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RESEARCH PAPER

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Incidence of mycotoxin producing fungi in sorghum sourced from different markets of Eritrea

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Key words: Sorghum, Mycotoxin, Survey, Fungicide, Contamination

Abstract

Sorghum serves as staple food for over 100 million people in Sub-Saharan African countries. It is the most important nutritional security crop. However, sorghum is susceptible to contamination by molds that produces aflatoxin that causes hepatoxin and carcinogenic effects on humans and animals. This study was conducted to survey sorghum storage conditions in relation to mycotoxin contamination and to determine the efficacy of neem against *Aspergillus flavus*. The survey was done through questionnaires in Asmara, Keren and Asmat. The survey determined that sorghum is stored together with other cereals in all the markets. It also determined that sorghum sold in Asmara, Keren and Asmat is obtained from different places such as; Anseba, Mendefara, Gashbaka and Halhale. The survey also determined that most of the sorghum in the markets has been in storage for between 3-12 months with very little being stored longer than 1 year. In terms of control, sorghum sellers use a combination of fungicide and local remedies to eliminate pests and diseases. The common fungicide reported to be used was Tanphos with the local remedies used being; chillies, neem, lime and ash. We identified mycotoxins such as *Fusarium* and *Aspergillus* spp. in sorghum seed obtained from different markets of Eritrea. Treatment with neem was found effective as it diminished the radial growth of *Aspergillus flavus*. The concentration of mycotoxins specifically *Aspergillus flavus* in all sorghum samples was found to be higher. Therefore, attention should be given by responsible authorities to mitigate the effects of the mycotoxins.

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth most important cereal crop that is shaped like a little ball coated with an edible hull. Sorghum is used as a major food and nutritional security crop for more than 100 million people in the Horn of Africa (Katile *et al.*, 2010). Ethiopia is one of the major centers of origin and diversity for Sorghum cultivation (Mekbib, 2009). The lives of Eritreans depend on Sorghum as a staple food crop. It's versatile: sorghum can be broken down into flour for baking, boiled to make a side dish, and popped like popcorn. The sorghum plant has a natural drought tolerance, which means it can grow just about anywhere it's cultivated. Sorghum is susceptible to many abiotic and biotic factors and among the biotic factors are diseases caused by fungal pathogens such as moulds. The greatest concern for mould growth in food crops is the production of mycotoxins that are harmful to human and animal health (Prom *et al.*, 2021; Wu, 2015). The mycotoxin producing fungi include; *Aspergillus* sp., *Penicillium sp.* and *Fusarium* sp. (Wogan, 2012).

Aflatoxins are naturally occurring toxic secondary metabolites of storage fungi (*Aspergillus flavus*) produced in agricultural production stored inappropriately and at high moisture and temperature. (Chulze *et al.*, 2010). The fungus is common in areas with stressful conditions like drought. *Aspergillus* sp. contains a diverse group of microorganisms producing different types of mycotoxins (Fung *et al.*, 2004). There has been a report of fungal contamination of cereal crops such as sorghum with aflatoxins worldwide. Bio-fungicides or biological pesticides are formulations made from naturally occurring substances that control pests by non-toxic mechanisms and in an ecologically friendly manner. Bio-fungicides have been defined as a form of pesticides based on microorganisms or natural products (Meena, 2021). Anonymous (2014), reported that plant extracts were likely the earliest agricultural bio fungicides. Farmers surveys carried out in Ghana have highlighted that many farmers do not use commercial synthetics (Belmain and Stevenson, 2001) and instead, use plant-based products. Many farmers in Asia and Africa have been using plant extracts such as neem (*Azadirachta indica*), wild tobacco (Calotropisprocera), wood ash and dried chillies among others for controlling and repelling some insect pests (Anukwuorji, *et al.*, 2012; 2013; Ahmed *et al.*, 2005).

Surveys of the disease in various African countries have shown high disease incidences sometimes resulting to deaths (Astoreca *et al.*, 2019). Mycotoxins such as aflatoxins in human and animal diets can lead to aflatoxicosis. There has been no report on incidences of mycotoxins in major cereals consumed in Eritrea such as sorghum. Therefore, it's important to screen sorghum grains for mycotoxins contamination. One of the most important mycotoxins is *Aspergillus flavus,* the causal agent of aflatoxins. Efforts to control aflatoxins have utilized different methods. However, most of the efforts have utilized synthetic chemicals that are not environmentally safe and can be toxic to human and animals. There is need for development of safe alternatives to control aflatoxins. Many botanicals have been shown to inhibit different fungal pathogens. These botanicals have been shown to be environmentally safe and non-toxic to human and animals. This makes them potential bio-fungicides in the management of aflatoxins in Eritrea.

