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Isolation of Fungi from Maize Samples Collected from Selected Counties in Kenya

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ABSTRACT

Background: Maize is the stable food in Kenya. However, its production has been jeopardized by the high prevalence of fungi in most developing countries and the whole world at large. Some fungi produce mycotoxins which threaten human lives.

Methods: This study aimed at isolating fungi from selected Counties in Kenya. Maize samples were ground using a kitchen blender and cultured on Potato Dextrose agar at 28°C for 7d. The fungal isolates were identified using morphological and cultural characteristics. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 software.

Result: The mean fungal isolates varied from 10.8±0.2 CFU/g in *Wallenia spp.* to 47.4±0.2CFU/g in *Aspergillus Spp.* The most common fungal isolates were *Aspergillus spp., Fusarium spp.* and *Penicilium spp.* The Mean fungal isolates from the selected Counties varied from Kitui (9.6±0.2 CFU/g), Machakos (9.7±0.3CFU/g), Bungoma (25±0.1CFU/g), Migori (25.3±0.3CFU/g), Kericho (25.3±0.2CFU/g), Kisumu (27.1±0.1CFU/g), Meru (27.1±0.2CFU/g), Kisii (28.5±0.1CFU/g) and Trans-Nzoia (30.1±0.3CFU/g). The number of spores in maize samples from baskets varied from 13.6±0.2-19.6±0.2, polypropylene (24.0±0.2-24.0±0.2), Jute (13.6±0.2-21.6±0.2) and polythene bag (48.0±0.2-72.0±0.2) CFU/g.

Conclusion: This study established that maize in the selected Counties is highly contaminated by pathogenic fungi. The most prevalent fungal spp. were *Aspergillus, Penicillium* and *Fusarium*. The best maize storage material for maize were baskets. There is need of identifying the most prevalent fungi up to the molecular level.

Key words: County, Fungi, Isolation, Maize, Samples.

INTRODUCTION

Maize is an important staple food in most parts of the world (Zhao *et al*., 2017). Its growth is expansive due to its adoptability to different farming systems (Samson *et al*., 2014). Besides, the crop is adjustable to different regions of the world (Paterson *et al*., 2017). Maize contributes to high food security especially in developing countries. With the rising human population all over the world, maize production needs to be supported by all means (Janˇciˇc *et al*., 2016).

In Kenya, farming is the key driver of the country's economy (Okioma *et al*., 2020). To small scale farmers, maize provides 60% of dietary calories and over 50% of proteins (Tobin-West *et al*., 2018). Small scale farmers grow about 2 million hectares annually with an average yield of 1.2-1.6 tons per annum (Odhiambo *et al*., 2013). The biggest drawback to maize production is infestation by fungi (Chaubey *et al*., 2015). Some fungi produce mycotoxins which impact negatively to human and animal health (Surendra *et al*., 2011). Mycotoxins are carcinogenic, mutagenic and teratogenic (Salau *et al*., 2017). In addition, they cause impaired growth in children and sometimes immunosuppression (Sangares *et al*., 2016). Mycotoxins cause leuco-encephalomalacia in horses, pulmonary oedema in pigs, liver and kidney cancer in mice and rats. Fungal growth is increased by high temperature, humidity, monsoons, low rains and flooded conditions (Affeldt *et al*., 2014). Poor harvesting and postharvest practices predisposes maize to fungal attack (Zajc and Gunde-Cimerman, 2018). The highest producer of mycotoxins are in the genera *Aspergillus, Fusarium* and *Penicillium*. The fungi are ubiquitous and cosmopolitan (Medina, 2017). Additionally, the fungi are filamentous proliferating in a vast range of environmental conditions (Krulj, 2019). Damage by fungi in maize is manifested by colour change, decrease in nutritional value and reduction in overall quality and quantity of maize (Negash, 2018). This study aimed at isolating fungi in stored maize in selected counties in Kenya.

