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# Detection and Enumeration of Moulds on Some Legumes and a Cereal Grain from Two Local Markets and Two Shopping Malls in the Accra Metropolis

**Andrew Amegbedzi Minamor[1*] and Beatrice Adu Appiagyei[1]**

*[1]Department of Science Laboratory Technology, Accra Technical University, P.O.Box GP 561, Accra, Ghana.*

***Authors' contributions***

*This work was carried out in collaboration between both authors. Author AAM conceived and designed the experiments. Authors BAA and AAM carried out the bench work and analyzed the data. Author AAM developed the structure and arguments for the paper. Authors BAA and AAM wrote the first draft of the manuscript. Author AAM made critical revisions and approved final version. Both authors read and approved the final manuscript.*

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## ABSTRACT

This study detected and enumerated moulds associated with seeds and cereal grains under different packaging systems displayed for sale at two different purchasing sites; open market and supermarket. Two popular open markets and supermarkets were used for the investigation. The moulds were isolated on two mycological media, Malt Extract Agar (Oxoid CM545) and Dichloran Rose Bengal Chloramphenicol Agar (CM 041) using decimal Serial Dilution and Pour Plate methods. Four fungal genera, *Aspergilus, Fusarium, Penicilium* and *Mucor* were isolated on maize, cowpea, peanuts and bambara beans purchased from the two sampling points. *Aspergilus* sp

**Corresponding author: E-mail: minamorandrew@yahoo.com;*

predominated with 31.9% isolation followed by Fusarium *verticillioide* (= *Fusarium moniliforme)* with 20.1%, *Penicilium digitatum*, 8.1% and *Mucor* spp 0.2% isolation on the samples from the open markets. A similar trend was observed on the samples from the supermarkets with much lower percentage occurrence; *Aspergillus* sp. 12.2%, *Fusarium* sp. followed with 3.3%, and *Penicilium* sp. with 0.6% isolation. *Mucor genus*, however, was isolated only on bambara beans from one of the open markets. Five *Aspergilus* species namely; *A. flavus* > *A. niger* > *A. fumigatus* >*A. ochraceus* > *A. sulphureus* in decreasing number of magnitude were isolated from the samples from the open markets. The samples from the supermarkkets also registered five *Aspergillus* sp. in this order of decreasing magnitude; *A. flavus* > *A. niger* =*A. fumigatus* > *A. sulphureus*> *A. ochraceus*. *Penicillium digitatum* and *Fusarium verticillioide* (=*Fusarium moniliforme)* were also isolated on the seed/grain from both open markets and the supermarkets. Most of the species isolated were mycotoxins producing moulds.

*Keywords: Aspergillus* sp*; Penicillium digitatum; Mucor; Fusarium verticillioide; isolation.*

## 1. INTRODUCTION

Cereals are grains or edible seeds of the grass family *Gramineae*. They are grown for their highly nutritious edible seeds, which are often referred to as grains [1]. Cereal grains are universally important as a major source of carbohydrate. They occupy a special position as products capable of long storage because they are stored after drying to a very low moisture level (not more than 8%). All the ancient civilizations were based on the cultivation of grains - Egypt on wheat, barley and rye; Rome, Greek and Europeans rather cultivated wheat. China and the Orient were noted for rice cultivation whereas Central America was noted for maize cultivation. When dried properly, the cereal grains can be transported long distances and they constitute one of the major components of International trade. In Ghana, the major cereal grains produced are maize, millet, sorghum and rice. Various seeds are produced in large quantities and are commercially very important. The major ones are legumes especially groundnuts, cowpea, bambara groundnuts, pigeon pea and to a lesser extent other seeds like *Citrullus* sp. Unfortunately, cereal grains and legumes all suffer spoilage to different degrees caused mainly by fungi.