Materials and methods

Survey of sorghum storage diseases

The survey was done in three places of Eritrea. Thirty five questionnaire were used, 15 in Asmara, 10 in keren and 10 in Asmat. The questionnaire included sixteen questions to collect information about ; gender , level of education ,cereals sold, years of experience ,sorghum storage location and conditions ,storage duration, challenges faced during storage, diseases affecting sorghum during storage and managements undertaken and others .Each and every seller was asked individually and different storage places were visited to check storage conditions.

Mycotoxin producing fungi isolation

Potatoes ,agar and sucrose were used to prepare medium potato sucrose agar medium. To prepare potato sucrose agar (PSA), briefly 200 g of potatoes were washed and cut into small pieces, boiled and the poato broth separated by muslin cloth into a clean beaker. To this broth, 20 g of sucrose was added followed by addition of 15 g of agar powder, mixed properly and then sterilized in a pressure cooker. The media was cooled slightly and then while still molten dispensed into petridishes. Sorghum bought from different sellers in different markets; Asmara, Keren and Asmat was placed in a petridish with the medium. This was done after the sorghum was sterilized with 10% clorox. After sterilization its put on the PSA and inserted in an incubation machine (water bath).Incubation was done for 3 days and the fungus growing was recorded using the colour of the mycelia.

Preparation on Aspergillus pure culture

Amongst the mycotoxins that grew on PSA containing sorghum grains based on their phenotypic characteristics was *Aspergillus flavus*. To prepare a pure culture of the fungus a streaking wire was used. The streaking wire was burnt until it become red hot for sterilisation. Then using the streaking wire a small colony of the *A.flavus* was transferred to a new petridish containing PSA media and kept in an incubator.

In vitro control of Aspergillus flavus by neem

To determine the in vitro growth inhibition potential of neem against Aspergillus flavus, 100 mg Fresh Neem leaves were crushed and juices extracted. 2 litres of Potato sucrose agar medium was prepared and sterilized in a pressure cooker. To 1 litre of the PSA medium 100 micro litres of the neem juice extract was added by a micro-pippete. This was throughly mixed and then dispensed into petridishes. To the the other 1 litre of PSA media, no neem was added. This PSA media was also poured into petridishes and formed the control. To petridishes ammedned with neem and those in the control, a small culture of Aspergillus was placed at the centre and incubated for 3 days. The radial growth diameter of the culture in the control and neem ammended were measured and recorded.

Data analysis

Data was analyzed by excel and presented in percentages and graphs. The mycotoxins data in sorghum was recorded as present or absent and then used to calculate percentages of infection.

Results

Demographics of sorghum sellers in different markets

A survey done to understand sorghum storage diseases from different markets showed that male sorghum sellers were the most with Asmara recording 87%, 90% in keren and 70% in Asmat. On the other hand few female sellers were reported with Asmara reporting 13% ,10% in keren and 30% in Asmat respectively (Fig. 1).

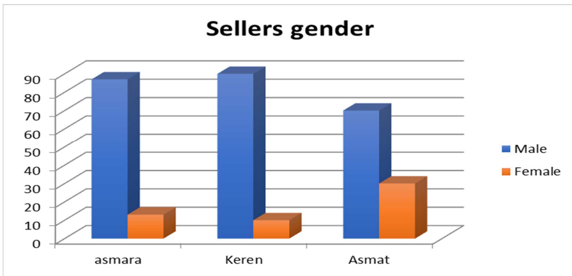

Fig. 1. Demographics of sorghum sellers in different markets

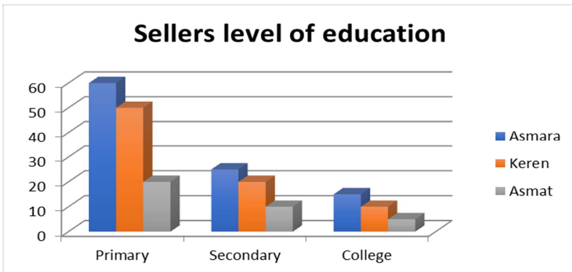

Fig. 2. Educational level of different sorghum sellers

Education levels of sorghum sellers in different markets

The survey also determined the educational level of the sorghum sellers as this has an influence on storage practices. From the results it was found that the educational level was as follows; Primary level and below 60% in Asmara , 50% in Keren and 20% in Asmat. With regards to Secondary level the following were the reported results; 20% in Asmara, 15% keren and 8% in Asmat. Lastly there were limited numbers of college educated sellers as follows; 15% in Asmara, 10% in Keren and 5% in Asmat (Fig. 2).

