Journal of Health, Medicine and Nursing
ISSN 2422-8419 An International Peer-reviewed Journal
Vol.109, 2023

Occurrence of Fungi of Public Importance in Rodents Trapped along and inside Grain Storage Facilities in Mbeya Municipal, Tanzania.

Anna P. Mollel[1*] Benign. A. Temba[2] Abdul S. Katakweba[3]

1. Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O BOX 3015, Chuo Kikuu, Morogoro Tanzania

2. Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O BOX 3021, Chuo Kikuu, Morogoro Tanzania

3. Institute of Pest Management, the Sokoine University of Agriculture P.O.BOX 3110, Morogoro Tanzania

*Email of the corresponding author: annaperia.rl@gmail.com

This work was supported by the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM and BTD) at the Institute of Pest Management of the Sokoine University of Agriculture.

Abstract

Rodents act as agents for the dispersal of pathogenic entities including fungi and enable their colonization in new areas. They interact with the human environment which acts as a route for the transmission of pathogens. A total of 210 rats were trapped in and along the storage facilities in selected wards in Mbeya city. Fresh fecal samples were collected from the intestines by dissecting the abdominal part of the rodent to obtain pellets. Samples were kept in clean envelopes and preserved at -20 ^{0}C at Mbeya National Research Institute (NIMR) for further laboratory analysis. Fungi were isolated by culturing in selective media and identification was done by colony morphology. Further confirmation of the isolated *Aspergillus flavus* was done by nested PCR to confirm the presence flR the gene in the isolates. Aflatoxigenicity of the isolated A. flavus was tested with a controlled experiment in which non-contaminated maize kernels were inoculated with the fungal spores and incubated for up to 15 days and accumulation of the aflatoxin analyzed by indirect competitive ELISA. *Aspergillus fumigatus* was the dominant fungal specie from the cultured samples, with a prevalence of 26% followed by *Aspergillus niger* and *Fusarium* species, both with a prevalence of 9%, *Aspergillus flavus* 3% and *Aspergillus ochraceus* 1%. Indirect competitive ELISA was performed on 10 maize samples that were infected with *A. flavus* isolates, 10 maize samples free from isolates contamination, and 4 pure isolates of *A. flavus* to check whether the isolates were potential producers of aflatoxins. The four pure isolates had a high concentration of aflatoxin compared to the samples contaminated with *A. flavus* isolates. These findings justify that rodents harbor pathogenic fungi in their intestinal tracts and act as dispersal agents of the fungi to foods and other human and animal premises. Effective control measures should therefore be applied in protecting foods and premises from rodents, especially mice and rats to minimize risks of disease spread.

Keywords: Pathogenic fungi, Rodents, Aspergillosis, dispersal, and mycotoxin-producing fungi.
DOI: 10.7176/JHMN/109-05
Publication date: July 30th 2023

1.0 INTRODUCTION

Rodents are among the mammals documented as pests due to several losses in agricultural crops including the stored grains in tropical areas (Singleton,2003). Despite losses in the agriculture sector rodents also impose serious risks to human and animal health as they interfere with food security and act as reservoirs of many pathogens and vectors that cause diseases (Meerburg et al., 2007; Ribas *et al*., 2016; Eisen et al 2018).

Apart from rodents and arthropods fungi are also among the organisms that inhabit stored grain environments (Frisvad and Samson, 1991). Urine from rodents may increase water activity in the affected area, which enhances the growth and development of molds due to nitrogen availability which in turn establishes storage fungi (Stejskal et al.,2005). Fungi are not only deleterious to stored crops but may also contaminate them with allergens and mycotoxins (Frisvad and Samson, 1991).

Most of the dispersal networks are accompanied by significant diverseness in species interactions, in such a way that some of the animal species attain more interactions compared to others making it more important to the structure of the dispersal community (Bascompte et al. 2006). Under numerous aspects, plants, and fungi depend on animals to ingest their fruits which led to the dispersal of propagules to new parts (Nathan et al. 2008).

Just like seed dispersal networks, mammals that consume fungi enable the dispersal of mycorrhiza fungi by consumption of fungal fruiting bodies, hence depositing spores through defecation (Stephens and Rowe, 2020), the pathogenic fungi introduced in new areas contaminate directly or indirectly foods, surrounding environments, humans, and animals which in turn contributes to major problems in the aspect of public health.
However, data on the isolation and identification of pathogenic fungi from various species of rodents found in or along the stored grain environment is currently not available. Therefore, this study aimed to isolate and identify pathogenic fungi from the feces of different rodent species captured inside and along the storage facilities in Mbeya Municipal, Tanzania. This will contribute to strategies for controlling the spread of pathogenic fungi to humans, animals, and plants.

2.0 MATERIAL AND METHODS

2.3 Description of the study area

This study was conducted in urban and peri-urban areas of Mbeya city in the southwest part of Tanzania. The city is located between latitudes -8.909401^0 South and longitudes 33. 460773^0 East and borders four districts which are Mbarali to the north, Ileje to the south, Rungwe to the East, and Mbozi to the west. Mbeya municipality is administratively divided into 36 wards, and the major economic activities in the area are commerce, agriculture, and livestock keeping, as well as small and large-scale industrial production (NBS, 2012). The wards covered in this study included Igawilo, Itende, Mwasenkwa, and Iziwa situated in peri-urban areas, and Iyela, Ruanda, Maendeleo, and Mabatini, all situated in the urban areas.
The pattern of rainfall in Mbeya is unimodally varying from 800 mm to 1800 annually, and temperature ranges between 16 °C and 20 °C (URT, 1997). Climate change includes wet and dry seasons; whereby dry seasons start from June to October while dry season starts from November to May.