The filamentous fungi that occur on cereal grains are divided into two categories; field fungi and storage fungi. Field fungi are those that invade seeds on the developing plants in the field when the grain is high in moisture. Some few example of such fungi are *Alternaria tenuis*, *Fusarium verticilliodes* (= *Fusarium moniliforme)* and *Epicoccum nigrum*. Storage fungi are those that invade grains in storage at lower moisture contents. Most of the storage mycobiota are species of *Aspergillus*, *Penicillium* and *Eurotium*. There are several other species of lesser frequency like *Curvularia* sp. *Sporendonema* sp. Fungal deterioration can be severe under prolonged storage at moderately high humidity. The major effects of fungal deterioration of grains include decreased germination, discoloration, development of visible mould growth, development of off-odour, dry matter loss, caking that is, large masses of kernel bound together and the production of mycotoxins in the grains [2]. Mycotoxins are toxic secondary metabolites produced by filamentous fungi or moulds [3,4] most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Mycotoxins exhibit a range of toxicological properties, including acute toxicity or poisoning, which often results in death, sub-acute or chronic toxicity, which though not fatal can gradually weakens or lowers the general health of the animal or human due to its effect on the immune system. Some mycotoxins are carcinogenic that is cancer-causing, others are mutagenic and are capable of causing mutations, they may also be teratogenic and embryo toxic, causing deformities and death in developing embryo [5].

Many factors such as humidity, high temperature, handling of products, like harvesting, shelling, transportation, storage and packaging promote fungal spoilage of cereal grains and legumes. The principal roles of food packaging are to protect food products from outside influences and damage, to contain the food and to provide consumers with ingredient and nutritional information [6]. Packaging must preserve product integrity by protecting the actual product against potential damage from climatic, microbiological and transit hazards [7]. In Ghana, as in many African countries, cereal grains are sold mainly in the local markets and supermarkets with different

storage conditions and kept through various packaging systems before reaching the consumers from different purchasing sites. In the local markets commodities particularly cereal grains and legumes are displayed openly in basins, pans, baskets, sacks the grains are exposed to dust and other possible contaminants [8].

In contrast to the local markets, commodities like cereal grains and legumes are well-packaged in sealed polythene bags and bottles arranged orderly on shelves to attract potential buyers. Cereal grains and legumes are staple foods in many countries and cultures they also serve as raw materials for many of our foods and certain beverages. Because of their extensive use as human foods and livestock feeds the microbiology and safety of cereal grains, legumes and their products is a very important area [2]. This paper sought to identify and enumerate moulds on cereal grains/seeds from two purchasing sites, the local market and the supermarket in the Accra metropolis to ascertain the effects of packaging and storage on the levels of mould contamination.

## 2. MATERIALS AND METHODS

### 2.1 Materials

One cereal grain and three legumes namely; Maize (*Zea mays*), groundnut (*Arachis hypogaea*), Bambara beans (*Vigna subterranea*) and (*Vigna unguiculata*) respectively were bought from two popular local markets Kaneshie and Makola and two popular Supermarkets, Shoprite and Marina Mall all in the Accra Metropolis. The samples were put into an ice-chest and transported to the Microbiology Laboratory for analysis. Two mycological media Malt Extract Agar (Oxoid CM545) and Dichloran Rose Bengal Chloramphenicol Agar (Oxoid CM041) were used for the isolation.

### 2.2 General Methods

#### 2.2.1 Sterilization of glassware

All glassware were properly washed in soapy water, thereafter rinsed in running tap-water. The Petri dishes, test tubes, and culture bottles were allowed to dry upside down on a laboratory work bench. The Petri dishes were then packed into appropriate canisters and then placed in a pre-heated hot -air oven at 160℃ for 1 hr.

#### 2.2.2 Preparation of media

The Malt Extract agar and Dichloran Rose Bengal Chloramphenicol Agar were prepared following the manufacturer's directions.

