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RESEARCH ARTICLE

Prevalence of *Aspergillus flavus* in maize-cultivated soils of Zanzibar

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ABSTRACT

Aspergillus flavus is known to infect maize while still in the field, resulting in contaminations that culminate into Aflatoxins in stored grains. Aflatoxins are reported to cause cancer, liver diseases, and death in humans. Determination of the levels of *A. flavus* in soils used for the production of maize in Zanzibar was still being determined despite the confirmed report on the occurrence of the contaminant in the soils. The present study quantified the *A. flavus* population in fields under maize cultivation in Zanzibar. The population of fungi isolates varied significantly ($p<0.05$) in different locations. Both *A. flavus* S-strain and L-strain were highly abundant in Bumbwisudi (5.767/g, 5.833/g) compared to other sites. They were lowest at Kiwani (0.567/g, 0.7/g) and Kibondeni (1.2/g, 0.867/g), *A. niger* was abundant at Kisauni (2.867/g) and lowest at Nyamazi (0.267/g) while *A. parasiticus* was abundant at Kisauni (2.633/g) and lowest at Mkokotoni (0.267/g). *Fusarium* spp. was highly abundant at Kitope (5.733/g) followed by Nyamazi (5.433/g), Mkokotoni (5.3/g), Bambi (5.267/g), Umbuji (5.2/g), Kisauni (5.133/g) while the lowest counts were at Bumbwisudi (0.7/g), Chimba (0.867/g), Kibondeni (1.133/g) and Kiwani (1.367/g). The results suggest that *Aspergillus flavus* will threaten maize production in Zanzibar.

Keywords: *Aspergillus flavus*, Aflatoxin, Colony Forming unit

INTRODUCTION

Aspergillus flavus fungi have been of consideration for centuries because of their harmful impact as degraders of agricultural products through contamination of food and feed by aflatoxins that harmfully affect human and animal health (Temba et al., 2016). Aflatoxins are a group of secondary fungal metabolites primarily produced by fungi belonging to *A. flavus,* which is the main species responsible for the aflatoxin contamination of crops (Mannaa & Kim, 2017). According to Senghor *et al.* (2020) *A. flavus* is

extensive in agricultural areas where crops susceptible to aflatoxin contamination are grown, including maize. Recent studies have demonstrated that the contamination of maize grain by *A. flavus* often occurs in the field preceding harvest (Yan et al., 2021). The source of inoculum for field contamination is the soils where these organisms commonly hibernate (Nesci & Etcheverry, 2002; Aminu & Keta, 2021). Spores in the soil could be spread by wind or insects to the contamination site on the standup crops (Palumbo et al., 2014). Biogeography and population dynamics of aspergilli as fundamental causes of mammalian toxicity have directed an increased essential to recognize where these fungi occur in nature (Klich, 2002). According to Monda et al. (2020) *A. flavus* is mainly a saprophytic fungus that exists in the soil and settles in numerous environments with rich carbon and nitrogen sources. Earlier studies have confirmed that numerous soil fungi are significantly influenced by cultural practices (Tan et al., 2017). According to Bei et al. (2018), fertilizer applications, crop rotations, and manure applications have all been revealed to disturb the soil fungal population. Kachapulula et al. (2017) reported that soils cultivated to maize may comprise populations of roughly 2 x 10^3 propagules per gram of soil. The difference in soil populations consequently shows that numerous factors tend to control the soil population of *A. flavus*. The existence of *A. flavus* in the soil is a significant determinant of whether the soil becomes a primary source of inoculum for field contamination (Nesci & Etcheverry, 2002). The composition of Aspergillus populations in a particular space affects the amount of contamination of maize with aflatoxins (Mutegi et al., 2018). According to Massomo (2020), risk plotting the *A. flavus* population might be translated into forceful gears to assist in decision-making about highlighting the strategic involvements to reduce aflatoxins contamination. Therefore, this study aimed to determine soils' *A. flavus* microbial profiles from maize-grown fields in Zanzibar's Unguja and Pemba Islands.