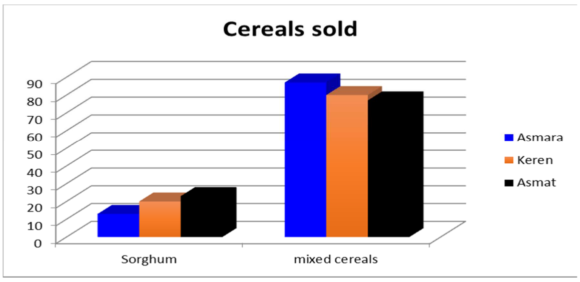

Fig. 3. Cereals sold in various markets from Eritrea

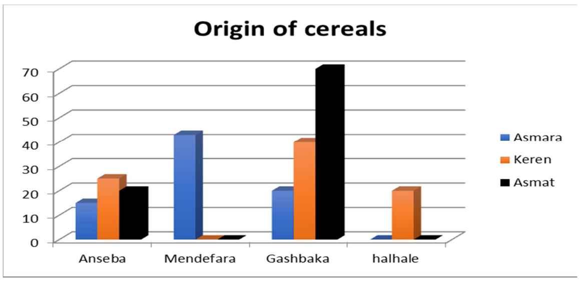

Fig. 4. Origin of cereals sold in various markets

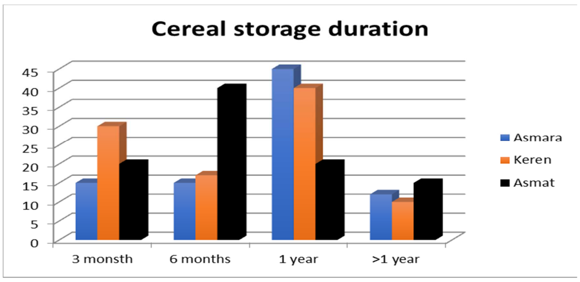

Fig. 5. Cereal storage duration

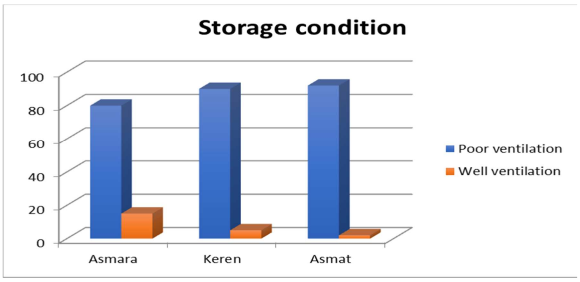

Fig. 6. Storage condition

Cereal types sold in various markets across Eritrea

To understand the nature of grains sold by the sellers, the following information was obtained showing that sorghum alone was sold by 13% in Asmara, 20% in Keren, 23% in Asmat while sorghum mixed with other grains was as follows; 87% in Asmara, 80% in Keren, 77 in Asmat. This shows that in most of the markets surveyed, the sellers sold multiple grains (Fig. 3).

Origins of the sorghum sold in the various markets

Information on the origin of sorghum sold in the different markets was sought. This is to understand the effect of different climatic conditions on sorghum storage and diseases development. The results showed that there were 4 mentioned origins of the grains sold. Anseba originated 13% of sorghum sold in Asmara, 25% of that sold in Keren and 20% of the sorghum sold in Asmat. On the other hand, Gashbarka originated 20% of the sorghum sold in Asmara, 40% of that sold in Keren and 70% of sorghum sold in Asmat. Additionally, Mendefera supplied 43% of the sorghum sold in Asmara and limited amounts of sorghum sold in Keren and Asmat (Fig. 4). As expected, Gashbaka supplied the most of the sorghum sold in Asmat due to close proximity as compared to Keren and Asmara. The study also showed that most of the sorghum sold in Keren comes from Halhale compared to Asmara and Asmat.