#### 2.2.3 Serial-dilution method and inoculation

A 10 g of each sample of seeds was weighed and transferred aseptically into 100 ml of 0.1% Peptone in 250 ml Erlenmeyer flasks and then shaken in Gallenkamp Model Orbitar shaker at 140 rev/min for 30 mins. From this stock suspension, decimal serial dilution method was employed up to $10^{-4}$. 1ml aliquot of each dilution level was inoculated into appropriately labeled Petri dishes containing either Malt Extract Agar or Dichloran Rose Bengal Chloramphenicol Agar. Two replicates of each dilution level were poured using Pour Plate method for each medium used. The Petri- plates were incubated at 28-31℃ until fungi grew (5-10 days). The objective of using two media is to recover a wide range of fungal species from the seeds.

#### 2.2.4 Identification of fungi

Fungi encountered in these investigations were identified by their colour, culture and morph ological characteristics using the conventional identification manual of fungi [9-11].

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Four fungal genera namely *Aspergillus, Fusarium, Penicillium*, *Muco*r were isolated from legumes and a cereal/grain from two markets and two shopping malls. The number and percentage occurrence of the fungal species are shown in the tables below.

### 3.2 Discussion

Being seasonal crops, particularly in sub-Sahara Africa, cereal grains/legumes are stored as dried grains and form enormous reserve of food for the lean seasons [12]. Many factors regrettably, encourage food spoilage in Ghana. For instance, high uniform temperature supporting growth of mesophiles, high atmospheric humidity, in addition, there is lack of standardization for drying of grains and seeds. Some are kept in storage with rather high internal moisture levels which support growth of microorganisms. Handling of the products such as harvesting,

shelling, transportation during which the products are bruised, wounded, cracked among others making them susceptible to wound infection. Quite considerable amount of the products are kept under un-protective conditions rendering them liable to contamination.

**Table 1. Number and percentage occurrence of fungi isolated from a cereal grain and some legumes bought from an open market (Makola) in the Accra metropolis**

| Fungal genera | Number (n) and occurrence (%) on indicated seeds from Makola Market | | | |
|---|---|---|---|---|
| | **Maize (n)(%)** | **Cowpea (n)(%)** | **Groundnut (n)(%)** | **Bambara beans (n)(%)** |
| *Aspergillus* | (53)(61.6)) | (18)(29.5) | (25)(47.1) | (21)(75.0) |
| *Penicillium.* | (4)(4.6) | (13)(21.3) | (9)(16.9) | (5)(17.8) |
| *Fusarium* | (29)(33.7) | (30)(49.1) | (19)(35.8) | (2)(7.1) |
| *Mucor* | - | - | - | - |
| **Total** | **86** | **61** | **53** | **28** |

**Table 2. Number and percentage occurrence of fungi isolated from a cereal grain and some legumes bought from an open market Kaneshie in the Accra metropolis**

| Fungal genera | Number (n) and occurrence (%) on indicated seeds from Kaneshie Market | | | |
|---|---|---|---|---|
| | **Maize (n)(%)** | **Cowpea (n)(%)** | **Groundnut (n)(%)** | **Bambara beans (n)(%)** |
| *Aspergillus* | (9)(42.8) | (31)(54.3) | (24)(85.7) | (20)(95.2) |
| *Penicillium.* | (4)(19.0) | (6)(10.5) | (3)(10.7) | - |
| *Fusarium* | (8)(38.0) | (20)(35.0) | (1)(3.5) | - |
| *Mucor* | - | - | - | (1)(4.3) |
| **Total** | **21** | **57** | **28** | **21** |