MATERIALS AND METHODS

Study Sites and Soil Sample Collection

Soil samples were collected from ten Unguja villages with 80 field soil samples and six Pemba villages with 50 field soil samples, totaling 130 field soil samples (Nesci & Etcheverry, 2002). Sampling points were geo-referenced with a Global Positioning System (Seetha et al., 2017), total soil field samples (n = 130) were collected depending on occurred fields grown with maize in a particular village and all soil field samples collected in a particular village were mixed to obtain composite site sample presenting such village (Nesci & Etcheverry, 2002). Sampled villages in Unguja included Bumbwisudi (6.058^{0}S, 39.258^{0}E), Umbuji (6.126^{0}S, 39.37^{0}E), Nyamazi (6.234^{0}S, 39.279^{0}E), Mkokotoni (5.883^{0}S, 39.241^{0}E), Mkwajuni (5.893^{0}S, 39.298^{0}E), Kitope (6.000^{0}S, 39.269^{0}E), Kisauni (6.250^{0}S, 39.234^{0}E), Kibondeni (6.220^{0}S, 39.260^{0}E), Bambi (6.415^{0}S, 39.213^{0}E) and Kizimbani (6.^{0}S, 39.^{0}E). Pemba sites include Kiwani (5.253^{0}S, 34.453^{0}E), Pujini (5.747^{0}S, 34.480^{0}E), Vitongoji (5.134^{0}S, 34.492^{0}E), Machengwe (5.045^{0}S, 34.445^{0}E), Chimba (4.586^{0}S, 34.453^{0}E) and Makangale (4.551^{0}S, 34.411^{0}E). All collected soil samples were sent to the Sokoine University of Agriculture Pathology laboratory for fungi isolation and quantification.

Determination of Soil pH

The pH meter was calibrated before pH measurement by washing the electrodes with water from a wash bottle. Gently drying the electrode with a tissue, followed by placing the electrodes in the pH 4 buffer solution, gently swirling the solution, and adjusting the meter to pH 4.0 using the buffer control (Rowell, 2014). Then, the electrodes were removed, rinsed in water, dried, and inserted into the pH 7 buffer. The pH meter was calibrated before measuring each composite sample. After the pH meter was calibrated, 10g of each soil sample was weighed into a bottle with a screw cap, 25 ml of water from a measuring cylinder was shaken for 15 minutes using the shaking machine, and the suspension was then stirred. Electrodes were swirled onto the suspensions, and pH was recorded after 30 seconds (Rowell, 2014).

Fungi isolation and CFU/g quantitation

For each composite site sample, 1 g was dissolved in 9 mL of autoclaved distilled water, serially diluted to 10−3, and repeated three times. As per Zhang et al. (2017), 100 µL of each dilution was distributed on Rose Bengal agar plates, sealed, and incubated at 28 °C for 7 days in darkness. Plates were tested for fungal growth, and then colonies were counted to determine the average soil CFU/g, depending on location after point-inoculation, incubated colonies on Potato Dextrose Agar (PDA) at 28 °C. Pure colonies were created by sub-culturing the developing fungal cultures. Morphological differences in colony color and conidial morphology revealed pure fungal colonies (Afzal et al., 2013). Weledesemayat et al. (2016) described *A. flavus* species as yellow-brown, brown to black, or shades

of green with dense erect conidiophores. Many tiny dark green sclerotia were seen in *A. flavus* S-strain isolates. *A. flavus* L-strain colonies were yellow to brilliant green without sclerotia (Thathana et al., 2017). Penicillium species produced blue spores, while *A. parasiticus* had dark green colonies and rough conidia (Klich, 2002). *A. niger* isolates had carbon black or dark brown spores (Weledesemayat et al., 2016).

Data Analysis

Data were transformed using the logarithm function (log4) before analysis. Analysis of variance and comparison of means for CFU/g were carried out using GenStat® Executable release 16 Statistical Analysis Software. The Tukey Honest Significant Difference test (HSD) compared the means at 5% probability. Analysis of variance (ANOVA) was used to validate the variability of isolates in different villages and pH levels. Regression (R^2) and correlation (r) between A. flavus L-strain and A. flavus S-strain were performed to examine the relationship between the two parameters using Excel (MS-2016) to evaluate their relations in terms of population in different locations and pH levels.