2.4 Study design

The study employed a cross-sectional design. It was conducted from January to March 2022 whereby laboratory activities were done at Sokoine University of agriculture.

2.5 Sampling

Sampling involved the collection of fecal droppings from within the storage facilities and fresh fecal pellets from dissected rats.

2.6 Rodent trapping, identification, and characterization

The rodents were trapped using Sherman traps and locally made wire loop traps (H.B. Sherman Traps Inc., Tallahassee, USA). The traps were set between 5.00 pm and 6.00 pm and checked the next morning (between 7.00 am and 9.00 am to collect entrapped rodents. Captured rodents were carried in ventilated sacs to a temporary laboratory for species/genus identification using anatomical and morphometric parameters including body weight, head-body length, tail length, hind foot length, and ear length (Skinner and Chimimba, 2005). The rodents were also characterized for important biological features including sex and reproductive status.

2.7 Collection, preservation, and transportation of fecal samples

Captured rodents and identified rats were anesthetized using diethyl ether, aseptically dissected and fresh fecal pellets were collected from the rectum. The pellets were kept in a sterilized paper envelope containing silica gel packs for the absorption of moisture. The envelopes were wrapped and placed inside sterilized zipped nylon bags and preserved at -20 ^{0}C at Mbeya National Research Institute (NIMR) for further laboratory analysis.

2.8 Isolation and identification of fungi of public importance

Both of the collected fecal pellets were ground using a motor and pestle to form a fine powder, which was mixed with 2 ml of normal saline (that was prepared by suspending 0.85 g of NaCl in 1000 ml of distilled water) and shaken vigorously.
The obtained mixture was poured into two Petri dishes with Saboraud Dextrose Agar (SDA in which the manufacturer recommended 65.0 g to be suspended in 1000 ml of distilled water and Potato Dextrose Agar (PDA in which the manufacturer recommended 39.0 g to be suspended in 1000 ml of distilled water). Chloramphenicol (0.8 ml for every 100 ml of the broth media) was added to the PDA to inhibit bacteria growth while SDA is a general media for the cultivation of all yeast, molds, and aciduric bacteria. and it naturally inhibits the growth of bacteria. The Petri dishes were incubated at 35 ºC for up to 7 days. After 7 days' plates with visible fungal growth were sub-cultured to obtain pure colonies and facilitate isolation as well as identification. The macroscopic identification involved studying the characteristics of the colonies and microscopic observation was done by preparing fungal smears (Pitt and Hocking, 1997). For microscopic identification, a wet mount preparation technique was used whereby a spore colony was picked using a mounting

needle and placed in a clean and dry microscopic slide containing a drop of Lactophenol Cotton Blue (LPCB). Coverslip was placed on top of the slide and the preparation was left for about five minutes before observation (Leck, 1999). The observation was done at x10 magnification for setting up and then at x40 for fine visualization of fungal morphologies.

Subculture of aflatoxin-producing suspects

Aflatoxin-producing suspects (*Aspergillus flavus and Aspergillus parasiticus*) from Saboraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were sub-cultured in Malt Extract Agar (Raper and Fennell, 1965) at 25 ^{0}C for seven days. There were 10 suspects of *Aspergillus flavus* whereby each one was sub-cultured in three replicates. After seven days' growth was observed.

Introduction of the aflatoxin-producing suspects into maize kernels

Dry, free from damage, and fresh maize from the farm during the period of harvest were sterilized in the oven. Each of the sub-cultured A. flavus in MEA was added mixed with sterilized distilled water and swirled to allow the spores and conidia to detach. The suspension was poured into a clean universal bottle and about 5 grams of adjacent maize kernels were added and soaked overnight. On the next day-soaked maize kernels were transferred to the petri dish containing wet cotton so as to retain moisture and provide the best condition for fungal activity. The negative controls of the infected maize were the adjacent maize from each of the infected samples, that is they were sterilized and placed on a sterile petri dish containing cotton wool moistened with sterile distilled water. The plates were incubated at 27-35 ^{0}C for seven days. The effect was observed from the third day to the seventh day.

Species confirmation of Aspergillus flavus by PCR

Pure fungi colonies of *Aspergillus flavus* suspects from rodent fecal pellets that were grown for about 3-7 days on MEA were subjected to molecular analysis. DNA extraction was performed using Quick-DNA™ Fungal/Bacterial Miniprep kit according to the modified manufacturer's (Zymo Research) protocol. The reaction mixture contained dNTPs, a DNA template, Taq DNA polymerase, and a pair of forward and reverse primers. A total volume of 25 µl was used in the amplification stage and other conditions required were optimized (Bintvihok et al., 2016).