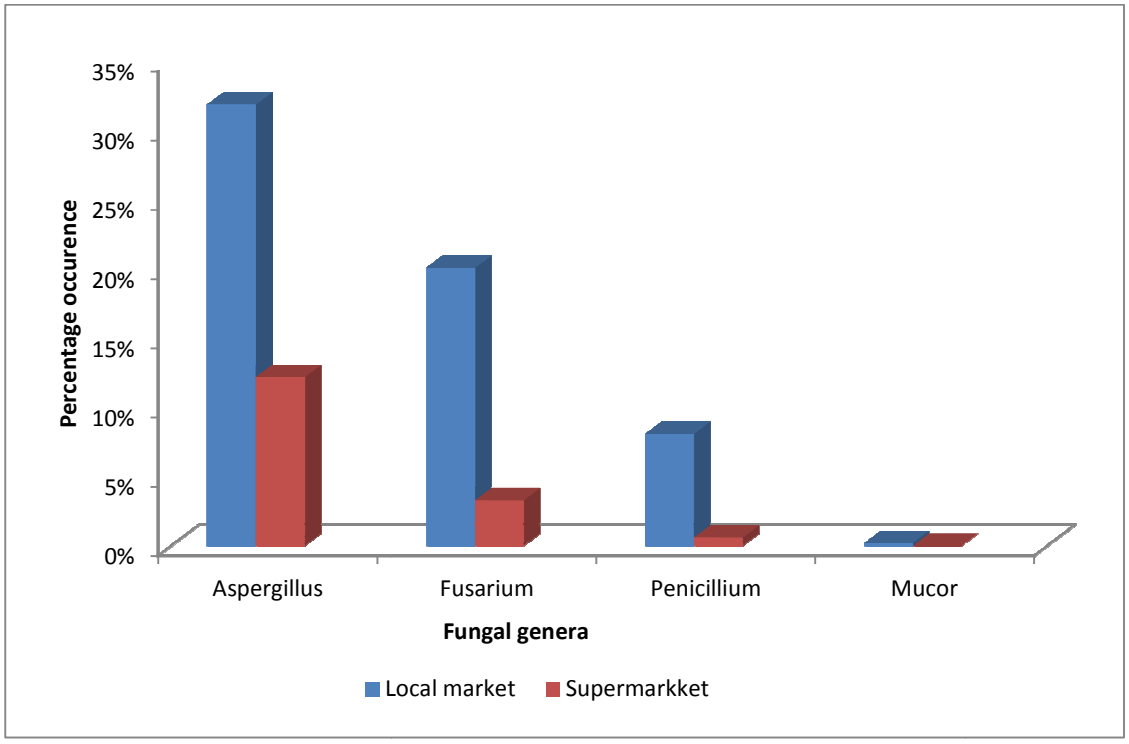


**Fig. 1. Pooled data of fungal genera isolated from a cereal grain and some legumes bought from two popular local markets and two supermarkets in the Accra metropolis**

**Table 3. Number and percentage occurrence of fungi isolated from a cereal grain and some legumes bought from a popular supermarket (A) in the Accra metropolis**

| Fungal genera | Number (n) and occurrence (%) on indicated seeds from supermarket (A) | | | |
|---|---|---|---|---|
| | **Maize (n)(%)** | **Cowpea (n)(%)** | **Groundnut (n)(%)** | **Bambara beans (n)(%)** |
| *Aspergillus* | (10)(76.9) | (18)(90.0) | (2)(5.0) | (13)(86.6) |
| *Penicillium.* | - | - | - | - |
| *Fusarium* | (3)(23.0) | (2)(10.0) | (2)(50.0) | (2)(13.3) |
| *Mucor* | - | - | - | - |
| **Total** | **13** | **20** | **4** | **15** |

**Table 4. Number and percentage occurrence of fungi isolated from a cereal grain and some legumes bought from a popular supermarket (B) in the Accra metropolis**

| Fungal genera | Number (n) and occurrence (%) on indicated seeds from supermarket (B) | | | |
|---|---|---|---|---|
| | **Maize (n)(%)** | **Cowpea (n)(%)** | **Groundnut (n)(%)** | **Bambara beans (n)(%)** |
| *Aspergillus* | (8)(53.3) | (5)(71.4) | (5)(71.4) | (5)(83.3) |
| *Penicillium.* | (2)(13.3) | - | (1)(14.3) | - |
| *Fusarium* | (5)(33.3)) | (2)(28.6) | (1)(14.3) | (1)(16.7) |
| *Mucor* | - | - | - | - |
| **Total** | **15** | **7** | **7** | **6** |

