would sustain the growing embryo.

King and Fields (1959), found that *Aspergillus flavus*, *Aspergillus ruber*, *Aspergillus restrictus*, *Aspergillus candidus*, and *Aspergillus amstelodami* caused a reduction in the germination capacity of Alaska and Alsweet pea seeds. Heavy rice grain infection by *A. padwickii* has been found to severely reduce germination (Ganguly, 1947; Mathur and Neergaard, 1970; Mathur et al., 1971). *Fusarium moniliforme* caused grain rot of sorghum (Mathur, et al., 1967). Maize grains infected by *Diplodia maydis* had low viability (Nemlienko and Grisenko, 1962).

Fungal species that do not damage the seed, may attack the seedlings which emerge after germination of the seed. Seed-borne *A. niger* caused crown rot of groundnut seedlings in various countries (Ashworth, et al., 1964; Gibson, 1953; Jackson 1962; Nema, et al., 1955). Danquah (1973), demonstrated similar effects by seed-borne fungi in seedlings of bambara groundnut by *Fusarium moniliforme*, *Fusarium equiseti*, and *Mucor phaseolina* in rice and in sorghum by *F. moniliforme*.

Loss of food and changes in food value due to infection could be tremendous. Total break down of the seed is possible under long periods of favourable conditions for fungal activity. Damaged germs of seeds are always associated with heavy invasion of the embryo by storage fungi (Christensen, 1955; Christensen and Kaufman, 1964; Kaufman, 1964). Flour milled from wheat that contains 20% discoloured kernels may yield bread of smaller loaf volume than normal (Sorger-Domenigg, et al., 1955) and of 'off ' flavour. The major chemical changes induced by seed-borne fungi in the seed are changes in the levels of total oil, free fatty acids, sugars, proteins, and changes in pH.

There has been considerable interest worldwide in mycotoxins ever since they were discovered with the attendant mycotoxicoses. The mycotoxins, particularly aflatoxins,

produced by *Aspergillus flavus* and *A. parasiticus* have been of interest to eliminate or reduce their incidence in order to provide safe food for man and his domesticated animals. Mycotoxins have been identified in various agricultural products both in the raw state as well as processed forms. Ambient environmental and meteorological conditions in the tropics are ideal for mould growth and mycotoxin production. When these conditions are available, they act in concert to promote mould growth and mycotoxin production in foods. These conditions have been clearly spelt out by Northolt (1979). However, the presence of mould spores in a particular food does not imply that mycotoxins are present.

Many known mycotoxins are fungal secondary metabolites, produced under conditions not optimal for growth (Bu'Lock, 1980). Nonetheless, substantial growth of toxigenic species would be expected to precede production of a significant amount of mycotoxin. Temperature, relative humidity, moisture, and growth substrate are factors known to generally influence fungal growth.

Most fungi can grow and remain metabolically active within a broad temperature range (Kendrick, 1985). Relatively high moisture and relative humidity generally are required for fungal growth. The *Aspergillus* and *Penicillium* spp., which proliferate on grain during storage, generally require moisture content of > 15% and a relative humidity of 70-90% (Ciegler et al., 1981).

Many common grains, nuts, leaves, and stems are good growth substrates for fungi, including toxigenic species (Ciegler et al., 1981, Butler, 1975).

However, it must be emphasized that recovery of toxigenic species from any environment does not substantiate the presence of a mycotoxin, since mycotoxin production is not a necessary result of fungal growth (Bu'Lock, 1980). Often, the conditions necessary for mycotoxin production are very different from those required

for growth. *Fusarium tricintum* was shown to produce a significant amount of T2 toxin at 15°C but little at higher temperatures (Ciegler et al., 1981). The aflatoxin produced by *A. flavus* increased with decreasing carbon dioxide levels, or with an increase from 1% to 5% . In contrast, ochratoxin production in a grain fermentor was found to occur only after the air in the fermentor was exhausted (Ciegler et al., 1981). Certain mycotoxins isolated from laboratory cultures were produced only when key nutrients levels in the medium became growth –limiting. Biosynthesis of the mycotoxin patulin was induced only when available nitrogen was limited, and ergot alkaloids were produced after phosphate limitation (Bu'Lock, 1980). Laboratory cultures of *A. parasiticus* showed good growth at 35 or 40°C but did not produce aflatoxins at these temperatures. At lower temperatures, however, aflatoxins were produced even though growth was significantly less (Bennett et al., 1981).

Factors also may interact to influence mycotoxin synthesis. Cultures of *A. parasiticus* produced greater amounts of aflatoxin in the light than in the dark at 20 or 25°C. Lesser amounts of aflatoxin were detected in cultures grown in light rather than in the dark at 30°C, however (Bennett et al., 1981). The effect of microbial consortia on mycotoxin production is not well understood. Two studies suggest that aflatoxin and ochratoxin production were influenced by the presence of other fungi (Ciegler et al., 1981).

Mycotoxins are toxic organic compounds produced by numerous fungi. They produce a wide range of acute and chronic systemic effects that cannot be attributed to fungal growth within the host (Northrup and Kilburn, 1978) or allergic reactions to foreign proteins (Schiefer, 1986). Approximately 50 identified fungal compounds are believed to cause mycotoxicoses in humans and animals. Ten of these compounds are

carcinogenic in laboratory animals or are associated with human cancer in epidemiological studies. A dozen others are suspected of being human mycotoxins, based on toxic effects observed in laboratory animals (Ciegler and Bennett, 1980).

Consumption of mouldy food has long been associated with sickness in humans and domesticated animals. In the seventeenth century, it was recognized that mouldy rye produced disease (Butler, 1975). Ergot alkaloids derived from fungi were identified as toxic agents in the eighteenth century (Ciegler et al., 1981). In the early part of the twentieth century, thousands of deaths were attributed to the consumption of mouldy grain or rice. A massive outbreak of haemorrhagic disease occurred during World War II in regions of Russia. This disease, alimentary toxic aleukia, later was determined to be caused by mycotoxins in food prepared from mouldy grain. Another human mycotocosis of significance, acute cardiac beriberi was a common disease in Japan, especially in the second half of the last century. The disease is characterized by difficulties with breathing, nausea and vomiting, and after 2 to 3 days, severe pain and distress. Progressive paralysis may lead to respiratory failure and death. Beriberi is the general name for vitamin deficiencies resulting from the consumption of mouldy rice. Careful work by Uraguchi (1971) showed that acute cardiac beriberi may not be a vitamin deficiency, but a toxicosis. In 1910, the incidence of acute beriberi suddenly decreased in Japan: Uraguchi points out that this coincided with the implementation of a government inspection scheme which dramatically reduced the sale of mouldy rice. The incidence of true beriberi, resulting from the consumption of polished rice, was unaffected. It is notable that victims of this acute cardiac beriberi were often healthy adults. Onyalai is an acute disease characterized by haemorrhaging lesions in the mouth. It has been endemic in Africa, especially in the southern Sahara regions for

at least 80 years (Rabie et al., 1975). It is much more common in rural than urban populations, since many of the people affected by onyalai subsist on millet, Rabie et al. (1975) suggested the possible role of mycotoxin in this disease. Toxigenic isolate *Phoma sorghina* were found to be common on millet consumed by affected populations, and Rabie et al. (1975) were able to reproduce many of the symptoms of onyalai in rats fed maize and wheat on which *P. sorghina* had been grown. In Japan, numerous deaths were attributed to " yellow rice disease " from consumption of mouldy rice. No specific mycotoxins were identified that could cause the disease, but several toxin-producing fungi were isolated from rice (Butler, 1975; Ciegler et al., 1981). The death of 100,000 turkey poults in England in 1960 which was traced back to a lot of groundnuts infected with *Aspergillus flavus* (Spensley, 1963) is one of the dramatic observations of the production of toxins by fungi grown on foodstuffs, which constitute a continuous health hazard to man and his domesticated animals. Secalonic acid D was reported to produce toxic effects in the rat lung and teratogenic effects in mice (Ehrlich et al., 1982). Peers and Linsell (1973) reported aflatoxin B_1 levels of 2.5 – 3.5g per kg in local bears in diets of Kikiyos and Kambas in the lower altitude Miu anga and adjourning Kamba areas in Kenya, where they also showed positive correlation between lever cancer and aflatoxin infection. Hundreds of mycotoxins have been isolated from various fungal cultures in the laboratory. Considered as one group, mycotoxins must be portrayed as structurally diverse complex organic compounds. However, distinct classes of mycotoxins are defined based on structural class or fungal origin. Twenty-one different mycotoxin classes, each containing a number of specific mycotoxins, have been described (Cole and Cox, 1981).

Conditions, which allow toxin production, are more restricted than those for growth. Many substrates are poor for production of some toxins while excellent for growth.

The knowledge of which toxins will be produced in which food would be based on knowledge of the specific mycoflora of foods, laboratory experiments, and data from chemical analyses for mycotoxins in foodstuffs under different conditions. It has been reported that nearly all known food-borne fungi have the ability to produce mycotoxins in pure culture on laboratory substrates (Frisvad, 1988). This supports the view of Janzen (1977) that secondary metabolites are of great ecological significance (protection of the genome against other microbes, insects and other animals).

Aflatoxins are secondary metabolites (highly oxygenated and heterocyclic compounds) produced by some strains of *Aspergillus flavus* (Link. ex Fries) and *Aspergillus parasiticus* (Speare). Four major structurally related aflatoxins have been identified, namely B_1, B_2, G_1, and G_2. However, aflatoxin B_1 is the primary form produced by most aflatoxin-producing species under most culture conditions (Ciegler and Bennett, 1980). Aflatoxin B_1 is most noted for its carcinogenic effect on rats and humans alike. Consequently, the majority of health effect studies have focused on aflatoxin B_1. Aflatoxin B_1 shows acute oral toxicity to laboratory animals. The liver is apparently the primary target organ (Butler, 1975). However, aflatoxin B_1 is most noted for its chronic toxicity, especially carcinogenicity. It has mutagenic, and teratogenic effects in mammals. Some studies show a close connection between hepatic cancer and ingestion of aflatoxin B_1 in grains (Ciegler et al., 1981). Rats given intratracheal doses of aflatoxin B_1 and G_1 for 30 weeks developed carcinomas of the liver, intestine, and kidney, presumably due to systemic absorption of the toxin (Northrup and Kilburn, 1978). Other studies showed that acute inhalation of aflatoxin B_1 caused tracheobronchial cell destruction in hamsters and guinea pigs (Northrup and Kilburn, 1978). Consequently, contamination of foods or feeds and their products by

aflatoxins is undesirable and a serious health hazard to man and his domesticated animals (Enomoto et al., 1992).