The PCR amplification was performed in two steps, of which the first step involved a reaction between forward and reverse primers which are aflR-1(AAC CGC ATC CAC AAT CTC AT) and aflR-2 (AGT GAC GTT CGC TCA GAA CA) respectively, the regulatory gene with a product size of 400 bp. The PCR product from aflR1 was used in round two as the template to perform PCR using the aflR2 primer as adopted and modified by (Manonmani et al., 2005)

Determining aflatoxigenicity of Aspergillus flavus Isolates

Aflatoxin-producing species that were confirmed by PCR were subjected to Indirect Competitive Elisa and samples were as follows; maize kernel infected with A. flavus, non-infected kernels, and Sterile plate containing MEA media without any sample (Negative control for MEA plates) were all dried in the oven. Each of the dried samples was separately ground to the particle size of fine instant coffee (95%) that was capable of passing passes through a 20 mesh screen. Extraction of samples and experiment was carried out according to the protocol of l *helica biosystems* total aflatoxin kit, 3310W.MacArthur Blvd. Santa Ana, CA 92704 USA.

Results were observed and recorded as they were shown by a Microtiter plate reader using a 450 nm filter. Concentrations of samples were calculated

2.9 Statistical Analysis

Age, Sex, Species, and physiological rank proportions of rodents were obtained by Descriptive statistics. A number of observed fungal groups, their corresponding wards, and rodent species were obtained through descriptive statistics by using tables and figures. Proportions of positive and negative samples from the ELISA test were observed and the corresponding concertation of aflatoxins was obtained using the standard curve.

3.0 RESULTS

3.1 Trapped rats

In the course of 3-5 consecutive nights, a total of 210 rodents were captured and identified as shown in table 3.0. The number of captured species are *Rattus rattus* 107(51.0%), *Praomys* 40(19.0%), *lophuromys* 29(13.8%), *Mastomys natalensis* 19(9.0%), *Mus musculus* 9(4.2%), *lemnicomys spp* 4(1.9%) as presented (in Tables3). Among them, 54.7% were females, whereby 28.1% were sexually active, 2.9% pregnant/ lactating and 23.7%

were not sexually active. Of the rats captured, 45.3% were males. About 25.9% were sexually active and 19.4% weren't. Generally, in all genders, 56.9% were sexually mature.

Table 1: Proportions of species, wards, and gender of various rodents captured in and along the storage facilities in Mbeya Municipal

Wards	***Rattus rattus***	***Mus musculus***	***Mastomys* spp**	***Praomys* spp**	***Lophuromys* spp**	***Lemniscomys* spp**	**Shrew**	**Total**
Iyela	22	1	7	0	0	0	0	30
Ruanda	18	3	2	3	0	0	0	26
Maendeleo	23	0	3	0	0	0	0	26
Mabatini	18	5	3	0	0	0	1	26
Iziwa	2	0	0	15	6	2	0	25
Igawilo	18	0	3	4	1	0	0	26
Mwasenkwa	6	0	1	7	9	0	1	24
Itende	0	0	0	11	13	1	0	26
Male	62	4	9	22	11	1	0	109
Female	45	5	5	18	18	0	2	93
Total	**107**	**9**	**19**	**40**	**29**	**4**	**2**	**210**

3.2 Isolated fungi of public importance

Isolates of pathogenic fungi were obtained from both fresh and old fecal samples, where the total number of fresh fecal samples was 210, and old fecal samples were 78, making a total number of 288 samples. From a total number of 210 trapped rats, positive proportions for known pathogenic fungi were as follows (table 3)

Table 2: Fungi species, rodent species, and their respective numbers and proportions Fungi species

Fungi species	**Count (%)**	**Percentage**
Fungi		
A. flavus	10	3
A. Niger	27	9
A. fumigatus	76	26
A. ochraceus	4	1
***Penicillium* spp**	8	3
***Fusarium* spp**	27	9
Rodents		
Rattus rattus	29	14
Mus musculus	2	1
***Mastomys* spp**	6	3
***Praomys* spp**	11	5
Shrew	4	2
***Lophuromys* spp**	1	1

Different forms of Fusarium and Penicillium *spp* were observed macroscopically). Identification to species level was possible for *Aspergillus flavus, Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium expansum*, of which it was achieved through the use of differential media which was Malt Extract Agar (MEA), colony diameter, color (Bandh et al.,2011), and observation under the microscope.