In this investigation, four genera namely, *Aspergilus, Fusarium, Penicilium* and *Mucor* were isolated on maize, cowpea, groundnuts and bambara beans purchased from two popular local markets and two supermarkets in the Accra metropolis. *Aspergilus* sp. predominated with 31.9% followed by *Fusarium verticillioides* (= *Fusarium moliniforme)* 20.1% then *Penicilium* sp. with 8.1% and *Mucor* sp. 0.2% on the samples from the local markets. A similar trend was observed on the samples from the supermarkets investigated with much lower percentage occurrence. *Mucor* sp. however, was only isolated on bambara beans purchased from Kaneshie market (Table. 2). The five *Aspergilus* species isolated were *A. flavus* > *A. niger* > *A. fumigatus* > *A. ochraceus*> *A. sulphureus* in decreasing number of magnitude were observed. The samples from the supermarkets also registered the following *Aspergilus* species; *A. flavus* > *A. niger= A. fumigatus*> *A. sulphureus* > *A. ochraceus* in decreasing magnitude. *Penicilium digitatum* and *Fusarium verticillioides* (= *Fusarium moniliforme)* were also isolated from the seeds/grains at the two sampling points (Tables 5 and 6).

The two genera *Aspergillus* and *Penicillium* encountered in this investigation are storage fungi while the *Fusarium* sp. is a field fungi [2]. A search through pertinent literature reveals that the *Mucor* sp. isolated in this investigation, has for the first time been isolated from bambara beans sampled from the Kaneshie Market in the Accra metropolis (Table 2).

Microbiological heating by invading insects or mites in stored grains and seeds coupled with the activities of storage fungi in grains contribute to damaging of the stored grains and seeds. Heating of grains and seeds has been discussed extensively by [13,14]. The effects of storage fungi on seeds and grains has been dealt with extensively by [15].

*Fusarium verticillioides* (= *F. moniliforme*) might have been carried from the field to storage in a symptomless state [16,17]. Respiration activities of storage fungi and invading insects and mites might have produced water sufficient enough to support the growth of *Fusarium verticillioides* (= *F. moniliforme*) to keep them in storage. The total percentage occurrence of *Fusarium verticillioides* (= *F. moniliforme*) from the open markets was 20.1% whereas the supermarkets registered 3.3% (Fig. 1). *Fusarium verticillioides (= Fusarium moniliforme)* is one of the most prevalent fungi associated with maize - a basic human and animal dietary staple [18]. Studies in South Africa [18] and in China [19-21] have shown that cultures of *Fusarium verticillioides (= F. moniliforme)* on maize and maize products can cause cancer in rats; thus, the danger to human and animal health of *Fusarium verticillioides (= F. moniliforme)* infested maize is self evident [22]. Furthermore, it is well

established that *Fusarium verticillioides (= F. moniliforme)* can be internally seed-borne in symptomless apparently healthy maize kernel [16,17]. At least two classes of mutagens are formed by *Fusarium verticillioides (= F. moniliforme* namely *Fusarin* C [23,24] and *Moniliformin* [25-27]). *Aspergillus ochraceus* forms *ochratoxin* A in maize [28] as well as other mycotoxins such as emodin, kojic acid, neospergilic acids, penicillic acid and secatonic acid. *Penicilium digitatum* form *patulin i*n cultures whereas *Aspergillus flavus* produces different kinds of *aflatoxins* which are carcinogenic.

Mycotoxins on seed/ grains is of global health concern which need to be tackled with all the seriousness it deserves. Unfortunately, these mycotoxin producing moulds were all isolated from the seeds grains from the two sampling points, local market and supermarket albeit with less percentage occurrence on the seed/grain from the supermarket.

## 4. CONCLUSION

In this study, four genera namely *Aspergillus, Fusarium, Penicillium*, and *Mucor* were isolated from maize, cowpea, groundnuts and bambara beans purchased from two local markets and two supermarkets in the Accra metropolis. *Aspergillus* spp predominated with 31.9% occurrence followed by *Fusarium* sp. 20.1%, *Penicillium* spp. 8.1% and *Mucor* sp. 0.2%. The *Mucor* sp. was isolated for the first time on bambara beans from the open market. A similar trend was observed on the samples from the supermarkets albeit with much lower percentage occurrences.