Three different kinds of investigations examined the association between inhalation of aflatoxin and human cancer. Epidemiology studies have reported the cancer rate among workers in commercial plants that may have generated aflatoxin-containing dust during processing of feed and/or nuts. Other studies reported cancer occurrence in individuals possibly exposed to airborne aflatoxin.

Farmers who clean out mouldy grains from storehouses suffer from burning of the eyes, nose, and throat, chills, fever and dry irritating coughs (Emanuel et. al 1975).

The threat to human and animal health owing to consumption of foods and feedstuff has led to many countries setting limits to the permissible level of aflatoxins in feeds and foodstuffs. Ghana is yet to set such limits although the FAO and the Codex Alimentarius Commission's Standard limits are often quoted. Work done on maize in Ghana, indicated unacceptable levels (Awuah and Kpodo,(1996). Aflatoxin level of 22,168ppb has been reported on damaged groundnut kernels in Ghana (Kpodo et al. 1996). However, there is limited information on the incidence of aflatoxins and resident mycoflora in cereals like millet and sorghum in Ghana. It is therefore imperative that the study should be extended to cover other grains and cereals, which enjoy considerable patronage in Ghana. Millet is a food commodity, which is very popular to the people of the three northern regions of Ghana. Incidentally, these regions are said to be the most deprived in the country. The evaluation of the aflatoxin levels as well as other mycotoxins will form a basis for the development of technologies to reduce if any, of these health hazards in foods made or prepared from millet. This will go a long way to benefit the most impoverished, healthwise as well as

economically and subsequently lessen their plight and help in the fight to reduce pove ty.

Food security and quality is a necessity for all nations the world over. With the increase in the world population, it makes it even more imperative that serious thought should be given to feeding the increased numbers.

Pearl millet (*Pennisetum glaucum* (L) R. Br.) was domesticated some four hundred years ago in what is now the heart of the Sahara desert. It is planted on some 14 million hectares in Africa and the same in Asia. Global production of the grain exceeds 10million tonnes per year. In Ghana, it enjoys considerable patronage by the peoples of the three deprived northern regions with a total population of 3,346,105 (2000-population census). The total national production from 1995 to 2000 is 1,037,000 metric tonnes. The estimated per capita consumption for the year 2000, is 17.5kg/head/year (Based on figures compiled by the Statistics, Research and Information Directorate (SRID) of the Ministry of Food and Agriculture for 1996-2000).

The grain is tear-shaped and smaller than those of wheat. The seed range in colour from white to brown, blue, or almost purple. Some thresh from glumes while others require husking. Approximately one third of the world's millet is grown in Africa with about 70% in West Africa (ICRISAT, 1987). The plant is generally sensitive to low temperatures at the seedling stage and at flowering. High daytime temperatures are needed for the grain to mature. In Africa's zone, temperatures are typically above 30°C, which is conducive to the plant. It does best in light well-drained loams, and cannot tolerate water-logging. It is tolerant to subsoils that are acidic and high in

aluminium content. Pearl millet is supremely adapted to heat and aridity. It thrives where habitats are harsh. Of all the major cereals, it is the most able to tolerate extremes of heat and drought. It yields reliably in regions too hot and too dry to consistently support good yields of maize.

The importance of water to agriculture cannot be over-emphasized. It's role in this venture is very crucial. Agriculture is usually a country's biggest user of water, so that crops that sip rather than gulp water are likely to be in greater demand. This is where millet could be a vital resource. The crop also suffers less from diseases compared to sorghum, maize and other grains (National Research Council (1996).

It also has fewer insect pests. Although it is one of the best means of sustaining life in the most desolate and difficult parts of the world, millet also grows well under what may be described as pampered conditions, that is, under irrigation for example. As a result it is often misconstrued as a crop not suitable for good lands. Like sorghum and maize, it has the C_4 photosynthesis. All in all, the ability to adapt to both good and bad conditions makes it a potentially outstanding food crop for vast areas of the green house afflicted world where climates may change wildly from decade to decade or even annually, and where more and more people must obtain food from hot dry soils (National Research Council, 1996).

The potential of millet for the tropics can be seen in Ghana, where early millet is extremely important to the rural folk. The type (*Pennisetum glaucum*) grown here normally matures at the peak of the rainy season, a time when farmers have exhausted their food stocks from the previous harvest. In Ghana, millet is gathered when the grains are in the dough stage and are soft and sweet. Usually, the freshly harvested heads are steamed, threshed, and dried. This process is the exact opposite of normal

practice. Probably, this makes it possible to recover the immature grains that would otherwise turn mush when threshed..

The world annual production of the crop exceed 10 million tonnes (National Research Council (1996) and in Ghana over one metric tonnes (1,0370,000) was produced between 1995 and 2000. This gave a per capita consumption in 2000 as 17.5kg/head (Based on figures compiled by SRID for 1996-2000).

The grain (is actually a superior foodstuff) contains 9% protein, and a good balance of amino acids. The grain has more fat and it's level of food energy (784 kilocalories/kg) is among the highest for whole grain cereals. It has more protein, and it's level of essential amino acid lysine is better than in most cereals. However, it may be low in threonine and the sulfur-containing amino acids. It has more oils than maize and is therefore, a "high-energy" cereal. Millet has neither the tannins nor the other compounds that reduce digestibility in sorghum (National Research Council (1996).

However, it has poor storage stability, which is overcome by parboiling and semi-wet milling. Millet is one of the more nutritious of the common cereals. A review research in India (CSIR, 1966) states that a diet based on millet and pulses is somewhat better at promoting human growth than similar diet based on wheat.

It may not be enough to worry about grain production or availability. What is perhaps more important is the amount and quality of the food that is consumed. It is estimated that 25% of each year's food production is either lost or rendered unfit for consumption. During handling and storage, high heat and humidity support moulds growth and deterioration of grains such as obtains in tropical Africa including Ghana.

The millet grain is used mainly as whole, cracked, or ground flour; dough, or a grain like rice. Foods prepared from it include fermented foods, unfermented bread, thin and thick porridges, steam-cooked dishes (couscous), non-alcoholic beverages and snacks.

Koko a porridge prepared by mixing millet flour with water into a fine paste, which is then kept in a warm place for a day or two to ferment. The resulting sour dough is then poured gently into boiling water to form a thin porridge of creamy consistency. Marsa, which is a favourite snack of Ghanaians especially for people of the Northern and Upper Regions, is made from millet. It is a deep-fried pancake prepared from the leavened butter of millet flour (National Research Council, 1996). Here in Ghana, as in India, millet is sometimes roasted and consumed like sweet corn.

The objectives of the present study are to update the list of resident mycoflora in millet and to determine the level of aflatoxin contamination in market samples. Ten (10) markets in the Greater Accra region of Ghana were selected and millet samples from these markets were examined for their mycoflora and aflatoxin contamination levels. Finally, the effect of heat treatment on the levels of aflatoxins in millet during the preparation of koko and Tuo zaafi was also studied

MATERIALS AND METHODS

Sample collection:

Samples were collected from ten (10) markets in the Greater Accra Region of Ghana namely, La, Osu, Makola, Madina, Maamobi, Nima, Mallam Atta, Agbogbloshie, Kaneshie, and Mambrobi. A total of 5kg of millet (*Pennisetum glaucum* (L)) was bought from each market centre. Five hundred grams of the sample was bought from each of an average of ten sellers at each market. The sellers were chosen at random from different locations of the markets. Samples for each market were pooled to represent a sample for a particular market. Samples were stored in sealed polythene bags and stored at 4°C in a refrigerator till analyzed. Milling of the grains prior to water activity measurement and aflatoxin analysis was carried out using a KCH – Universalmühle M2 miller. Milling time was set at 2 minutes.

Seed/ Grain moisture determination

This was determined in accordance with J.AOAC (1984)

Water Activity

The water activity (a_w) of the samples was determined using the Durotherm (Wert-Messer) water activity meter. Ten grams of millet flour was placed in the two receptacles for 24hr, after which readings were taken. Prior to this, the instrument was calibrated using a standard solution (90% R.H. Barium chloride solution).

Isolation and identification of fungal flora

The direct plating method was used. Twenty grains of sample were placed on Potato Dextrose Agar and incubated for six days at 28±2°C. One set of grain was surface sterilized whilst the other set was untreated. Surface sterilization was achieved by immersion in 10% sodium hypochlorite for 1-2 minutes and then rinsed in three changes of sterile distilled water. There were six replicates for each sample.

The various fungal species were mounted in lactophenol on glass slides and identified according characteristics outlined by Samson and van Reenen-Hoekstra (1988):

Alternaria alternata

Colonies were black. Conidiophores 1-3 septate, simple, straight with one or more apical pores. Conidia in long branched chains, ellipsodal, with short conical beak not exceeding a third of the conidial length, with up to eight (8) transverse and longitudinal septa and smooth-walled.

Cladosporium herbarum

Colonies greenish black. Conidiophores arising laterally or terminally from the hyphae, with terminal and intercalary swellings and smooth-walled. Conidia in long branched chains, ellipsoidal to cylindrical with rounded ends, and two or more celled.

Paecilomyces variotii

Conidiophores consisted of dense whorls of verticillatedly arranged branches, each bearing two to seven phialides. Phialides solitary or in whorls, consisting of a cylindrical to ellipsoidal basal portion tapering abruptly to a long, cylindrical narrow neck. Conidia in long divergent chains and one celled.

Fusarium moniliforme

Micro-conidiophores mostly unbranched, formed on aerial mycelium, with 1-3 simple elongated phialides. Micro-conidia in long chains, no septa, clavate with truncate base. Macro-conidiophores formed as lateral branches on hyphae, bearing 2-3 phialides. Macro-conidia slender, mostly 3 or 5 septate, slightly curved, fusiform, thin-walled with elongated curved apical cell and pedicellate basal cell, 3-septate.

Aspergillus flavus

Colony greenish. Vesicles globose to subglobose. Phialides borne directly on the vesicle or on the metulae. Conidia globose to subglobose.

Aspergillus niger

Colony black. Conidiophore stipes smooth-walled. Vesicles globose to subglobose. Phialides borne on metulae. Conidia globose to subglobose, ornamented with irregular warts, spine and ridges.