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MATERIALS AND METHODS

The study areas

The current is study was carried out in Egerton University,

Department of Biological Sciences laboratories in the year 2020. Maize samples were collected from Trans Nzoia, Kisii, Kisumu, Bungoma, Migori, Kericho, Machakos, Kitui and Meru Counties. Recently, the counties had reported mycotoxin infections (Okioma *et al*., 2020). Trans-Nzoia county is located in Western part of Kenya. The county lies at 2,100 m above sea level. It experiences a temperatures range of 9.05°C-26.85°C (Mutegi *et al*., 2018). Kisii County lies 0°41' 0 N 34°46' 0 E. The temperatures varies from 16°C-27°C. The County has a population of 1,152,282 (Okioma *et al*., 2020). Kisumu County covers an area of 2085.9 km^2. The County is located at longitudes 33° 20'E and 35°20'E and latitudes 0°20'South and 0°50'South (Josephat *et al*., 2015). Bungoma County has an area of 2,069 km^2 and is located at 00°34˙00¨N, 34°34'00''E (Odhiambo *et al*., 2013). Migori County is located in Western Kenya and its temperature varies from 24°C to 31°C (Birgen *et al*., 2020). Kericho County lies at 35° 02' and 35° 40' E and 0 23' S with an altitude of about 2002m above the sea level (Odhiambo *et al*., 2013). Machakos County is found at latitudes 0°45' S to 1°31' S and longitudes 36° 45' E to 37° 45' E. The county lies at an altitude of 1000 to 2100 m above sea level (Josephat *et al*., 2015). Kitui County is found in Eastern Kenya at co-ordinates 1°22' 0" South and 38° 1' 0" East and a temperature range of 26°C to 34°C (Birgen *et al*., 2020). On the other hand, Meru County is located in Eastern Kenya at 0.047035° N and 37.649803° E. The County has a temperature range of 8° C-32°C (Mutegi *et al.,* 2018).

Collection of maize samples

Maize samples were collected from baskets, polypropyne, jute and polythene bags from the selected Counties. The sample size was calculate at 95% confidence level at a precision of 5% as proposed by Nleya *et al*. (2018).

$$n = \frac{Z^2 pq}{e^2}$$

Where

n=sample size, Z= confidence level at 95% (1.96), p= estimated proportion of the sample population, q= (1-p) and e= desired level of precision at 5% with a standard value of 0.05. Substituting the values in the formula above, the sample size was determines as follows;

$$n = \frac{1.96^2 (200)\,(1+200)}{0.05^2} = 130$$

Samples were placed in sterile *khaki* bags and stored at 4°C in the Department of Biological Science laboratories of Egerton University.

Isolation of fungi from maize samples

The maize samples were surface sterilized using 70% ethanol for 2 minutes. The samples were rinsed with distilled water to remove ethanol on the surface (Chukwudi *et al*., 2021). The Maize samples were blot dried (Pemingo *et al*., 2016). A dry mill kitchen blender (BL335, Kenwood, UK) was used in grinding one kilogram of each maize sample. One gram of each ground maize sample was separately placed in 9mL of sterile distilled water, shaken using an orbital shaker and serially diluted up to10^{-2}. Aliquots of 0.1mL were each plated on Potato Dextrose Agar (PDA) and incubation at 28°C for 7d (Menza and Muturi, 2018). The fungal isolates were sub-cultured on PDA. The fungal isolates were enumerated using a formula provided by Mezzomo *et al*. (2018);

$$\frac{CFU}{g} = \frac{Number\ of\ coclonies\ of\ a\ fungal\ speceis}{Amount\ platedx\ Dilution\ factor}$$

Identification of the mycotoxin producing mycoflora

Macroscopic and microscopic characteristics were used in identifying the fungal isolates (Kumar and Kalita, 2017). Length and type of spores were used to morphologically characterize the isolates while cultural characteristics considered colour and margins of the colonies (Lee *et al*., 2013). In addition, the fungal isolates were characterized using fungal identification keys (Abdel-Sater *et al*., 2017).

Spores count of the fungal isolates

Fifty grams of each maize sample from baskets, polypropyne, jute and polythene bags was weighed and placed in a glass beaker containing 200mL of double distilled water and stirred for two minutes (Okayo *et al*., 2020). Using a teat pipette, the spore suspension was transferred into a heamocytometer. The number of spores from each sample was determined with the help of a microscope (Abe *et al*., 2015).

Data analysis

Data on the number (CFU/g) of fungal isolates from the selected Counties, mean fungal isolates and spore count per gram of maize sample were subjected to analysis of variance (ANOVA) using PROC ANOVA procedure of Genstat Discovery 2 statistical software version 25.0. The means were compared using Fisher's protected LSD test at 5%.