Five *Aspergillus* species (*A. flavus, A. fumigatus, A. ochraceus, A. sulphureus*, *Penicillium* spp. *Fusarium verticillioides* (= *F. moniliforme*) all at varied degree of occurrences at the two sampling sites.

Higher number of fungi were recorded in samples from the local markets than the supermarket. This may be due to the open exposure of the grains/seeds to dusts, high temperatures high relative humidity etc compared with well-packaged grains/ seeds normally found in the supermarkets. The data in this paper indicate that proper packaging and storage may drastically reduce the number of storage fungi on grains/seeds displayed for sale.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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# APPENDICES

## Appendix 1a

**Table 5. Various fungal species isolated on indicated seeds bought from two popular local markets in the Accra metropolis**

| Site | Fungal species isolated on indicated seeds | | | |
|---|---|---|---|---|
| | **Maize** | **Cowpea** | **Groundnut** | **Bambara beans** |
| Kaneshie market | *Aspergillus flavus*<br>*A. sulphureus*<br>*A. niger*<br>*Fusarium* sp<br>*Penicillium* sp | *A. niger*<br>*A. flavus*<br>*A. ochraceus*<br>*A. fumigatus*<br>*Fusarium* sp<br>*Penicillium* sp | *A. flavus*<br>*A. sulphureus*<br>*A. niger*<br>*A. fumigatus*<br>*Fusarium* sp<br>*Penicillium* sp | *A. niger*<br>*A. ochraceus*<br>*A. flavus*<br>*Mucor* sp |
| Makola market | *A. flavus*<br>*A. fumigatus*<br>*A. niger*<br>*Fusarium* sp<br>*Penicillium* sp | *A. niger*<br>*A. flavus*<br>*A. ochraceus*<br>*A. fumigatus*<br>*Penicillium* sp | *A. flavus*<br>*A. niger*<br>*A. fumigatus*<br>*A. sulphureus*<br>*Fusarium* sp<br>*Penicillium* sp | *A. niger*<br>*A. flavus*<br>*Fusarium* sp<br>*Penicillium* sp |

## Appendix 1b

**Table 6. Various fungal species isolated on indicated seeds bought from two popular shopping malls in the Accra metropolis**

| Site | Fungal species isolated on indicated seeds | | | |
|---|---|---|---|---|
| | **Maize** | **Cowpea** | **Groundnut** | **Bambara beans** |
| Shopping Mall A | *Aspergillus flavus*<br>*A. sulphureus*<br>*Fusarium* sp | *A. niger*<br>*A. flavus*<br>*A. ochraceus*<br>*A. sulphureus*<br>*A. fumigatus*<br>*Fusarium* sp | *A. fumigatus*<br>*A. flavus*<br>*Fusarium* sp | *A. niger*<br>*A. flavus*<br>*Fusarium* sp |
| Shopping Mall B | *A. flavus*<br>*A. fumigatus*<br>*A. ochraceus*<br>*A. sulphureus*<br>*Fusarium* sp<br>*Penicillium* sp | *A. niger*<br>*A. flavus*<br>*A. ochraceus*<br>*A. fumigatus*<br>*Fusarium* sp | *A. niger*<br>*A. fumigatus*<br>*A. sulphureus*<br>*Fusarium* sp<br>*Penicillium* sp | *A. flavus*<br>*A. niger*<br>*Fusarium* sp |