Rhizopus spp.

Colony brownish. Sporangia many-spored. Columella globose or half-globose . Spores short-ellipsoidal, irregularly angled and striate.

Germination Test/ Seed viability:

Thirty surface-sterilized grains were placed in sterile Petri dishes (9cm) containing moistened filter paper and incubated at 30±2°C for seven days in a dark cabinet (incubator). Surface sterilization was achieve by immersion in 10% sodium hypochlorite for 1-2 minutes and then subsequently rinsed in three changes of sterile

distilled water. Glass Petri dishes were sterilized in canister by heating in a Gallekamp oven 300 series set at 160°C for 24hr.

Germination experiment was carried out for the measurement of root lengths of the germinated seeds. In this experiment, 20 seeds were placed in Petri dishes with moistened filter paper and kept in cupboards. There were five replicates. Measurements were carried out on 60 grains on the average for each market sample, and the means taken. Measurements were taken after ten days with a ruler.

Media, Preparation

Potato Dextrose Agar (PDA)

Two hundred grams of peeled potatoes chopped into bits was boiled in 500ml of distilled water for twenty minutes. This was then allowed to cool and strained using a muslin cloth. 20g of dextrose and 15g of agar were added. The resultant solution was heated to melt the agar after which, distilled water was added to make it up to a litre. This was then autoclaved at 121°C and 1.05cm/kg pressure for 20minutes and stored in medicinal flasks in a refrigerator until needed. The PDA was amended with chloramphenicol (500ppm) prior to plating.

Consumables:

Aflatoxin standard used was from Sigma Chemical Co. Ltd (St. Louis, Mo USA). The stock solution contained 5µg/ml B_1, 1.5µg/ml B_2, 5µg/ml G_1, and 1.5µg/ml G_2

Aflatoxin working solution:

1000µl of aflatoxin stock solution (Sigma A 1023) was dissolved in benzene: acetonitrile, in the ratio 98:2 (v/v) and transferred to a volumetric flask and slowly evaporated under nitrogen flow. The residue was redissolved in 10ml of acetonitrile to

give 0.5µg/ml B_1, 0.15µg/ml B_2, 0.5µg/ml G_1, and 0.15µg /ml G_2 . This was stored at –18°C and protected from daylight.

Aflatoxin standard:

1000µl of the aflatoxin working solution above, was transferred to a screw-cap vial and slowly evaporated under nitrogen flow. The residue was redissolved in 5ml of HPLC mobile phase I consisting of 10% methanol, 30% acetonitrile, and 60% deionized water to give 0.1µg/ml B_1, 0.03µg/ml B_2, 0.1µg/ml G_1, and 0.03µg/ml G_2. This was also stored at –18°C and protected from daylight.

Reagents for HPLC separations were of HPLC grade (Merck Chemicals). All other chemicals and reagents used were of analytical grade. The mobile phase solution was filtered through a 0.45µm Millipore HV disc filter and degassed before use through a Millipore filtration unit (Millipore Corp. Bedford)

Preparation of Koko

Three hundred grams of millet grains (Makola sample) were first steeped in 1500ml of water overnight and milled. This was then mixed with 1500ml of water and allowed to ferment for two days and subsequently used for preparing koko.

The koko was prepared by mixing millet flour with water into a fine paste 1:5w/v, which is then put aside in a warm place for a day or two to ferment. The resulting sour dough is then dropped in boiling water to form a thin porridge of creamy consistency. Hundred grams of koko was used in the extraction for aflatoxins.

Preparation of Tuo Zaafi (TZ)

This is a stiff porridge prepared by adding the sour dough to boiling water as in the case of koko described above. However, there is no steeping of grains in this instance. After ten minutes, dry dough is added proportionally to obtain the desired stiffness.

Extraction and HPLC analysis:

Extraction and clean-up procedure for aflatoxins in millet:

The extraction procedure was based on that of Pons (1979). Twenty-five grams of millet flour was weighed into a Waring Commercial Blender. Fifty millilitres of 10% sodium chloride and 200ml of methanol were added. This was followed by homogenizing by blending at low speed for 1minute and high speed for 3 minutes. The resultant solution was allowed to cool off to room temperature and then filtered through a Büchner funnel equipped with filter paper and a thin layer (1-2cm) of celite. Hundred millilitres of the filtrate was transferred into a 250ml beaker after which 100ml zinc acetate was added gradually while stirring. After 5minutes, 10g of celite was added and after stirring filtered through a fluted funnel. A 100ml of the resultant filtrate was transferred into a 250ml separating funnel. Twenty-five millilitres of dichloromethane was then added and shaken for one minute with occasional release of pressure from the funnel. This was then allowed to stand for 10 minutes for separation of the phases. The lower dichloromethane phase was drained off into a chromatography tube, which had previously been filled with a 4cm layer of fibrous cellulose and a 2cm layer of sodium sulphate (anhydrous). The eluate was collected in a 250ml round bottomed flask. Addition of 25cm was repeated and drained off as previously done. Lastly, the chromatography tube was rinsed with 25ml of dichloromethane. The dichloromethane was evaporated until approximately 2 ml was left using the rotary evaporator.

A narrow chromatography tube was filled half way up with eluent A (50% of diethylether, 50% pentane (vol/vol) and degassed by tapping. After letting a small portion of the eluent out through the cock, the column was packed by adding in small

portions 2g of deactivated silica gel while circling the tube. Afterwards, the inner surface was rinsed with approximately 5ml of eluent A.

When the packing settled, approximately 1.5g of anhydrous sodium sulphate was added gradually. The eluent was drained out so as to make the surface of the liquid align the surface of the sodium sulphate. It was of great importance that there were no air pockets in the column material and that the surface did not dry out in the course of the analysis.

The residue arising from evaporation with the rotary evaporator was then transferred to the top of the packing material. The cock on the tube was adjusted to give a flow rate of approximately 1.5ml/min with the aid of a measuring cylinder and drained to the sulphate layer. The flask was rinsed twice with 2ml dichloromethane, and after each transfer drained to the surface of the sulphate layer.

Twenty-five millilitres of eluent B (90% toluene, 10% acetic acid vol/vol) was passed followed by 50ml of eluent A through the column. When changing eluent, draining was to the surface of the sulphate layer and flow readjusted to approximately 1.5ml/min.

Aflatoxins were eluted by passing 60ml of eluent C (80% dichloromethane, 20% acetone (vol/vol) through the column and collected in a 250ml round bottomed flask.

The eluate was evaporated with the rotary evaporator until approximately 2ml was left. The residue was transferred with dichloromethane to a 10ml volumetric flask.

Five millilitres of the resultant solution was pipetted into each of two ReactiVials and allowed to evaporate to dryness. The vials were then screw-capped and stored at –18°C. The final residue was redissolved in 0.1-1ml HPLC mobile phase I and used for HPLC analysis.

Extraction and clean-up procedure for koko and Tuo zaafi was as above but with few modifications.

The HPLC instrument system used for all HPLC analyses, was from Waters Associates (Milford, MA, USA) and included a Model 501 solvent delivery system fitted with Rheodyne model 7125 injector with a 20µl fixed volume loop, a temperature control system consisting of a temperature control module and two column heaters, a model 470 scanning Fluorescence detector, and a model 746 Data model.

Identification and quantification of aflatoxins was by the reversed-phase liquid chromatography with post-column iodine derivatization. Separation of aflatoxins was carried out on a Spherisorb S5 ODS-1 column of dimensions 25x 4.6mm packed with 5µm particles (Phase Separations Inc. Norwalk, USA) maintained at 35°C. HPLC mobile phase flow rate of 1.0ml/min and post-column iodine derivatization of aflatoxins B_1 and G_1 was achieved using saturated iodine solution (Shepherd and Gilbert, 1984) pumped at a flow rate of 0.5ml/min using an Eldex precision metering pump (Eldex Laboratories Inc., San Carlos, USA). The derivatization tube consists of stainless steel tubing (5m x 0.3mm) maintained at 75°C.

The excitation and emission wavelengths used were 360nm and 440nm, respectively. The aflatoxins were identified by their retention times, and peak areas were used to determine their concentrations in the samples by reference to standard curves obtained by chromatography of pure aflatoxin standard solutions under identical conditions. To compensate for any day-to-day variation, standards were routinely run at the beginning, in-between, and after samples had been run.

RESULTS AND DISCUSSION

The most important prerequisite for any action program to mitigate aflatoxin contamination of food is to know which of the commodities grown in any region are susceptible to fungal damage and at what stage or stages they are vulnerable to fungal infection leading to mycotoxin formation. Such information is only obtained by comprehensive survey programs designed to identify;

(a) High risk crops and commodities

(b) High risk regions

(c) High risk populations and age groups.

.World-wide surveys carried out over the past decade indicate that aflatoxins can contaminate many staple foods consumed by humans, particularly those plant foods that contribute protein and calories such as groundnut, tree nut, corn kernels, soybeans, cotton seed, grains and rice.

Results from this present study show that millet grains purchased from the ten markets varied considerably in their resident mycoflora profiles. Eight (8) different fungal species belonging to seven genera were encountered (Table 1). *A. flavus* was encountered in 8/10 markets (80%) and *F. moniliforme* (8/10) 80% of the markets. The millet samples obtained from the La market did not harbour any fungal species. Kaneshie, Mallam Atta, Maamobi, Osu, and Nima market millet samples harboured seven species of fungi (Table 1). *A. flavus* constituted 3.3-13.3% of the total mycoflora isolated from the grains from the different markets. Recently, Okyere (2002) also isolated twenty-one (21) fungal species from millet grains purchased from the Maamobi market. *Aspergillus* species (*A. clavatus*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. oryzae*, *A. sulphureus*, *A. tamarii*, *A. terreus*) predominated followed by *Penicillium* species (*P. brevicompactum*, *P. citrinum*, *P. digitatum*, *P. glabrum*),

Table 1.

PERCENTAGE OCCURRENCE OF INDICATED FUNGAL SPECIES ON MILLET GRAINS AT 30^{0}C.