RESULTS

Colony forming unit in a gram of soil across Unguja and Pemba maize cultivated soils

There was a significant difference among villages on fungal CFU/g ($p<0.001$). Kizimbani, Kisauni, and Kitope had significantly high CFU/g compared to other villages, followed by Bumbwisudi, Umbuji, and Bambi. Kibondeni and Kiwani had low CFU/g **(Figure 1).**

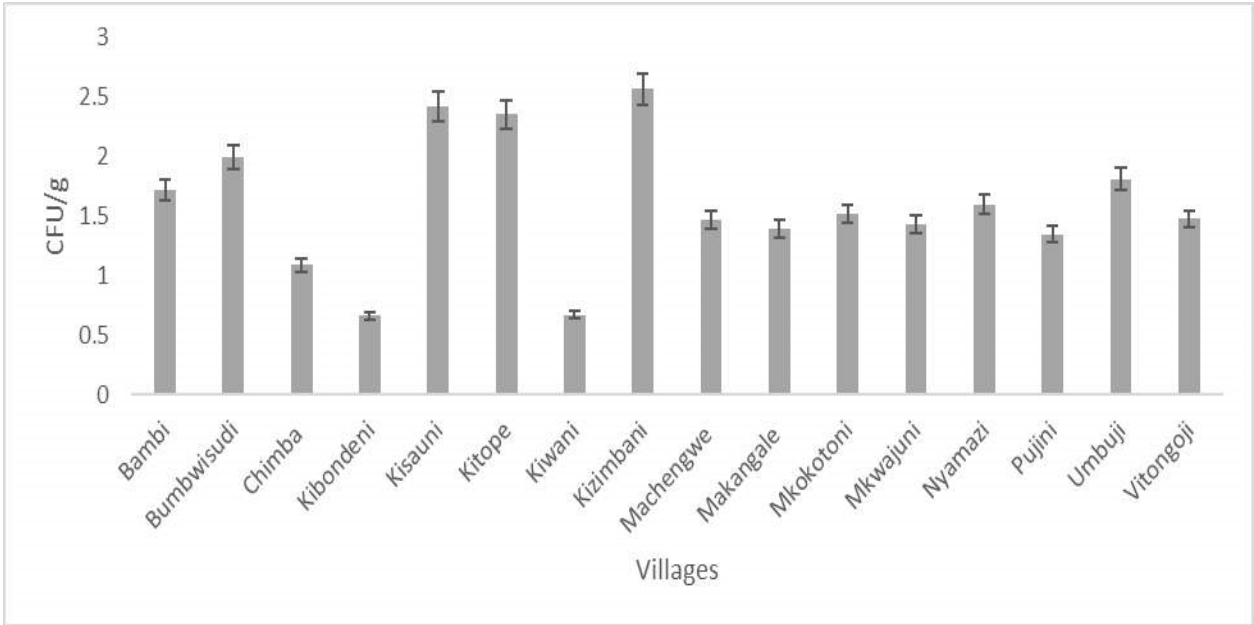

Figure 1. Average Fungi Colony Forming Unit per gram of soil in maize-cultivated villages.

Occurrences of recovered fungi isolate across Zanzibar maize-cultivated villages

The population of fungi isolates varied significantly ($p<0.05$) in different locations except for Cladosporium, which had a low population number and was almost in all locations ($p>0.05$) (Table 1). Both *A. flavus* S-strain and L-strain were abundant in Bumbwisudi (5.767/g, 5.833/g) compared to all sites. They were low at Kiwani (0.567/g, 0.7/g) and Kibondeni (1.2/g, 0.867/g), *A. niger* was abundant at Kisauni (2.867/g) and low at Nyamazi (0.267/g) while *A. parasiticus* were abundant at Kisauni (2.633/g) and low at Mkokotoni (0.267/g). Fusarium was highly abundant at Kitope (5.733/g), followed by Nyamazi (5.433/g), Mkokotoni (5.3/g), Bambi (5.267/g), Umbuji (5.2/g), Kisauni (5.133/g) and were low at Bumbwisudi (0.7/g), Chimba (0.867/g), Kibondeni (1.133/g) and Kiwani (1.367/g). On the other hand, Penicillium was high at Bumbwisudi (0.467/g) and low at Kiwani (0.1/g) **(Table 1).**