**Appendix 2a**

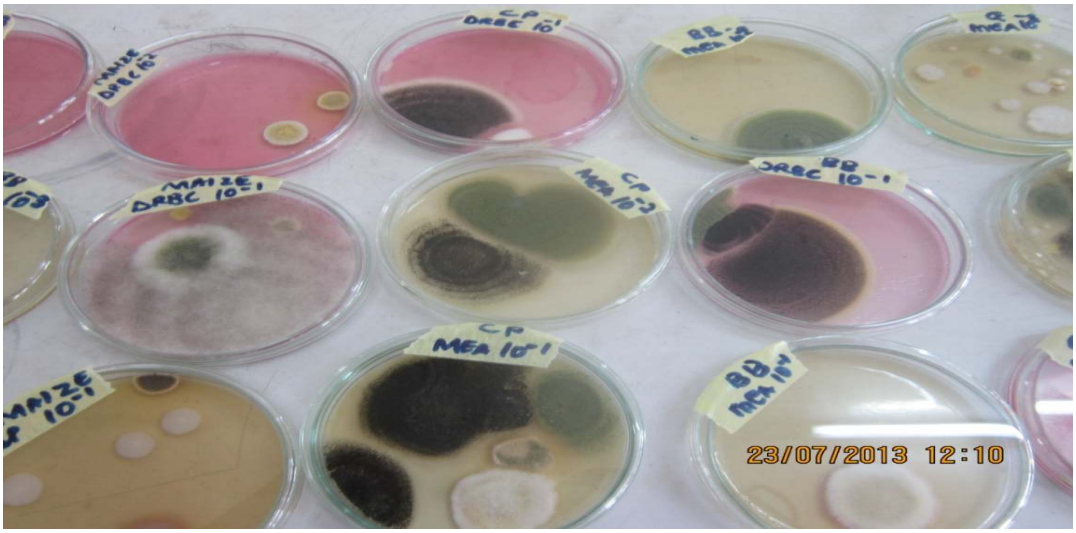


**Fig. 2. Growth of some moulds from cereals and grains bought from the supermarkets on DRBC and MEA incubated at 31±2℃ after seven (7) da y**

**Appendix 2b**

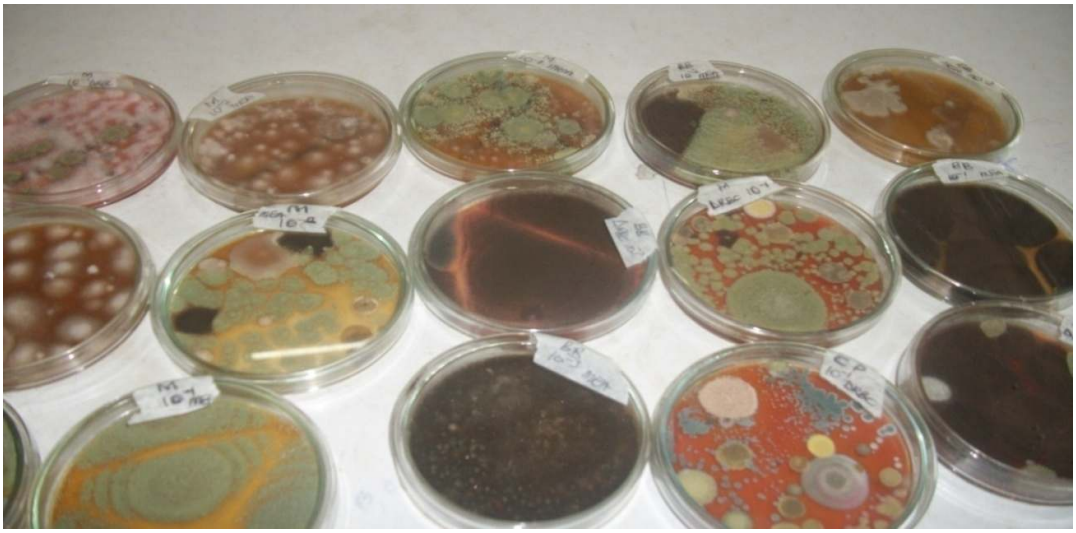

**Fig. 3. Growth of some moulds from cereals and grains bought from the Local markets on DRBC and MEA incubated at 31±2℃ after seven (7) da ys**



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