Market	*A.niger*	*Helmintho sporium* spp	*Rhizopus* sp.	*A.flavus*	*Alternaria alternata*	*Paecilomyces variotii* sp.	*Cladosporium* sp.	*Fusarium moniliforme*
Mallam Atta	6.7	6.7	3.3	3.3	6.7	3.3	-	3.3
Kaneshie	6.7	13.3	-	3.3	10.0	3.3	26.6	3.3
Madina	6.7	-	-	3.3	6.7	13.3	23.3	3.3
Maamobi	20	3.3	3.3	10.0	-	3.3	3.3	6.6
Osu	30	13.3	3.3	6.7	10.0	-	6.7	3.3
Mamprobi	-	10.0	-	3.3	3.3	3.3	3.3	-
Nima	23.3	3.3	3.3	6.7	13.3	-	50	3.3
La	-	-	-	-	-	-	-	-
Makola	16.7	-	-	13.0	3.3	6.7	46	-
Agbogbloshie	-	3.3	-	-	-	-	6.7	3.3
Total no. of markets with indicated fungi	7	7	4	8	7	6	8	8

Cladosporium (*C. herbarum*, *C. fulvum*,), *Fusarium moniliforme*, *Mucor hiemalis*, *Paecilomyces variotii*, *Bipolaris=Helminthosporium maydis*, and *Rhizopus oryzae*. At a storage humidity of 75% and above, *A. flavus* constituted 9.1-61.6% of the total population of fungi (Okyere, 2002).

The percentage infection of the millet samples from the ten markets also varied considerably. While La samples were apparently free of external contamination by fungi, 6.7% of the Agbogbloshie samples were infected and the highest infection was recorded in the Makola samples (86.7%) (Table 2). According to Dewey and Wayne (2000), safe moisture content for storage of millet at 70-75% ERH is 11-12% moisture content. The moisture content of the flour of millet from the ten markets varied from 8.6% moisture content (Mamprobi) to 12.3% moisture content (Makola). Grains with less than 10% moisture content do not provide a favourable environment for insect activity (Christensen and Kaufman, 1969). However, germination capacity of the grains was also variably affected. About 60% of the surface-sterilized millet grains from La market germinated as compared to 73.3% of the Madina market samples (Table 3) and 56% of the Osu market samples. Surface sterilization precludes fungi that are not seed-borne leaving those that have ramified the interior portion of the seeds (Neergaard, 1983).

In cereal grains in general, dark embryos are designated as 'damaged' and wheat with such characteristics are referred to in grain trade as 'sick' indicating that indeed such embryos are in fact dead and decayed. Damaged germs are always associated with heavy invasion of the embryo by storage fungi attended by reduced germination capacity (Christensen, 1955; Christensen and Kaufman,, 1964; Kaufman, 1964). Damage of seed embryos by fungi, particularly the storage species, has been reported by several workers (Halloin, 1974, Sorger-Domenigg et. al., (1955).

Table 2. Relationship between moisture content and percentage infection of millet grains at 30^0C for 7 days.

Market	Percentage infection of Millet seeds (%)	Moisture content of Millet flour (%)
Mallam Atta	33.3	10.7
Madina	76.7	12.0
Osu	86.7	9.6
Maamobi	66.7	10.5
La	0	11.6
Nima	90	11.0
Kaneshie	83.3	10.2
Makola	86.7	12.3
Mamprobi	63.3	8.6
Agbogbloshie	6.7	10.8

Table 3. Influence of surface-sterilization on the germination capacity of millet grains infected with varying storage mycoflora.

Market	Percentage Germination(%)	
	Sterilized Grains	Non-sterilized Grains
Mamprobi	36.6	63.3
Madina	73.3	66.6
Mallam Atta	50	73.3
Kaneshie	50	43.3
Maamobi	60	75
Nima	63.3	50
Agbogbloshie	66.6	63.3
La	60	55
Osu	56	70
Makola	60	53

The pathogenecity of the metabolites of *Aspergillus restrictus*, *A. niger*, *A. tamarii*, *A. flavus* and *Bipolaris=Helminthosporium maydis* on millet seedlings has been clearly demonstrated recently by Okyere (2002). These fungi reduced root length, leaf width, height of plant and chlorophyll a & b contents as well as total chlorophyll of millet seedlings. This confirms data in Table 4 where both percentage germination and root length of seedlings were considerably affected. Rather curiously, the water activity (the new term for the environmental condition that makes water available for fungal activity) values were low but still there was decline in germination capacity (Table 5). In many instances, the seed-borne fungus from the field damages the germ before drying take place and although the seed may seem relatively fungus-free (as in the case of the millet from La market) germination was still depressed (68.6%) (Table 4).

Surface mycoflora isolated on the millet samples included *Aspergillus flavus*, which is responsible for the production of aflatoxin B_1, and B_2. Other fungi encountered were *Helminthosporium*(=*Bipolaris*) spp., *Cladosporium herbarum*, *Aspergillus niger*, *Alternaria alternata* and *Fusarium moniliforme* (Table 1). These fungi are capable of producing other mycotoxins which may be harmful to man and his domesticated animals. *Aspergillus niger* was recorded in 7 out of the 10 market samples. Mycotoxins produced by this fungus include kojic acid, malformins, naphthoquinones, and nigraginin. *Alternaria alternata* is known to produce alterneriols, altertoxins and tenuazonic acid; *Cladosporium herbarum* produces epi- and fagi-cladosporic acid (Frisvad, 1988). Epidemics of *Fusarium* head blight and maize ear rot are chronic in cereal-growing areas of Asia as well as parts of Africa and South America. Public health authorities in these areas reported acute human toxicosis

from deoxynivalenol (Bhat *et al.*, 1989). The effect of chronic exposure to trichothecenes (which are immunosuppressors) is not clearly known. *F. moniliforme* produces under various conditions, fusarins and fumonisins which are of public health importance. It also produces moniliformin, and gibberellins. A number of other known or suspected mycotoxins are produced by various species of *Fusarium*, *Aspergillus*, and *Penicillium*. Zearalenone is produced by several *Fusarium* species and may be classified as a *Fusarium* toxin (Ciegler and Bennett, 1980; Cole and Cox, 1981). Zearalenone is recognized for its estrogenic effects in swine. These effects may include low fertility, reduced litter size, malformation of offspring (Ciegler et al., 1981). It is in this light that the isolation of the fungus may be worrying. The isolation of *Paecilomyces variotii*, which produces the mycotoxin patulin, is also worth noting.

The isolation of *F. moniliforme* agrees with isolation of the same fungus on millet in other countries such as the USA,(Luttrell, 1954;Luttrel, 1955; Wells and Winstead, 1965; Wilson et al., 1993). *Helminthosporium* (=*Bipolaris*) spp by Wilson et al., (1993), and *Rhizopus* sp by Luttrel, (1955). These fungi have been isolated on millet in India, by Konde et al., (1980), Girisham et al, (1985), Mishra and Daradhiyar, (1991), and Ingle and Raut, (1994). Onesirosam,(1975) isolated *Rhizopus* on millet in Nigeria. The isolation of *Alternaria alternata*, *Helminthosporium* spp, and *Rhizopus* sp also isolated in India (Mishra and Daradhiyar, (1991) have also been reported in Nigerian specimens. *F. moniliforme* occurrence on millet is also reported in neighbouring Nigeria (Onyike et al., 1991, Onesirosam, 1975) and Zimbabwe (Onyike et al., 1991). However, in the USA, *Alternaria alternata*, *A. niger*, *A. flavus*, and *Cladosporium* spp. were not encountered.

Table 4. Relative germination capacity and length of emerging roots(mm) in millet grains purchased from the indicated markets.

Market	Percentage Germination(%)	Average length of roots (mm)
Mamprobi	56	85.3
Madina	78	96.2
Mallam Atta	81	84.7
Kaneshie	67	65.2
Maamobi	59	52.1
Nima	72	86.1
Agbogbloshie	66.6	79.7
La	68.6	72.3
Osu	73.3	71.4
Makola	71.3	83.7

Table 5. Relative water activity and room temperature of the indicated market samples.

Market	Water activity(a_w)	Room Temperature (^oC)
Madina	0.67	30
Makola	0.65	28
Mallam Atta	0.73	28
Mamprobi	0.65	30.5
Maamobi	0.65	29.5
Agbogbloshie	0.63	29
Kaneshie	0.69	33.5
Osu	0.68	29
La	0.68	29.5
Nima	0.66	28

Table 6 show aflatoxin levels of millet flour samples obtained from the ten (10) markets in the Greater Accra Region of Ghana. Samples from 70% of the markets (7/10) contained two or more types of aflatoxins. The levels ranged from 1.3µg/kg (Kaneshie) to 79.8µg/kg (Makola). No types of aflatoxins were detected in the La and Agbogbloshie markets samples (Table 6) and were apparently aflatoxin-free. Madina and Mamprobi samples contained total aflatoxins level of 38.8µg/kg and 34.2µg/kg respectively. Apart from Nima market sample, which contained 5.7µg/kg of aflatoxin G_1, none of the other samples from the rest of the markets contained aflatoxin G_1 and G_2.

The results give room for concern since aflatoxins have been reported to pose as a very serious health hazard to man and his domesticated animals given the fact also that millet is a staple for the poor and most deprived the world over especially in Africa. Aflatoxin B_1, the most toxic compound, is usually produced concurrently by many *A. flavus* strains with aflatoxin B_2. Aflatoxins are usually formed by both *A. flavus* and *A. parasiticus*. In this study, *A. parasiticus* was not encountered. Aflatoxins G_1 and G_2 are formed by *A. parasiticus* (Klich and Pitt,1988) and some strains of *A. flavus* NRRL 5906. Aflatoxins cause liver cancer. They are also immunosuppressive making one susceptible to other health disorders. They act very slowly and after prolonged consumption can lead to liver cancer. A person's chances of contracting the disease are compounded significantly if he also carries the Hepatitis B virus, which causes jaundice (ICRISAT, 1987).

Aflatoxins have been implicated in human diseases including liver cancer, Reye's syndrome, Indian childhood cirrhosis, chronic gastritis, kwashiorkor and certain occupational respiratory diseases in various parts of the world, particularly in Africa

and Asian countries. In China, the Philippines, Thailand, Kenya, Swaziland and Mozambique, higher levels of aflatoxins in the food supply have been correlated with aflatoxins and their derivatives in human fluids which may be associated with liver cancer (Palmgren and Hayes, 1987).

Campbell (1983) has shown that in rats a high protein diet after exposure to aflatoxin caused a higher rate of cancer than a low protein diet. Thus it seem that nutrition can play a vital role in the induction of cancer by aflatoxin. Kwashiorkor, a disease of children in North Africa and elsewhere in undernourished populations, which is usually attributed to nutritional deficiencies, may also be related to aflatoxin intake (Hendrickse et al. 1982). The disease is common in Ghana especially in deprived communities. Aflatoxin-induced liver damage may make these children less able to cope with the high protein diets usually recommended as the cure for kwashiorkor (Newell, 1983).