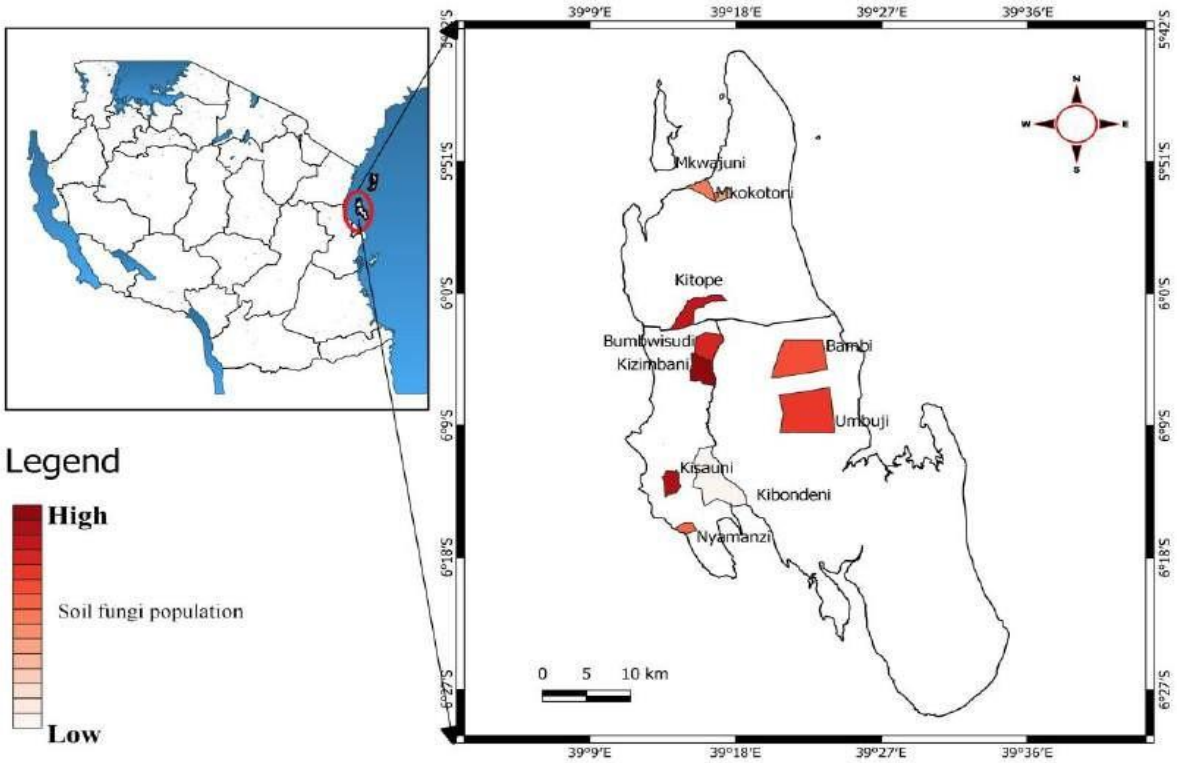

Figure 2. Map showing fungi isolates population in Unguja

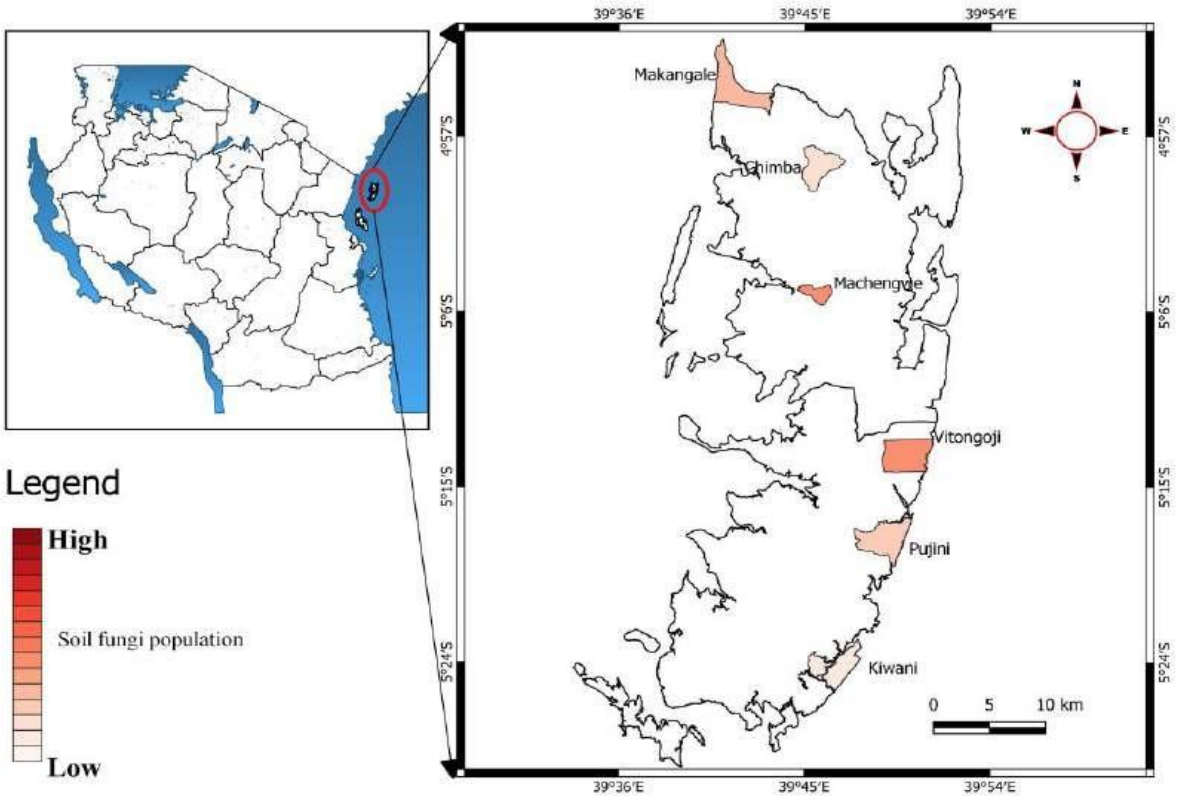

Figure 3. Map showing fungi isolates population in Pemba Villages

Table 1. Recovered Fungi isolates CFU/g in Zanzibar maize-cultivated villages

Villages	AFL	AFS	AN	AP	Cl	Fs	Pn
Bambi	2.200bcde	2.267bc	0.567abc	1.400d	0.133a	5.267fgh	0.200ab
Bumbwisudi	5.767g	5.833g	0.667abc	0.500ab	0.067a	0.700a	0.467b
Chimba	2.467cde	2.667cd	0.667abc	0.600abc	0.167a	0.867a	0.200ab
Kibondeni	1.200ab	0.867a	0.600abc	0.633abc	0.033a	1.133a	0.167ab
Kisauni	3.100e	3.00cde	2.867e	2.633f	0.067a	5.133fgh	0.167ab
Kitope	4.533f	4.267f	0.733abc	0.633abc	0.233a	5.733h	0.367ab
Kiwani	0.567a	0.700a	1.100c	0.867bc	0.000a	1.367ab	0.100a