RESULTS AND DISCUSSION

Fungal isolates from the selected counties

The fungal isolates from Trans-Nzoia varied from 12±0.3 CFU/g to 63±0.3 CFU/g, Kisii (11±0.2-62±0.3), Kisumu (11±0.2-59±0.2), Bungoma (12±0.1-55±0.1), Migori (13±0.2-54±0.3), Kericho (11±0.2-59±0.2), Machakos (4±0.1-15±0.2), Kitui (5±0.3-14±0.1) and Meru (6±0.2-57±0.3) CFU/g (Table 1). The mean fungal isolates varied from 10.8±0.2 CFU/g in *Wallenia spp.* to 47.4±0.2CFU/g in *Aspergillus Spp.* The most common fungal isolates were *Aspergillus spp., Fusarium spp.* and *Penicilium spp.* (Fig 1). The number of fungal isolates from the selected counties varied significantly (F=1.987 P=0.0484). These results differed with a previous study carried out by Tola and Kebede, (2016). The difference could be attributed to variation in fungal control methods used by farmers in the study sites. Marroquin-Cardona *et al*. (2014) asserted that some farmers use long term fungal control methods which influence the fungal species that

infect maize. In addition the most common fungi are known to produce a variety of mycotoxins (Al-Defiery and Merjan, 2015). This may explain the high cases of mycotoxins poisoning outbreaks reported in the study areas (Njoroge *et al*., 2016).

Morphological and cultural characteristics of the fungal isolates

Identification of the fungal isolate was carried out using colony morphology (cell size, shape, pigmentation and arrangements) and cultural characteristics (Table 2). Use of morphological and cultural characteristics in identification of fungi is the oldest and most widely used tool for fungal identification (Tai *et al*., 2020). The morphological and cultural characteristics concurred with a previous study carried out by Mousa *et al.* (2016). This may be attributed to isolation of the same fungal species (Ncube *et al*., 2021). Okayo *et al*. (2020) successfully isolated and characterized maize fungi using morphological and cultural means.

Mean fungal isolates from the selected counties

The Mean fungal isolates from the selected Counties varied from Kitui (9.6±0.2 CFU/g), Machakos (9.7±0.3CFU/g), Bungoma (25±0.1CFU/g), Migori (25.3±0.3CFU/g), Kericho (25.3±0.2CFU/g), Kisumu (27.1±0.1CFU/g), Meru (27.1±0.2CFU/g), Kisii (28.5±0.1CFU/g) and Trans-Nzoia (30.1±0.3CFU/g) (Fig 2). The mean fungal isolates between the selected counties varied significantly (F=1.877

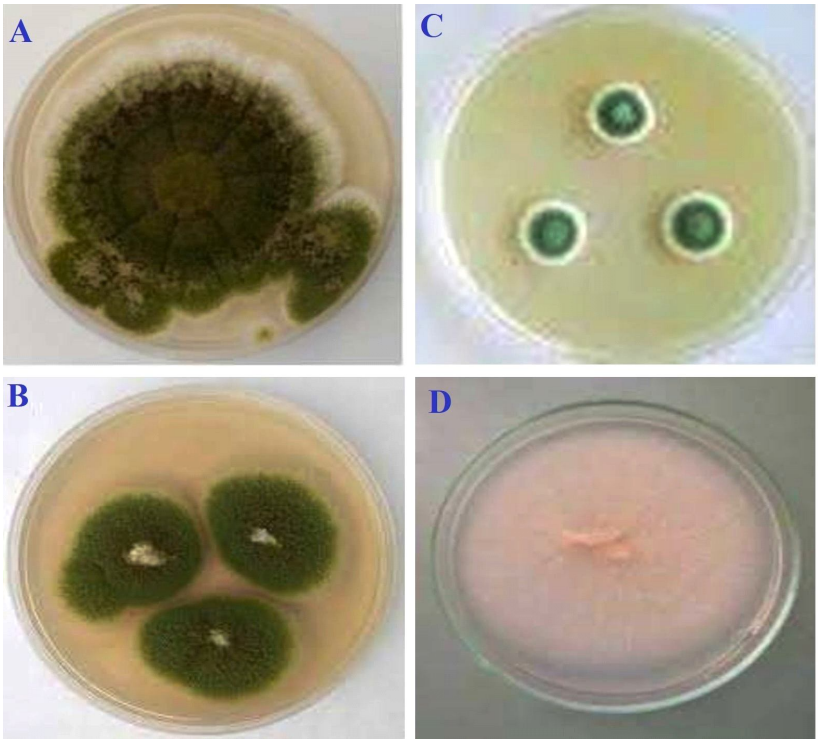

Fig 1: Most common fungal isolates from maize samples from the selected Counties; A and B; *Aspergillus spp*., C; *Penicillium spp*. and D; *Fusarium spp.*