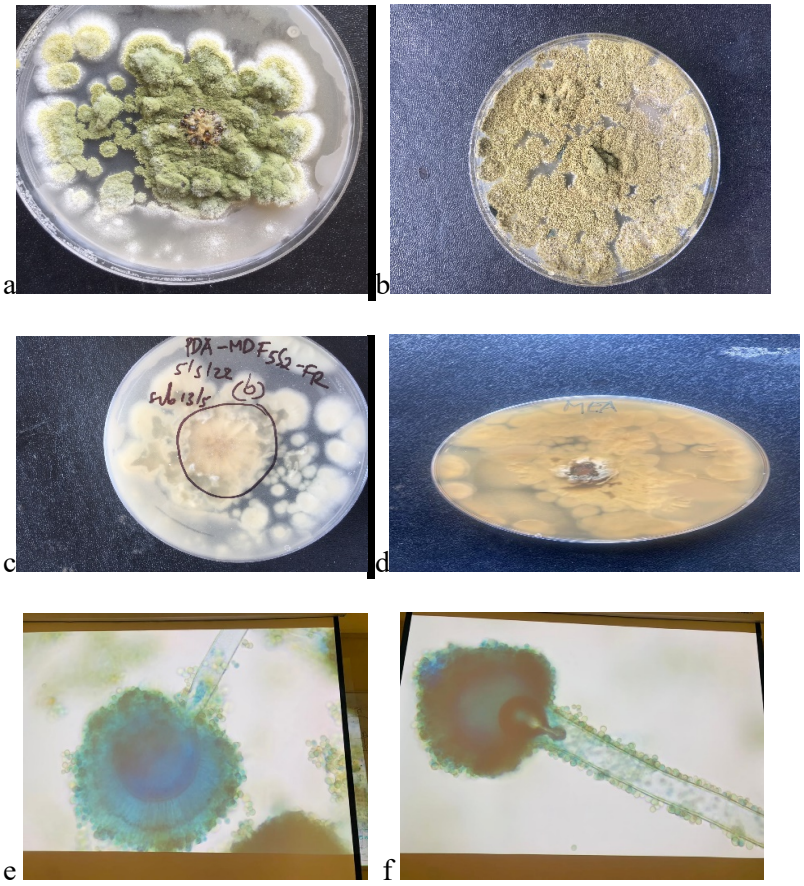
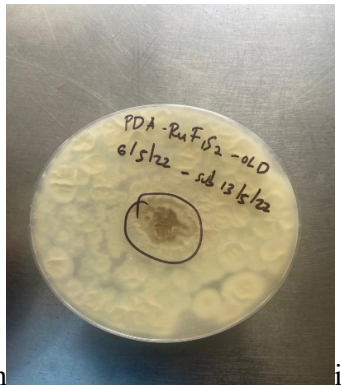


Figure 2.0: *Aspergillus flavus* on Potato Dextrose Agar front view(a) the reverse view(c) and under the microscope(e). *Aspergillus flavus* on Malt Extract Agar (b), reverse part (d) and under the microscope (f).


g


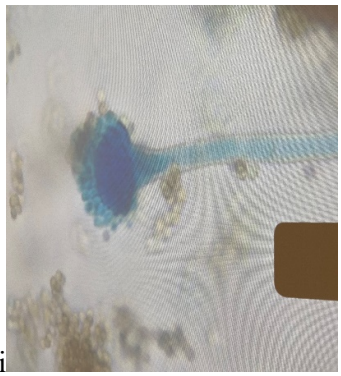
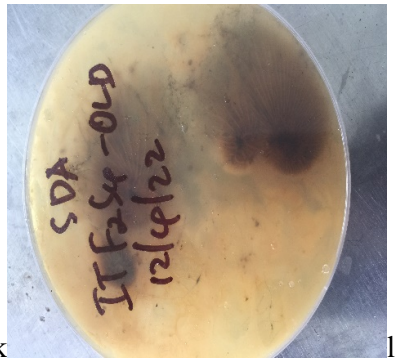
h

i

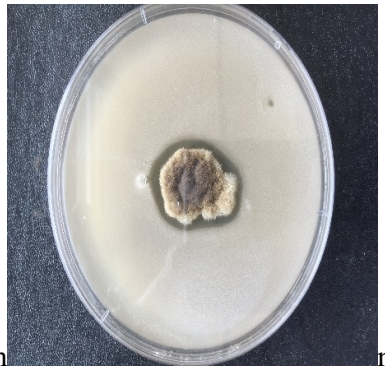
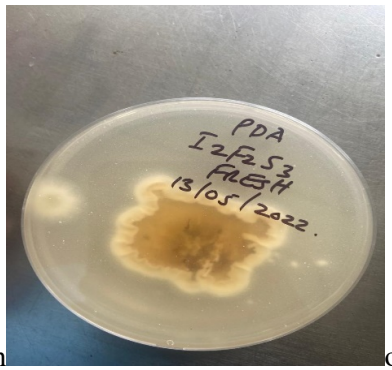
Figure 2.1: *Aspergillus fumigatus* on Potato Dextrose Agar front view(g) the reverse view(h) and under the microscope(i).


j


k

l

Figure 2.2: *Aspergillus niger* on Saboroud Dextrose Agar front view(j) the reverse view(k) and under the microscope(l).


m


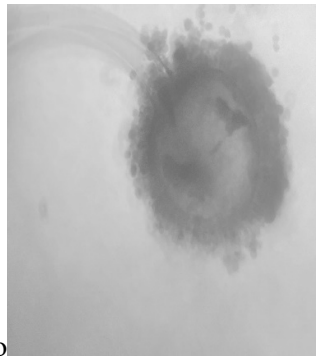
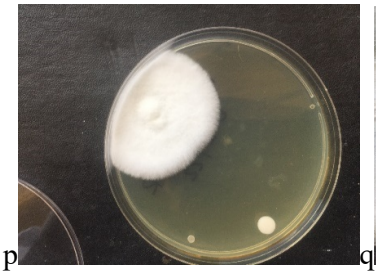
n

o

Figure 2.3: *Aspergillus ochraceus* on Potato Dextrose Agar front view(m) the reverse view(n) and under the microscope(o).

p  q  r 

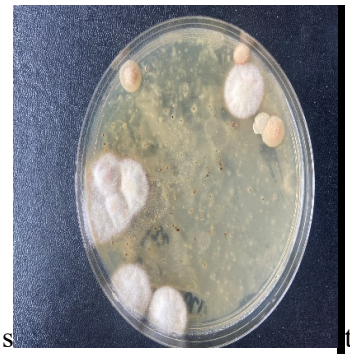
s  t 

Figure 2.4: Different *Fusarium spp* on Potato Dextrose Agar and Saboraud Dextrose Agar front view (p-t).