Kizimbani	5.167fg	4.133ef	1.733d	2.133e	0.033a	4.533efg	0.233ab
Machengwe	2.467cde	2.567cd	0.533abc	0.400a	0.067a	4.067e	0.167ab
Makangale	2.700cde	2.400bc	0.400ab	1.000cd	0.133a	2.967cd	0.167ab
Mkokotoni	2.633cde	1.333ab	0.5abc	0.267a	0.167a	5.300fgh	0.400ab
Mkwajuni	3.000de	0.867a	0.967bc	0.333a	0.167a	4.400ef	0.300ab
Nyamazi	2.433cde	2.300bc	0.267a	0.433ab	0.1333a	5.433gh	0.200ab
Pujini	1.933bcd	2.233bc	0.467ab	0.667abc	0.033a	3.800de	0.300ab
Umbuji	2.133bcde	3.600def	0.733abc	0.633abc	0.133a	5.200fgh	0.267ab
Vitongoji	1.867bc	4.733fg	0.6abc	0.500ab	0.067a	2.367bc	0.200ab
Grand mean	2.8	2.7	0.8	0.9	0.1	3.6	0.2
SED	0.3	0.31	0.16	0.12	0.07	0.28	0.09
LSD	0.62	0.63	0.34	0.25	0.14	0.57	0.18
cv	3.9	4.9	7.9	2.8	18.7	2.7	12.8
p-value	<.001	<.001	<.001	<.001	0.079	<.001	0.013