Table 7 & 8 show the effect of heat on aflatoxin B_1 and B_2 levels during the cooking of Koko and Tuo zaafi respectively. There was 42.2% and 83.3% reduction in aflatoxin B_1 and B_2 levels respectively after boiling Koko for 10 minutes (Table 7). There was 47.6% and 68.9% reduction in B_1 and B_2 respectively in the preparation of Tuo zaafi after 20minutes. This confirms the higher thermolabile nature of aflatoxin B_1 previously observed by Kamimura (1989) and Kpodo et al., (1996)

In recent studies at the central Food Technological Research Institute, Mysore, India, destruction of nearly 70% of aflatoxins was recorded upon cooking of rice under steam pressure (Fashiha et.al.,unpublished data). Cooking at atmospheric pressure can destroy about 50% of the toxin. Pressure cookers are now being extensively used for cooking food, particularly in urban households. This can help to minimize the hazards of this toxin to an appreciable degree.

Table 6: Aflatoxin levels of dry millet flour from ten markets in Gt. Accra Region

MARKET	Aflatoxin content (µg/ kg)				Total Aflatoxins (µg/kg)
	G_1	G_2	B_1	B_2	
AGBOGBLOSHIE	ND	ND	ND	ND	ND
MAAMOBI	ND	ND	19.4	3.0	22.4
MADINA	ND	ND	35.2	3.6	38.8
MAKOLA	ND	ND	75.1	4.7	79.8
MALLAM ATTAH	ND	ND	ND	ND	ND
MAMPROBI	ND	ND	25.3	8.9	34.2
KANESHIE	ND	ND	0.9	0.4	1.3
NIMA	5.7	ND	20.4	1.4	27.5
LA	ND	ND	ND	ND	ND
OSU	ND	ND	9.3	1.4	10.7

ND= Not detected

Detection limits: B_1 and B_2 = 0.04µg/kg

G_1 and G_2 = 0.06µg/kg

Table 7

Effect of heat treatment on aflatoxin B_1 & B_2 levels in millet koko (Heating was at 100°C)

Cooking time (minutes)	*Aflatoxin content (µg/kg)	
	B_1	B_2
0	4.5±0.1	1.2±0.1
5	3.7±0.2	0.5±0.02
10	2.6±0.4	0.2±0.02

* Values are means of two determinations ± S.D. on as is basis.

Table: 8

Effect of heat treatment on aflatoxin B_1 & B_2 levels in millet Tuo zaafi (Heating was at 100°C)

Cooking time (minutes)	*Aflatoxin content (µg/kg)	
	B_1	B_2
0	1.91±0.20	0.45±0.06
5	1.02±0.06	0.19±0.01
10	0.97±0.06	0.11±0.01
15	1.55±0.04	0.20±0.03
20	0.99±0.10	0.14±0.02

*Values are means of two determinations ± S.D. on as is basis.

Dry roasting and oil roasting of groundnuts reduces aflatoxin levels to a significant degree. About 65% reduction in aflatoxin B_1 level by oil roasting and about 69 percent reduction by dry roasting has been reported (Eschor et al., 1973). Dry roasting was found to reduce by 40 – 50% the aflatoxin B_1 content originally present (Waltking, 1971).

The distribution of aflatoxins among various product fractions when the toxin contaminated maize is milled has been carried out by Yahl et.al, (1971). Grits contained only one tenth of the aflatoxin concentration in the lot of whole grain levels from which they were produced. Aflatoxin B_1 level of meal was 13-16% of that in whole maize and for flour the level was about 30-70% depending on initial aflatoxin concentration.

It will be interesting to find an effective way of reducing or preventing mycotoxin contamination especially in millet and other cereals in Ghana.

Fermented millet dough is used to prepare a number of foods including Marsa, Koko, and Tuo zaafi. Koko is porridge served for breakfast. Its importance lies in the fact that millet koko is used as a weaning food for babies especially in the Northern Regions of the country. Tuo zaafi is a stiff porridge product, which is kneaded during cooking for about 20 minutes. It is served with soup usually for supper in the Northern Regions of Ghana..

Ghanaian maize and maize products contain relatively high levels of mycotoxin namely aflatoxins and citrinin (Kpodo et al. 1996). Although aflatoxins are known to be relatively stable, cooking has been found to reduce the levels in some foods and this has been attributed to the formation of water adducts, aflatoxins B_2 and G_2 (Pohland et al., 1968).

Studies have shown that aflatoxins persist throughout the traditional steeping and fermentation processes as practiced in Ghana. However, during the 3 hours cooking of Ga kenkey (an end product of fermented maize) aflatoxin B_1 and G_1 were reduced by 80% whilst aflatoxin B_2 and G_2 were reduced by 35% and citrinin was no longer detectable (Kpodo et al., 1996).

From this study, it was observed that the process of millet koko preparation leads to some appreciable reduction of aflatoxin levels (Table 7).

Wet-milling of maize is used to produce starch, oil and other products. Studies on aflatoxin contamination during processing show that starch, oil, and most other products are aflatoxin-free and that 80-90 percent of the aflatoxin is concentrated in the gluten feed fraction (steepwater, fibre and spent grain) which must be discarded or diverted to suitable uses (Yahl et. al., 1971). The possibility of loss of some level of aflatoxin can not be ignored in the light of the above findings.

Comparing millet with maize, phosphorus (average 339mg) is half as much, iron (average 9.8mg) is more than three times, and calcium (average 37mg) is more that five times as much. Traces of barium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, silver, strontium, tin, titanium, vanadium, zinc and iodine are found in millet (India CSIR, 1966). The trace amount of zinc is particularly of interest. This is because the element is reported to enhance the production of aflatoxins by *A. flavus*. This may be a contributory factor in the recorded levels of aflatoxins in the samples analyzed which are relatively low as compared to levels reported for maize by Kpodo (1996).

The nature or composition of the soil, the variety of the plant, and environmental conditions could influence the type of mycoflora to be encountered from region to

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The nature or composition of the soil, the variety of the plant, and environmental conditions could influence the type of mycoflora to be encountered from region to

region. This study should therefore be extended to cover the other regions in the country. Moulds and mycotoxin (aflatoxin B_1 and B_2) contamination of millet should stimulate increased concern for appropriate harvesting and storage of the commodity in this country.

The potential role of aflatoxins and other mycotoxins in cancer should be sufficient incentive for further investigations, especially in Africa, particularly Ghana where the occurrence and significance of mycotoxins has not yet been fully assessed. While it may be possible to produce a food supply completely free of mycotoxins, improvements in storage and handling of millet grains and other commodities can minimize mould growth, and consequently reduce the risk of mycotoxin contamination in food supplies.

SUGGESTIONS FOR FURTHER WORK

1. It may be prudent to investigate the storage structures and materials used in the storage process to ascertain their suitability or otherwise.