Storage duration of sorghum sold in different markets

Storage duration has an influence on the growth of fungal molds common in cereals including sorghum. From our study, it was revealed that the storage period varied across the three sampled areas. Storage of sorghum for a period of three months was highest in Keren with 30% followed by 20% in Asmat and 15% in Asmara, while storage duration of six months was as follows; 15% in Asmara, 20% in Keren, 40% in Asmat. With regards to storage period of one year the highest was 45% in Asmara, 40% in Keren and 20% in Asmat while for periods greater than one year the study showed they were almost similar reporting 12% in Asmara,10% in keren,15% in Asmat (Fig. 5).

Storage conditions for sorghum in different markets

Storage conditions such as humidity influence mold fungi development in stored grains. From this study we determined that the storage conditions of the sorghum under poor ventilation were reported as 75%

in Asmara, 90% in Keren and 92% in Asmat. On the other hand, well ventilated conditions were reported as follows; 25% in Asmara, 10% in Keren, 8% in Asmara (Fig. 6).

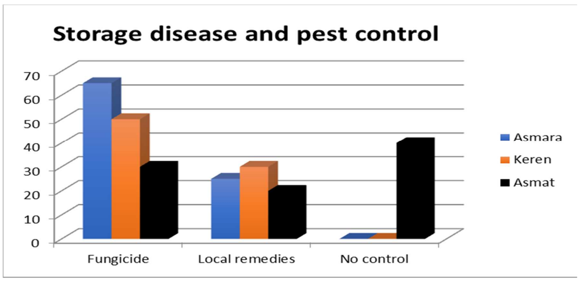

Fig. 7. Control measure

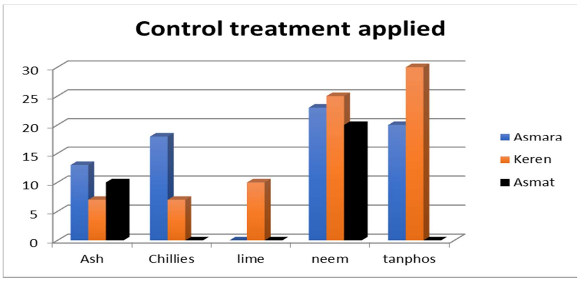

Fig. 8. Control treatment applied

Fig. 9. Pure culture of *Aspergillus flavus*

Table 1. Mycotoxin presence on the sorghum varieties

Sorghum variety	Market gathered from	*Aspergillus flavus*	*Fusarium* sp.
Senadil	Keren	75%	-
Codon	Asmara	45%	5%
Bazenay	Keren	35%	20%
Tebeldia	Breeder`s variety	90%	25%
Seare	Breeder`s variety	50%	15%
Embulmbul	Asmat	40%	20%

Different control measures implemented by sellers the sampled markets

Different control measures are done to inhibit fungal growth by sellers. From the study, sellers in Asmara practiced highest percentage of fungicidal usage which is 65% while slightly lower in Asmat and Keren which is 50% and 30% respectively. But the report on the local remedy usage of the three places was almost similar for Asmara it was 25%, Keren and Asmat 30%. The study also determined that sellers from Keren and Asmara practice more control mechanisms while Asmat farmers give little attention to control mechanisms (Fig. 7).

Specific remedies used in the control of sorghum diseases in the various markets

In this study it was determined that different botanicals and chemicals were used by the sellers and farmers. The chemical use was the highest in Keren with 30% followed by Asmara with 20%. On the other hand, ash was highly used by sellers in Asmara at 13% while the lowest was in Keren with 7%. Chillies were highly used by Asmara sellers with lime being predominantly used by Keren sellers. There was a high usage of nee min all the surveyed places (Fig. 8).

Mycotoxin isolation producing fundi isolated from different sorghum varieties

Sorghum is susceptible to mycotoxin infection in storage. A study on 6 different sorghum varieties (senadil, codon, bazenay. tebeldia, and seare) obtained from different markets was done. Tebeldia, the breeder`s variety, was found to have the highest percentage of *A. flavus* (Fig. 9) attack which is 90%. And Bazenay from keren was found to have lowest A.flavus attack which is 35%. Percentage attack of other varieties were as follows; senadil from keren 75%, codon from Asmara 45%, Seare, breeder`s variety 50%, from asmat 40% . *Fusarium* sp. attack was relatively lower than the *Aspergillus flavus* attack. Here too the Tebeldia variety was found to have relatively higher *Fusarium* sp. attack of 25% and codon from Asmara was found to have the lowest percentage that is 5%. And others lie in between (Table 1).

In vitro growth inhibition of Aspergillus flavus by neem

Neem, had an inhibitory effect against *A.flavus* as shown low radial growth (Fig. 10) of *Aspergillus* was recorded but in the unamended (control) relatively larger radial growth was noted (Fig. 11).