Note: *AFL=Aspergillus flavus L-strain, AFS=Aspergillus flavus S-strain, AP=Aspergillus parasiticus, AN=Aspergillus niger, Fs=Fusarium, Pn=Penicillium, Cl=Cladosporium*

A significant difference was observed in the relationships between *A. flavus* L-strain and *A. flavus* S-strain ($p<0.001$, $r= 0.666$, $R^2= 0.443$) (**Figure 3**). With increased abundance in L-strains, there was a reasonable increase in S-strains.

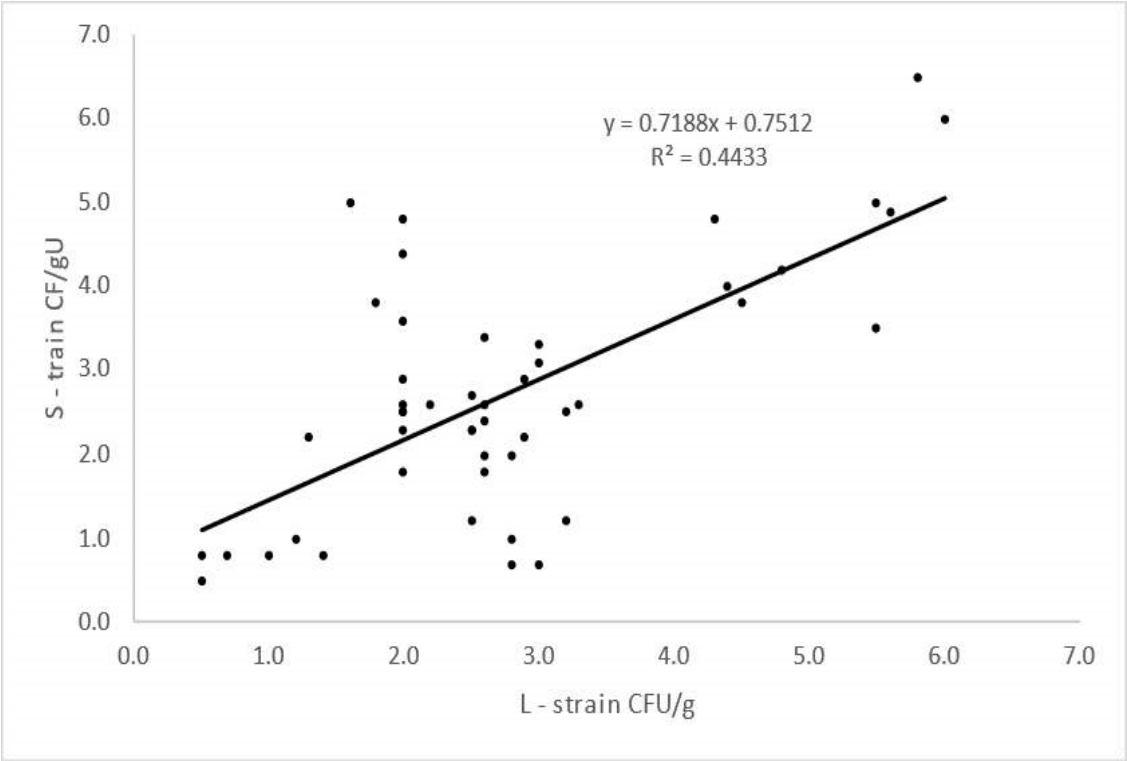

Figure 4. Relationship in soil occurrence between *Aspergillus flavus* L-strain and Aspergillus flavus S-strain.