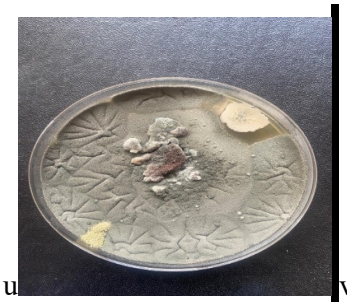
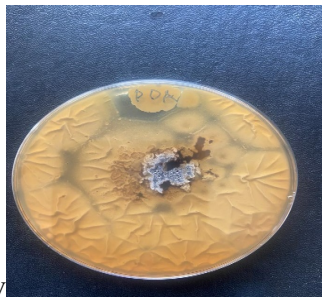
u  v 

Figure 2.5: *Penicillium expunsum* on Potato Dextrose Agar front view(u) the reverse view(v).The culture-positive samples gave similar results for aflR gene molecular detection as Figures 3.0 and 3.1 shows

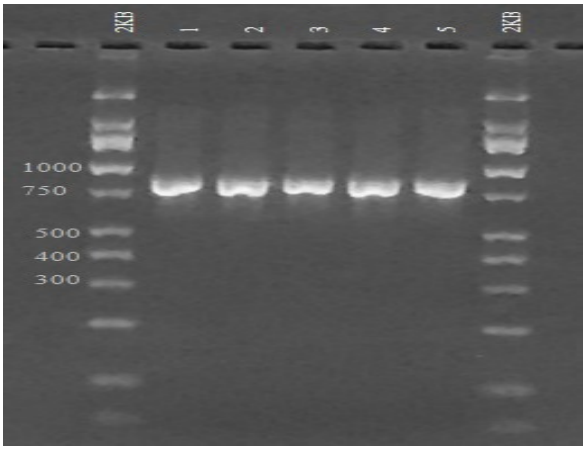


Fig 3.0: **Gel picture of PCR amplification for round one of aflR gene.**

Lane 1-5 represents that of aflR at an expected size of 800bp.

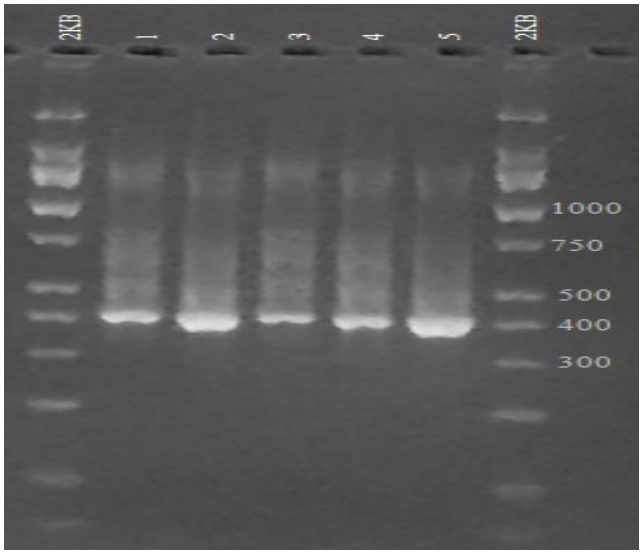


Fig 3.1: **Gel picture of PCR amplification for round two of aflR gene.**

Note: Lane 1-5 represents that of aflR at an expected size of 400bp.