2. Increasing cooking times for koko and Tuo zaafi and the consequences on other properties is worth considering.

3. The study should be extended to cover the other regions to give it a national outlook.

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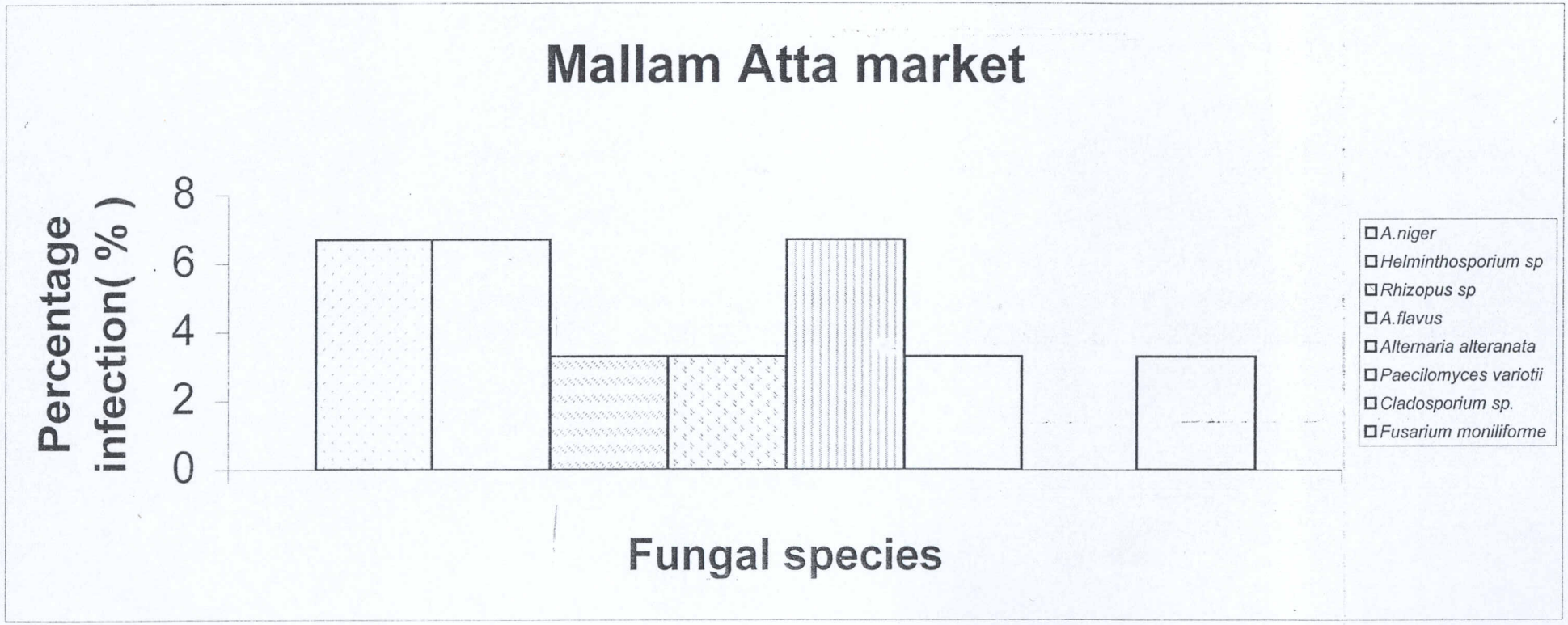
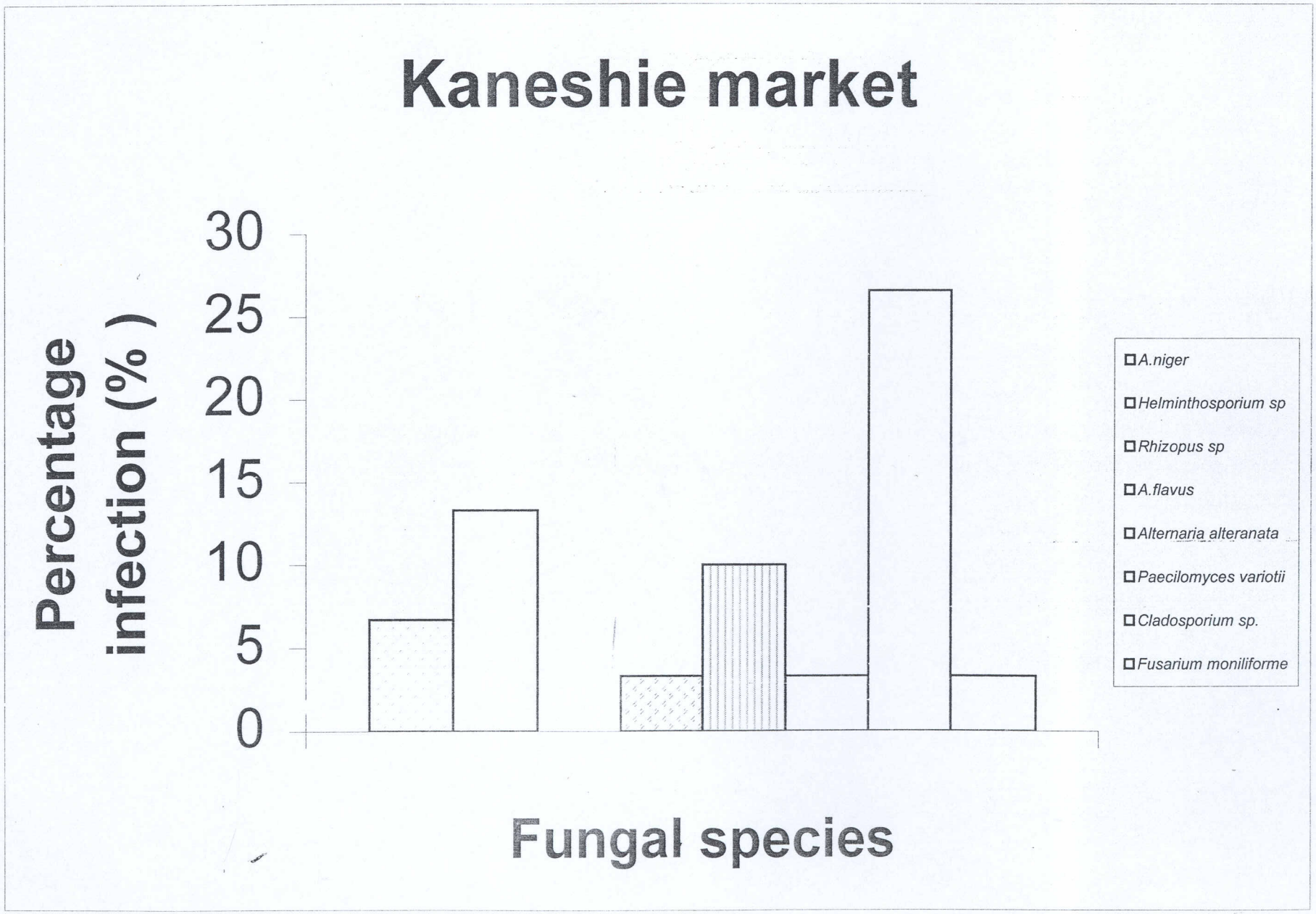
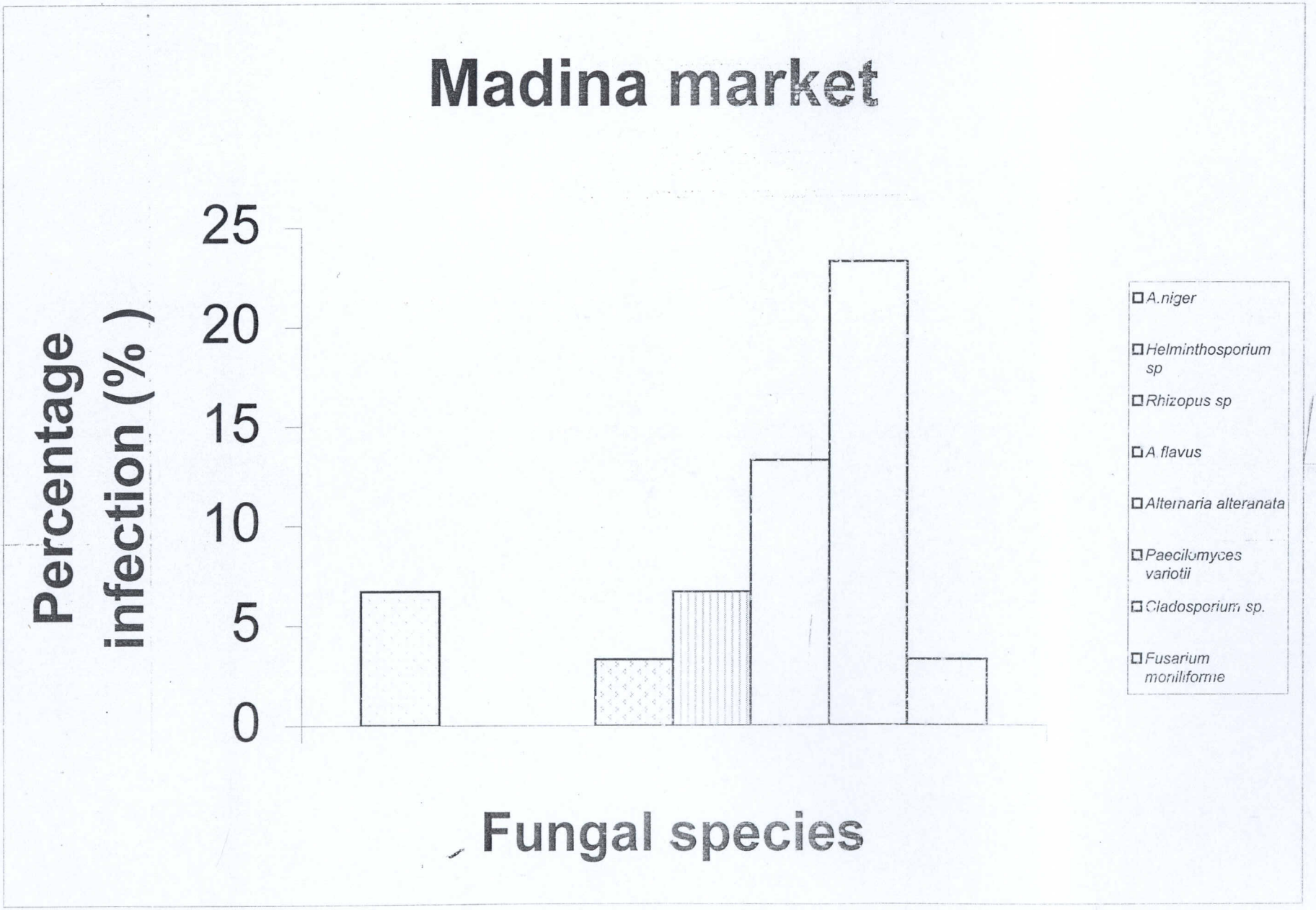
Appendix 1. Percentage infection of specific fungal species