Table 2. Effect of cropping practices on *Aspergillus flavus* S and L strain in Zanzibar maize cultivated villages

Villages	*A. flavus* L- strain	*A. flavus* S - strain	Cropping Practices
Bambi	2.2	2.267	Maize + Other cereals
Bumbwisudi	5.767	5.833	Maize
Chimba	2.467	2.667	Maize + Vegetables
Kibondeni	1.2	0.867	Maize + Vegetables
Kisauni	3.1	3.00	Maize + Vegetables
Kitope	4.533	4.267	Maize + Vegetables
Kiwani	0.567	0.7	Maize + Vegetables
Kizimbani	5.167	4.133	Maize
Machengwe	2.467	2.567	Maize + Vegetables
Makangale	2.700	2.400	Maize + Vegetables
Mkokotoni	2.633	1.333	Maize +Vegetables
Mkwajuni	3.0	0.867	Maize + Vegetables
Nyamazi	2.433	2.3	Maize + Vegetables
Pujini	1.933	2.233	Maize + Vegetables
Umbuji	2.133	3.6	Maize + Vegetables
Vitongoji	1.86	4.733	Maize + Other cereals

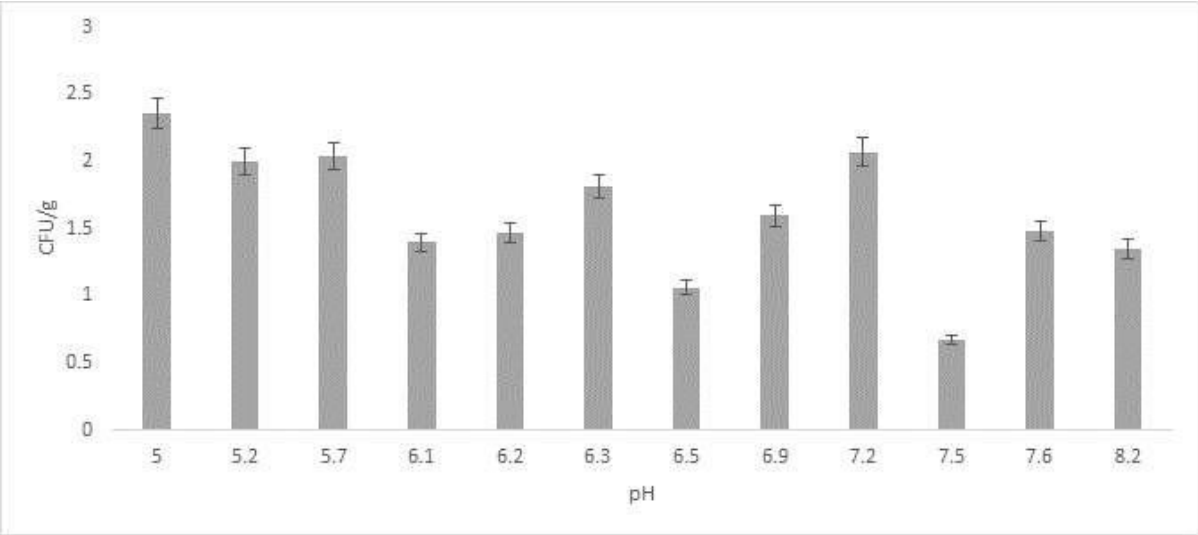

Figure 5. Effect of pH on Colony-forming Unit in gram

The fungal growth trend for most isolates showed a decrease in growth with a pH increase. Cladosporium and Penicillium were not significantly affected by pH change, while both A. flavus S-strain and L-stain had reduced CFU with increased pH.