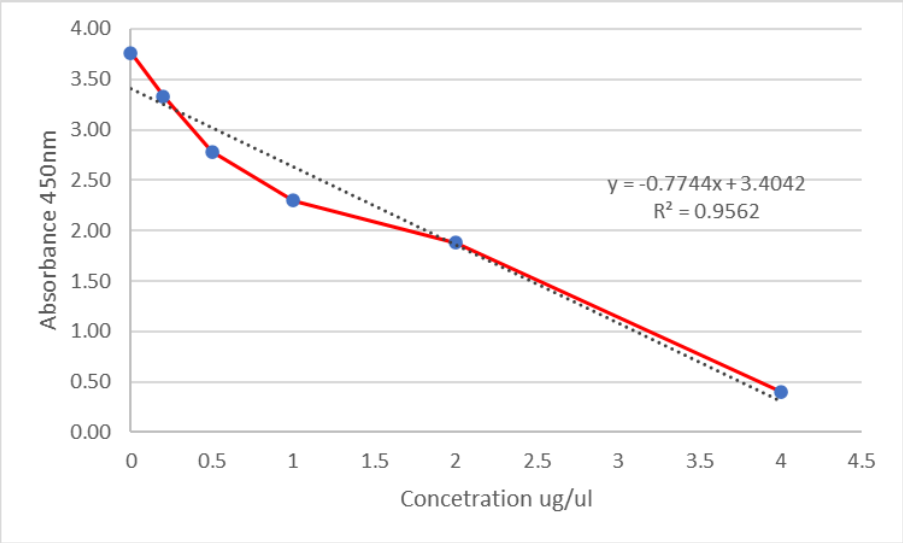


Fig 3.2: Standard curve of different concentrations of total aflatoxin

4.0 DISCUSSION

The study conducted found eight different species of rodents, of which some were captured within a parameter of about 3 m along the storage facilities and others inside the storage facilities. From a total number of 210 trapped rodents *Rattus rattus* (Black rats) was shown to be a dominant species inside the storage facilities than along the storage. These findings are corresponding to many studies conducted in different parts of the world. For example, studies conducted by several authors revealed that *Rattus rattus* was a dominating species inside the storage facilities (Chattopadhyay et al., 2009; Shiels et al., 2014; Feng and Himsworth, 2014; Htwe, 2021).
As shown in table 1, the most dominating rodent specie in urban areas was *Rattus rattus* (Hancke and Suárez, 2008) whereas in peri-urban areas were *Praomys and Lophuromys spp* (Meliyo *et al.,* 2014) followed by *Rattus rattus.* The reason behind this is most of the assessed wards located in per-urban areas were surrounded by farms and bushes/forest nature. Pathogens such as bacteria, parasites, and fungi can be transmitted from animals to humans through fecal droppings (Dufour and Bartram, 2012). Occurs by direct contact or indirectly through contamination of food, soil, or water (Penakalapati et al., 2017).
Pathogenicity and non-pathogenicity of dispersed fungal spores are an integral part of public health (Warnock, 2006). Studies concerning rodents acting as dispersal media for fungi spores have been carried out, but this study has gone far to isolate fungi of public importance from different rodent species that were trapped inside and outside the storage facilities. (Three fungal genera were able to be discovered from eight rodent species which were *penicillium, fusarium, and aspergillus* and their respective species were *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus,* and *Penicillium spp* and recovered from fecal samples of different rodent species as shown in table 2.
Aspergillus fumigatus was the dominant fungal specie with a prevalence of 26% (76/288), this corresponds with a study done on mice to assess the source and extent of fungal contamination (Mayeux *et al*., 1995) followed by *Aspergillus niger* and *fusarium species* with a prevalence of 9% (27/288), *aspergillus flavus* 3% (10/288) and *aspergillus ochraceus* 1% (4/288), isolated from both fresh and picked fecal samples.
A. fumigatus specie is among the airborne saprophytic fungi, of which is naturally found in the environment predominantly in soil, in which it causes invasive aspergillosis in humans (Kwon-Chung and Sugui,2013).
Since are naturally present in the environment rodents consume spores which are passed out of the gut to the nearest environment via fecal droppings. This implies that an increase in the rates of patients with aspergillosis can be contributed to an increase in the dispersal of *A. fumigatus* by domestic rodents habituating nearly or in contact with a human. According to Segal (2009), acute invasive aspergillosis is a rapidly progressive, frequently fatal disease that occurs in highly immunocompromised persons.
In the past years *A. fumigatus* was considered as a weak airborne pathogen (Dixon and Walsh,1992), in that case, it received relatively low attention. As a result of climate change a pathogen is currently the prevalent airborne pathogen causing invasive infections (aspergillosis) in immunocompromised patients (Andriole,1993). Individuals who are at risk of the disease are those with prolonged neutropenia, patients receiving hematopoietic

stem-cell transplants, and those with advanced acquired immunodeficiency syndrome (Segal and Walsh, 2006). Diagnosis of the disease is still challenging making clinical manifestation difficult, radiology can be employed but not all information will be obtained at the time (De Pauw *et al*., 2008). Allergic reactions can be induced in immunocompetent individuals by aspergillus species whereby it tends to manifest as asthma and sinusitis (Stevens *et al.,* 2003). Cough, difficulty breathing, and fever are frequent symptoms. According to Bodey et al (1992), invasive aspergillosis was responsible for about 30% of fungal infections in patients dying of cancer.

A group of mycotoxin-producing fungi isolated were; *A. flavus, A. Niger, A. ochraceus, penicillium expunsum,* and *fusarium species*. Of which they contributed about 3%, 9%, 1%, 3%, and 9% respectively. A study done on *mus musculus* that employed isolation of fungi from fecal samples found the same fungi species except for *fusarium spp and A. fumigatus* (Stejskal, *et al* 2005). Aflatoxin occurrence in grains is worldwide well documented, it occurs in areas characterized by high temperature and humidity (Ayejuyo et al., 2011). According to Kabak et al (2006), the problem of mycotoxin contamination in food and feed is a worldwide problem. The isolates of mycotoxin-producing fungi from fecal samples of rodents can aid in emerging of mycotoxins contamination in places that have never encountered the problem due to the dispersal behavior of rodents.