Table 1: Number (CFU/g) of fungal isolates from the selected Counties.

Isolate	County									Mean
	T. Nzoia	Kisii	Kisumu	Bungoma	Migori	Kericho	Machakos	Kitui	Meru	
Alternaria spp	21±0.3	20±0.1	17±0.3	16±0.2	14±0.2	15±0.3	4±0.1	6±0.3	21±0.2	14.9±0.2
Fusarium spp	59±0.2	56±0.2	49±0.1	52±0.3	47±0.1	53±0.3	9±0.3	10±0.2	57±0.3	43.6±0.1
Epicoccum spp	23±0.1	19±0.3	15±0.2	10±0.1	13±0.2	12±0.2	9±0.2	8±0.1	14±0.3	13.7±0.3
Wallenia spp	12±0.3	14±0.1	11±0.2	12±0.3	15±0.3	14±0.1	8±0.2	5±0.3	6±0.2	10.8±0.2
Penicillium spp	61±0.2	62±0.3	59±0.2	49±0.1	50±0.3	48±0.1	10±0.1	13±0.2	51±0.3	44.8±0.1
Xeromyces spp	14±0.3	11±0.2	15±0.1	12±0.2	14±0.1	11±0.2	11±0.3	14±0.1	18±0.3	13.3±0.2
Mucor spp	20±0.1	15±0.2	21±0.3	12±0.1	16±0.1	13±0.3	9±0.2	10±0.3	17±0.1	14.8±0.2
Aspergillus spp	63±0.3	60±0.1	57±0.2	55±0.1	54±0.3	59±0.2	12±0.2	11±0.1	56±0.2	47.4±0.2
Rhizopus spp	13±0.1	15±0.3	13±0.2	16±0.1	17±0.2	13±0.3	15±0.2	10±0.3	13±0.1	13.9±0.2
Nigrospora spp	15±0.2	13±0.3	14±0.1	16±0.2	13±0.3	15±0.1	10±0.2	9±0.3	18±0.2	13.7±0.1

T. Nzoia; Trans-Nzoia.

P=0.0474). Although these results indicated that fungi can grow in a wide range of ecological conditions, Kitui presented the lowest fungal isolates while Trans-Nzoia County presented the highest. This disagreed with other studies curried out elsewhere (Maryam, 2017; Okayo *et al*., 2020; Tai *et al*., 2020) which can attributed to differences in humidity conditions of various study sites (Medina, 2017). According to Ncube *et al*. (2021), fungal growth is favoured by warm, moist and humid conditions.

Spores count of the fungal isolates

The number of spores in maize samples from baskets varied from 13.6±0.2-19.6±0.2, polypropylene (24.0±0.2-24.0±0.2), Jute (13.6±0.2-21.6±0.2) and polythene bag (48.0±0.2-72.0±0.2) CFU/g (Table 3). The number of spores obtained from maize stored in baskets, polypropylene, jute and polythene bag varied significantly (F=3.815 P=0.03945). The results were in agreement with a previous study by Nyongesa *et al.* (2015). Dube and Mutewa (2015) observed that baskets allows further drying of maize in storage conditions. According to Peremingo *et al.* (2016) maize is in most cases harvested when moisture content is still high (Njoroge *et al*., 2016). Tai *et al.* (2020) recommended storage of maize in well aerated containers to further reduce the moisture content in the grains. Further, Negash *et al.* (2018)