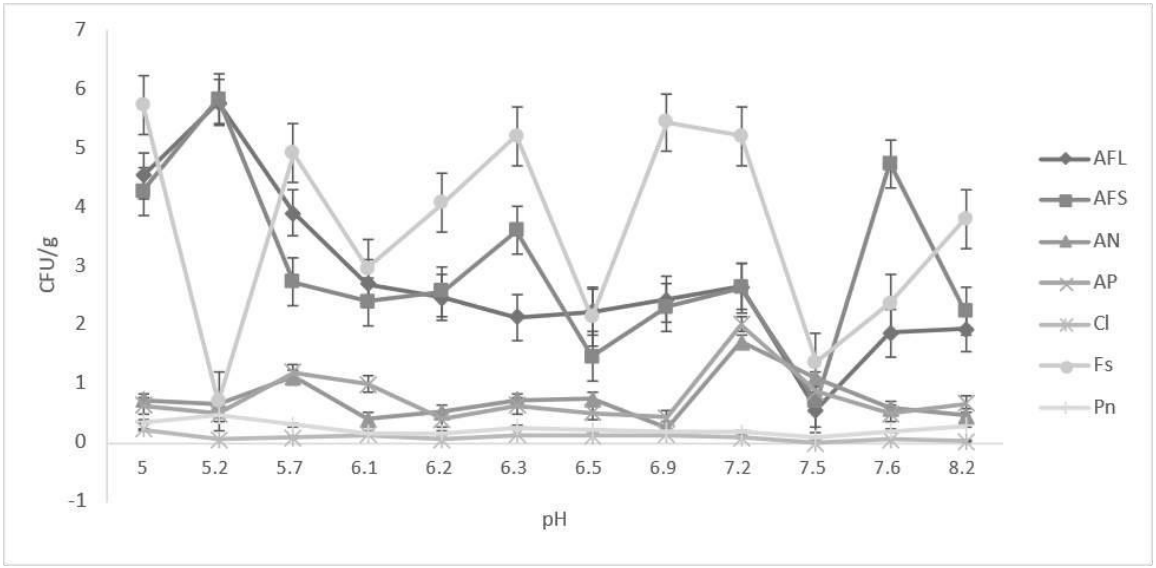

Figure 6. Effect of soil pH in isolated fungi CFU/g

Notes: *AFL = Aspergillus flavus L-strain, AFS = Aspergillus flavus S-strain, AP = Aspergillus parasiticus, AN = Aspergillus niger, Fs = Fusarium, Pn = Penicillium, Cl = Cladosporium*

DISCUSSION

Occurrence of Aspergillus flavus regarding cropping practices

Okoth et al. (2012) suggest that fungal ecosystems vary by species. *A. flavus* colonizes particular crops and others. Therefore, soils with such cropping techniques have more of it (Seetha et al., 2017). Consequently, plant detritus in the soil may impact fungal ecologies and be crucial to their population

(Nesci & Etcheverry, 2002). *A. flavus* is found in soil and decaying plants and contaminates crops (Chalivendra et al., 2017). Due to its dependence on organic matter, A. flavus, a saprophytic fungus, has been found to survive and reproduce in soil and plant waste. The results show that maize residues are substantial sources of *A. flavus* inoculum Monda et al.

(2020). A. flavus propagules were more abundant in previous-season maize cobs than in field soil (Jaime-Garcia and Cotty (2010)). Jaime-Garcia and Cotty (2004) found considerable amounts of *A. flavus* in maize cobs after two years in the soil, indicating that they enhance the fungus's survival and proliferation. Aspergillus contamination can be high in maize monoculture or rotation with vulnerable crops (Munkvold, 2014). *Aspergillus flavus* in maize decreased with cassava, tomato, and pepper intercropping (Hell et al., 2003). Cardwell et al. (2000) discovered that sorghum-intercropped maize increased A. flavus infection. This suggested that intercropping maize with certain crops enhances A. flavus contamination in maize kernels (Hell et al., 2003). Zanzibar villages, a hotbed for Aspergillus in soil, often cultivate maize, suggesting secondary inoculum from crop leftovers may increase fungus.

Occurrence of Aspergillus flavus and other fungi isolates regarding soil pH

A significant difference ($p<0.05$) was observed between recorded soil pH on the influence of fungal CFU/g with deviance to some fungal isolates, having a high number of populations in high pH (above 7), most fungi exhibited a high population between pH 5 and 7.2 *A. flavus* strain and fusarium are only fungi showed high population beyond 7.2 **(Figure 3).**

CONCLUSION

This study counted *A. flavus* in Zanzibar maize fields. The fungus isolates population varied greatly by location. Bumbwisudi has more *A. flavus* S- and L-strains than other sites. *A. niger* was plentiful at Kisauni and lowest at Nyamazi, whereas *A. parasiticus* was abundant at Kisauni and lowest at Mkokotoni. *Fusarium* spp. was most prevalent in Kitope, followed by Nyamazi, Mkokotoni, Bambi, Umbuji, Kisauni, and a low count at Bumbwisudi, Chimba, and Kibondeni. The data indicate that *Aspergillus flavus* could threaten Zanzibar maize production.

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DISCLOSURE STATEMENT

The author declares no competing interests.

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