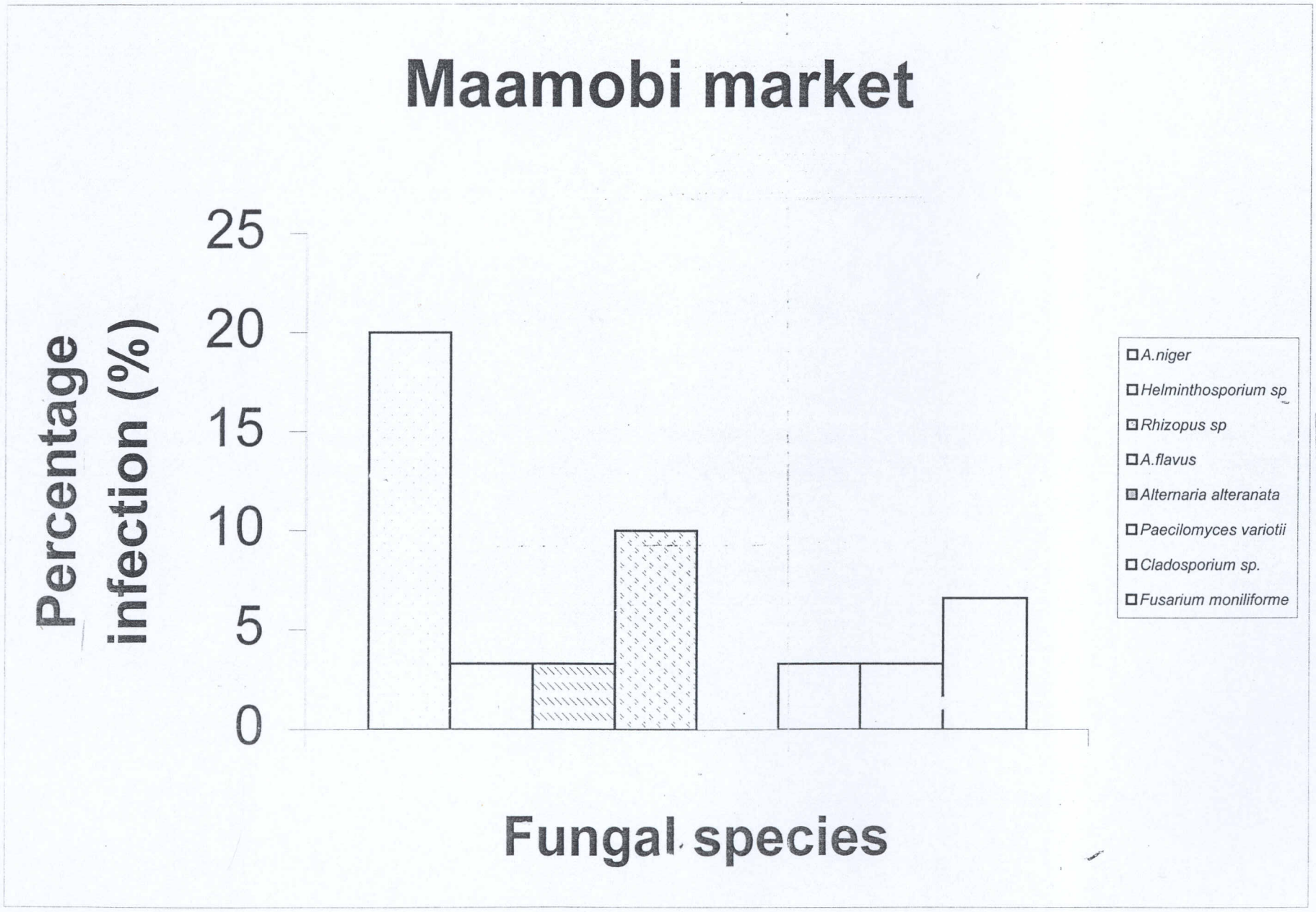


Appendix 2. Percentage infection of specific fungal species

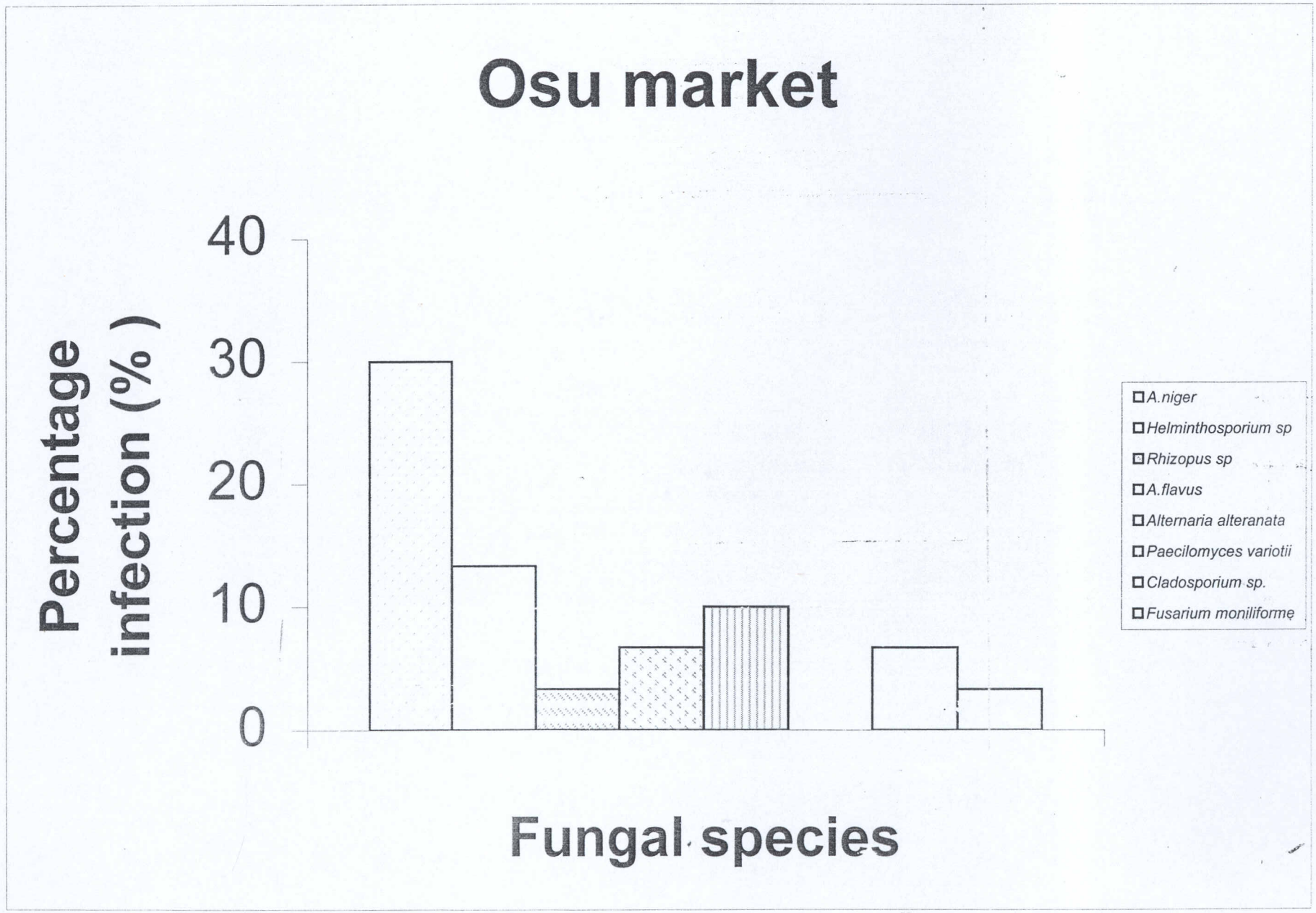



Madina market
Percentage infection (%)
25
20
15
10
5
0
Fungal species
A.niger
Helminthosporium sp
Rhizopus sp
A flavus
Alternaria alteranata
Paecilomyces variotii
Cladosporium sp.
Fusarium moniliforme

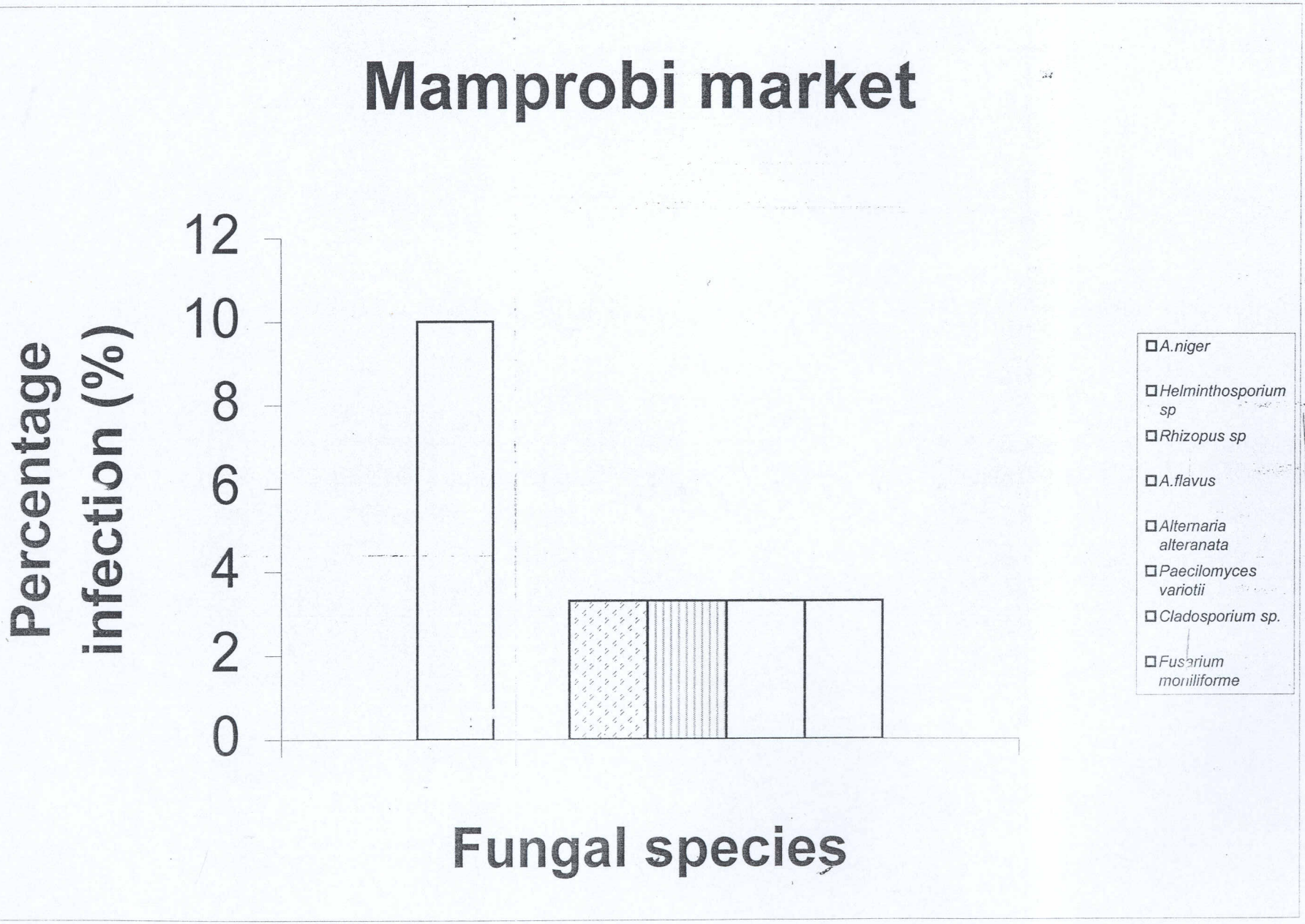
Appendix 4. Percentage infection of specific fungal species