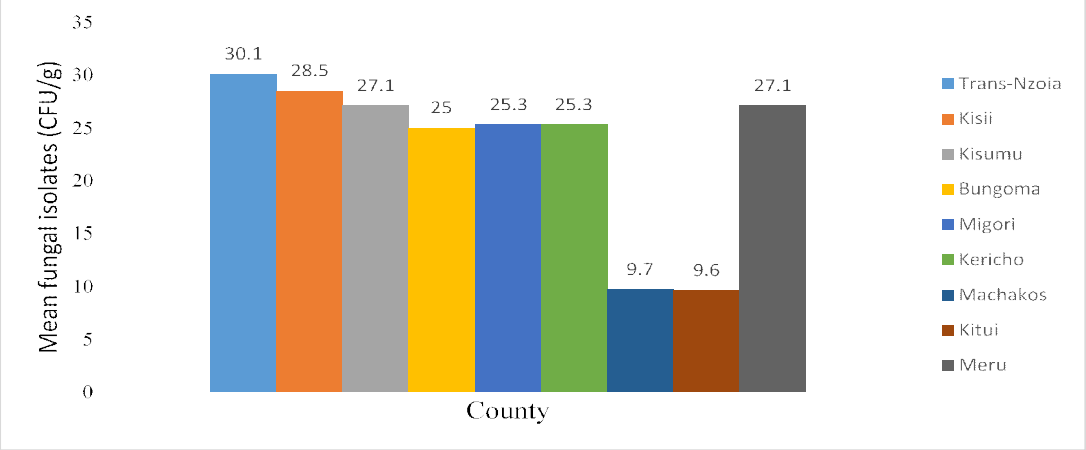

Fig 2: Mean fungal isolates from the selected Counties.

Table 2: Morphological and cultural characteristics of the fungal isolates.

Isolate	Morphological characteristics	Cultural characteristics
Alternaria spp.	Long chains of septate hyphae with dark brown conidiophores.	Black coloured colonies with irregular margins.
Fusarium spp.	Many celled distinct sickle shaped macro conidia.	Rapidly growing wooly to cottony, lemon and yellow coloured colonies.
Epicoccum spp.	Septate yellow to brown hyphae forming dark coloured, warted and spherical blastoconidia.	Felty colonies that appear in bright shades of yellow, orange and red, brown or black.
Wallenia spp.	Transparent hyphae, forming a compact mycelium with conidiphores.	Small brown colonies with a fine velvety texture, with long rows of spores that round up and become free at maturity.
Penicillium spp.	Coenocytic branched hyphal enlarge at the apex forming cornidophores that produce brownish black conidiain chains.	Large fluffy white colonies covering the whole agar surface.
Xeromyces spp.	Hyphae having closed fruiting bodies and D-shaped ascospores.	Low, transluscent, glistering, colourless faintly brown colonies.
Mucor spp.	Sporangium emanating from the hyphae without stolon or rhizoids.	Large fluffy white colonies almost covering the whole agar surface
Aspergillus spp.	Conidia designed in 360 arrangements covering the upper conidiophores.	Green colonies some with whitish margins colonies.
Rhizopus spp.	Coenocytic hyphae with up right sporagiophore bearing sporangiaconnected by stolon and rhizoids.	Large fluffy white milky colonies which later turned black with age
Nigrospora spp.	Septate hyphae bearing conidiophores that are straight or slightly curved producing brownish black, oblate spheroid and single celled conidia.	Rapidly growing colonies and appear hairy or woolly.

Table 3: Number of spores per gram of maize sample.

Replicate	Storage material			
	Basket	Polypropylene	Jute	Polythene bag
1	13.6±0.2	24.0±0.2	13.6±0.2	48.0±0.2
2	17.6±0.1	30.4±0.3	14.4±0.1	68.8±0.1
3	18.4±0.3	30.4±0.1	21.6±0.2	72.0±0.2
4	19.6±0.2	31.8±0.1	20.6±0.3	58.8±0.1
5	16.5±0.1	28.3±0.3	16.5±0.2	62.9±0.2
Mean	17.14±0.1	28.98±0.3	17.34±0.2	62.1±0.2

established that post-harvest practices of most farmers expose maize to infection by fungi prior to storage.

CONCLUSION

This study established that maize in the selected Counties is highly contaminated with fungi. The most prevalent fungal spp. were *Aspergillus, Penicillium and Fusarium*. The best maize storage material for maize were baskets. There is need of identifying the most prevalent fungi up to the molecular level. The mycotoxins produced by the most prevalent fungal spp. need to be established.

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