Insects and pests' distraction are one among the predisposing factors whereby injury sites act as the space for which fungal species invade seeds in the stores and field areas (Chandra et al., 2013). Techniques of molecular biology are currently used for studying the DNA of Aspergillus flavus (Woloshuk *et al*.,1995). Molecular confirmation of *Aspergillus flavus* suspect by nested PCR was carried out to identify the specific gene coding for aflR *A. flavus* suspect since it is sensitive and accurate. The aflR gene was confirmed, and the bands were observed at 800kb for the first round and 400kb for the second round. This is typically consistent to other studies relating confirmation of aspergillus flavus isolate by PCR method (Bintvihok et al., 2016). The difference is the isolates were recovered from rodent fecal samples. To justify whether the detected *A. flavus* was an aflatoxin producer competitive Elisa was incorporated. From 24 samples that were being tested in competitive Elisa of which there were 10 maize samples that were sterile and free from A. flavus isolate, 10 maize samples contaminated with *A. flavus* isolate, and 4 dried culture isolates of pure A. flavus. One of the maize samples that was not contaminated with *A. flavus* isolates had total aflatoxin concertation of 6.52 ppb which is the permissible level according to the FDA aflatoxin concentration level of cereals (FDA, 1994). Out of four tested pure isolates of *A. flavus* two of them had total aflatoxin concentrations of (7.35 and 7.22) ppb. These concentrations exceed the permissible level for milk and milk products which is 0.5 ppb (FDA, 1994), this implies that contamination of milk and milk products by fecal samples of rodents might cause the raise in the level's aflatoxin

5.0 CONCLUSION AND RECOMMENDATION

Since this study reported on rodents acting as the dispersal of pathogenic fungi which impact public health, proper control strategies and measures should be considered. Dispersal communities of pathogenic fungi can be reduced through strategic control of rodents. As the study indicated that there is a higher prevalence of *Aspergillus fumigatus* from fecal samples of rodents, this implies that storage facilities and households with accessibility for rodent infestations are the first lines for risk of contamination. *A. fumigatus* affects grain handlers, consumers, and immunocompromised individuals especially patients suffering from tuberculosis as affects the lungs.

Therefore, intense care and interventions such as community awareness implementation using one health approach, proper hygiene, and maintenance of storage facilities should be employed on a regular basis and Integrated rodent control approaches.

6.0 ACKNOWLEDGMENT

This work was supported by the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM and BTD) at the Institute of Pest Management of the Sokoine University of Agriculture. The authors would like to give special thanks to Mr. Erick Osward, Ms. Martha Adrian, Ms. Amina Kinyogoli, and Mr. Fadhil Mabruki for their technical assistance.

References

Andriole, V. T. (1993). Infections with Aspergillus species. *Clinical Infectious Diseases*, *17*(Supplement_2), 481-486.

Ayejuyo, O. O., Oluwo, R. A., Agbaje, T. O., Atamenwan, M., & Osundiya, M. O. (2011). Enzyme-linked immunosorbent assay (ELISA) of aflatoxin B1 in groundnut and cereal grains in Lagos, Nigeria.

Bascompte, J., Jordano, P., & Olesen, J. M. (2006). Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, *312*(5772), 431-433.

Bandh, S. A., Kamili, A. N., & Ganai, B. A. (2011). Identification of some Penicillium species by traditional approach of morphological observation and culture. *Afr. J. Microbiol. Res*, *5*(21), 3493-3496.

Bintvihok, A., Treebonmuang, S., Srisakwattana, K., Nuanchun, W., Patthanachai, K., & Usawang, S. (2016). A rapid and sensitive detection of aflatoxin-producing fungus using an optimized polymerase chain reaction (PCR). *Toxicological Research*, *32*(1), 81-87.

Bodey, G., Bueltmann, B., Duguid, W., Gibbs, D., Hanak, H., Hotchi, M., ... & Van't Wout, J. (1992). Fungal infections in cancer patients: an international autopsy survey. *European Journal of Clinical Microbiology and Infectious Diseases*, *11*(2), 99-109.

Chandra, H., Bahuguna, J., & Singh, A. (2013). Detection of aflatoxin in Zea mays L. from Indian markets by competitive ELISA. *Octa Journal of Biosciences*, *1*(1).

De Pauw, B., Walsh, T. J., Donnelly, J. P., Stevens, D. A., Edwards, J. E., Calandra, T., ... & Bennett, J. E. (2008). Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. *Clinical infectious diseases*, *46*(12), 1813-1821.

Dixon, D. D., & Walsh, T. J. (1992). Aspergillus, biology and industrial application. *Journal of Human pathogenesis*, 249-267.

Dufour, A., & Bartram, J. (Eds.). (2012). *Animal waste, water quality and human health*. IWA publishing.

Eisen, R. J., Atiku, L. A., Boegler, K. A., Mpanga, J. T., Enscore, R. E., MacMillan, K., & Gage, K. L. (2018). An evaluation of removal trapping to control rodents inside homes in a plague-endemic region of rural northwestern Uganda. *Vector-Borne and Zoonotic Diseases*, *18*(9), 458-463.

Feng, A. Y., & Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (Rattus norvegicus and Rattus rattus). *Urban Ecosystems*, *17*, 149-162.