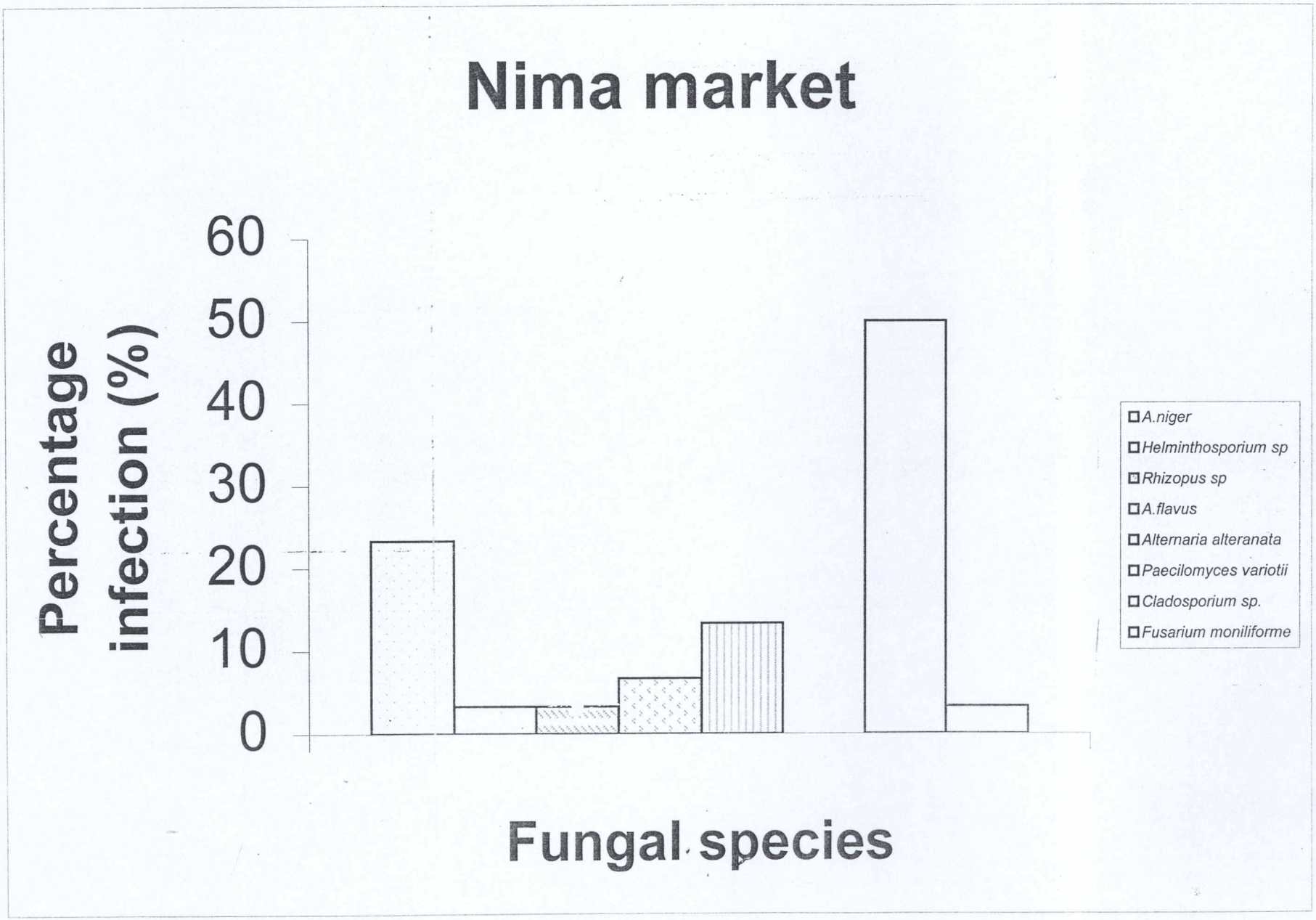
Appendix 5. Percentage infection of specific fungal species



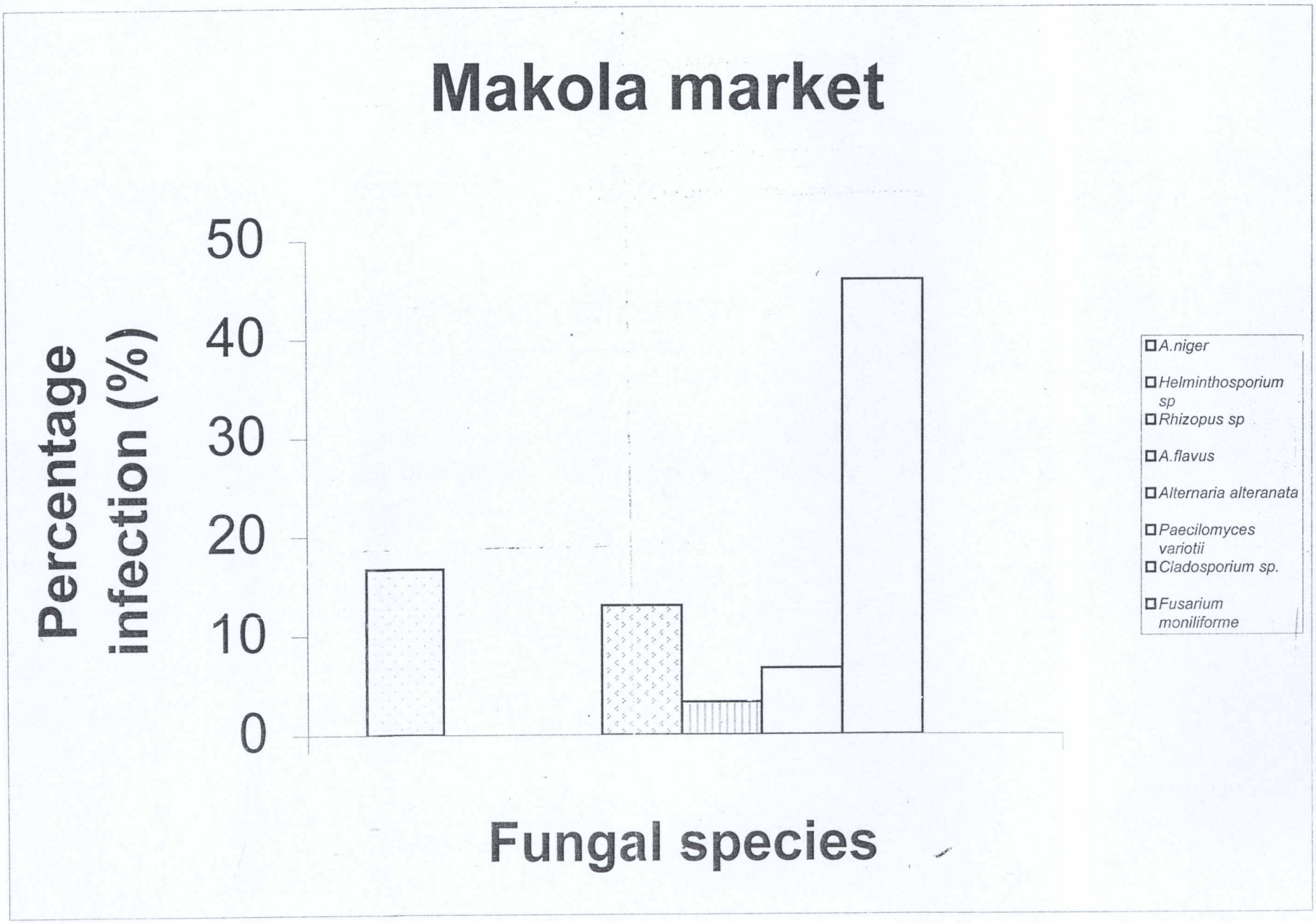
Appendix 6. Percentage infection of specific fungal species



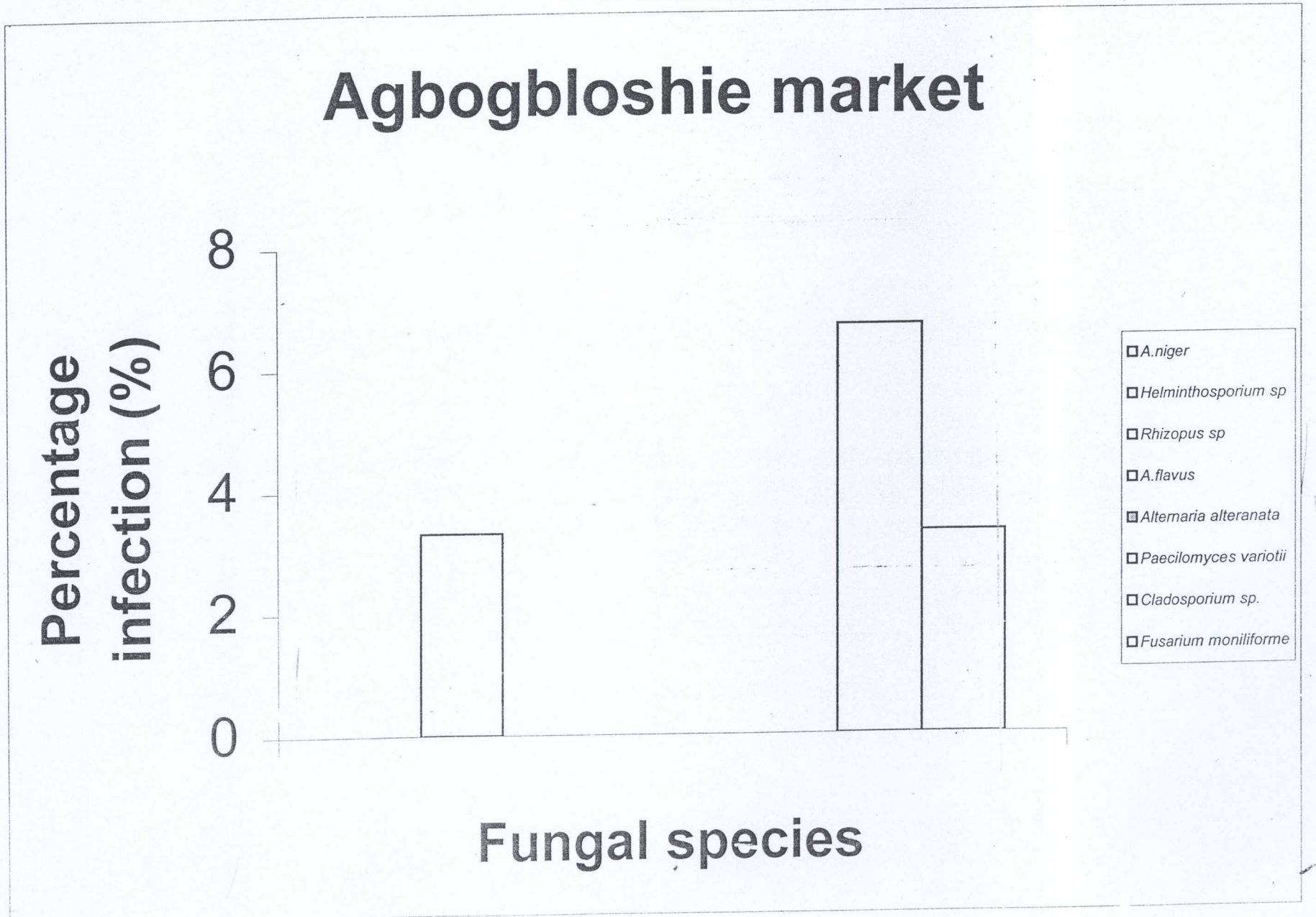
Appendix 7. Percentage infection of specific fungal species



Appendix 8. Percentage infectionof specific fungal species



Appendix 9. Percentage infection of specific fungal species



Appendix 10. Percentage infection of fungi of the various markets