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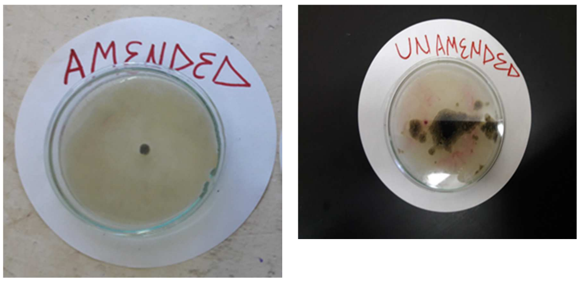

Fig. 10. Control of *Aspergillus flavus* by neem

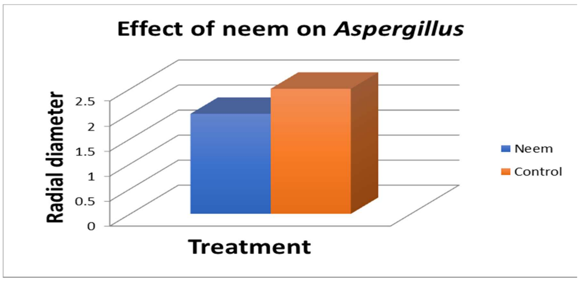

Fig. 11. Effect of neem on *Aspergillus flavus*

Discussion

Despite the importance of sorghum-based products in food and feed, in Eritrea little is known about mycotoxin-producing fungi isolated from sorghum kernels. Our study analyses sorghum samples collected from various locations in Eritrea specifically Asmara, Keren and Asmat. This work represents one of the first study about the occurrence of mycotoxin-producing fungi in sorghum grain in Eritrea. Our study showed that most Secondary level and college educated sorghum sellers were found in Asmara with the least educated being reported in Asmat. The study also showed that most of the sellers were male as compared to the female. Additionally, it reported that the origin of sorghum sold in the markets of Keren and Asmara were from Gash barka and Anseba regions. It has been reported that regions with temperature of 17-30^{o} C can support mycotoxin proliferation (Geremew *et al.*, 2016).

The sorghum sold in the markets surveyed was stored for 3 months, 6 months and 1 year. There were limited sellers who stored the sorghum above 1 year. In Ethiopia it has been reported that storage of sorghum for more than 1 year leads to higher level of aflatoxin contaminations (Geremew *et al.*, 2016). The study also showed that mycotoxin contamination can occur in sorghum at any period of storage if the storage conditions are poor. Under high temperature and moisture, the small amount of inocula fungi can increase rapidly. Storage conditions of the three places Asmara, Keren and Asmat were mostly of poor ventilation. It has been reported that storage facilities lacking proper airflow encourage the proliferation of mold fungi such as *Aspergillus flavus* in infected grain (Kange *et al.*, 2015). Storage diseases and pest control mechanisms were found to be higher in Asmara and Keren but almost none existent in Asmat.

Isolation studies on fungi growing medium showed that all the sorghum samples were found to be contaminated by different mycotoxins with variety Tebeldia being highly contaminated by *Aspergillus flavus*. This result is in agreement with Monica *et al.*, (2007) who reported infection of sorghum with *Aspergillus flavus, Aspergillus niger* and *Aspergillus parasiticus*. The presence of mycotoxin-producing fungi in sorghum kernels is a high risk due to the role of sorghum in food and feed chain. This study also showed inhibitory roles of neem against *Aspergillus flavus*. Different parts of the neem tree *(Azadirachta indica)*, a native tree from the drier regions of Asia and Africa that is considered a very important medicinally (Gupta *et al.*, 2017). Bhatnagar and McCormick (1988) found that using 10% concentration of neem leaf extract reduces 98% of aflatoxins.

Conclusion

This study showed that sorghum originates from different places in the surveyed markets. Sorghum sellers were found to practice both the use of fungicide and local remedies in the control of storage diseases and pests. In accordance to our study sorghum was found contaminated with several mycotoxin producing fungi. Those mycotoxins can cause many health-related problems. Among the reported mycotoxins from our study included; *Fusarium* spp. and *Aspergilus* spp.

Our study was also able to inhibit the growth of *Aspergillus flavus* through the use of neem at a concentration of 100 mg/ml. The findings indicate a need to minimize the infection levels in sorghum grains in the storage to avoid the mycotoxin contamination of sorghum kernels. This could ensure food and feed safety during post-harvest periods.

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