Frisvad, J. C., & Samson, R. A. (1991). Mycotoxins produced by species of Penicillium and Aspergillus occurring in cereals. *Cereal grain: mycotoxins, fungi and quality in dr,ying and storage.*, 441-476.

Hancke, D., & Suárez, O. V. (2018). Structure of parasite communities in urban environments: the case of helminths in synanthropic rodents. *Folia Parasitologica*, *65*, 1-9.

Htwe, N. M., Sarathchandra, S. R., Sluydts, V., Nugaliyadde, L., Singleton, G. R., & Jacob, J. (2021). Small mammal communities, associated damage to rice and damage prevention in smallholder rice storage facilities in Sri Lanka. *Crop Protection*, *145*, 105638.

Kabak, B., Dobson, A. D., & Var, I. I. L. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Critical reviews in food science and nutrition*, *46*(8), 593-619.

Kwon-Chung, K. J., & Sugui, J. A. (2013). Aspergillus fumigatus—what makes the species a ubiquitous human fungal pathogen?. *PLoS pathogens*, *9*(12), e1003743.

Leck, A. (1999). Preparation of lactophenol cotton blue slide mounts. *Community Eye Health*, *12*(30), 24.

Manonmani, H. K., Anand, S., Chandrashekar, A., & Rati, E. R. (2005). Detection of aflatoxigenic fungi in selected food commodities by PCR. *Process Biochemistry*, *40*(8), 2859-2864.

Meliyo, J. L., Kimaro, D. N., Msanya, B. M., Mulungu, L. S., Hieronimo, P., Kihupi, N. I., ... & Deckers, J. A. (2014). Predicting small mammal and flea abundance using landform and soil properties in a plague endemic area in Lushoto District, Tanzania. *Tanzania journal of health research*, *16*(3).

Nathan, R., Schurr, F. M., Spiegel, O., Steinitz, O., Trakhtenbrot, A., & Tsoar, A. (2008). Mechanisms of long-distance seed dispersal. *Trends in ecology & evolution*, *23*(11), 638-647.

NBS. 2012. The 2012 population and housing census: population distribution by administrative areas. National Bureau of Statistics, Ministry of Planning, Economy and Empowerment United Republic of Tanzania. Dar es Salaam, Tanzania.

Penakalapati, G., Swarthout, J., Delahoy, M. J., McAliley, L., Wodnik, B., Levy, K., & Freeman, M. C. (2017). Exposure to animal feces and human health: a systematic review and proposed research priorities. *Environmental science & technology*, *51*(20), 11537-11552.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Vol. 519, p. 388). New York: Springer.

URT (United Republic of Tanzania). 1997. Mbozi District Socio-economic Profile.

Raper, K. B., & Fennell, D. I. (1965). The genus Aspergillus. *The genus Aspergillus.*

Ribas, A., Saijuntha, W., Agatsuma, T., Prantlova, V., & Poonlaphdecha, S. (2016). Rodents as a source of Salmonella contamination in wet markets in Thailand. *Vector-borne and zoonotic diseases*, *16*(8), 537-540.

Segal, B. H. (2009). Aspergillosis. *New England Journal of Medicine*, *360*(18), 1870-1884.

Segal, B. H., & Walsh, T. J. (2006). Current approaches to diagnosis and treatment of invasive aspergillosis. *American journal of respiratory and critical care medicine*, *173*(7), 707-717.

Shiels, A. B., Pitt, W. C., Sugihara, R. T., & Witmer, G. W. (2014). Biology and impacts of Pacific island invasive species. 11. Rattus rattus, the black rat (Rodentia: Muridae). *Pacific Science*, *68*(2), 145-184.

Singleton, G. (2003). *Impacts of rodents on rice production in Asia* (No. 2169-2019-1613).

Skinner, J. D., & Chimimba, C. T. (2005). *The mammals of the southern African sub-region*. Cambridge University Press.

Stejskal, V., et al. "The influence of the type of storage on pest infestation of stored grain in the Czech Republic." *Plant Soil and Environment* 49.2 (2003): 55-62.

Stejskal, V., Hubert, J., Kubatova, A., & Váňová, M. (2005). Fungi associated with rodent feces in stored grain environment in the Czech Republic/Pilze assoziiert mit Kot von Nagetieren im Umfeld von Lagergetreide in der Tschechischen Republik. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 98-102.

Stephens, R. B., & Rowe, R. J. (2020). The underappreciated role of rodent generalists in fungal spore dispersal networks . *Ecology*, *101*(4), e02972.

Stevens, D. A., Moss, R. B., Kurup, V. P., Knutsen, A. P., Greenberger, P., Judson, M. A., ... & Participants in the Cystic Fibrosis Foundation Consensus Conference. (2003). Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clinical Infectious Diseases*, *37*(Supplement_3), S225-S264.

Warnock, D. W. (2006). Fungal diseases: an evolving public health challenge. *Medical Mycology*, *44*(8), 697-705.

Woloshuk, C. P., Yousibova, G. L., Rollins, J. A., Bhatnagar, D., & Payne, G. A. (1995). Molecular characterization of the afl-1 locus in Aspergillus flavus. *Applied and Environmental Microbiology*, *61*(8), 3019-3023.