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ISOLATION AND IDENTIFICATION OF ASPERGILLUS FLAVUS FROM SPOILT TOMATOES VENDED IN GWAGWALADA MARKET, ABUJA

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ABSTRACT

Aspergillus flavus is an important food borne pathogen reported to have caused serious respiratory and gastrointestinal illness in humans and animal worldwide including Nigeria. This necessitated a microbial study on spoilt tomatoes sold at various stands at the Gwagwalada market. Randomly 120 samples were collected for mycological studies. Samples were inoculated on PDA agar as on a petri dish for 3-7 days at 30^0C. Positive isolate of *A. flavus* were further confirm using morphological identification and microscopy. The overall prevalence of *A. flavus* for this study was 28.2%. The prevalence was distributed in the varoius tomato stands in the market. Antifungal sensitivity testing of 6(5%) positive *A. flavus* isolates was carried out using antifungal drugs like the susceptibility discs, comprising of Nystatin (100iu) Itraconazole (50µg), posaconazole (5µg), Caspofufungin(5µg), Voriconazole (1µg), Fluconazole (100µg) , Griseofulvin (10µg) , Metronidazole (50µg), Ketoconazole (15µg). The result indicated that the *A. flavus* isolates were sensitive to Posaconazole, Itraconazole, Caspofufungin, Voriconazole, and Nystatin as such are still potent drugs. However resistant to Fluconazole, Griseofulvin, Metronidazole, Ketoconazole and Amphotericin B. The study provides information on *A. flavus* as a potential food borne pathogen. Therefore it is recommended that public should be properly educated on antifungal drug use and also on the dangers of consuming spoilt tomatoes contaminated with *A. flavus*.

1. Introduction

Aspergillus flavus represents a considerable aflatoxin-generating fungal species that is ubiquitously found in soil and on a multitude of agricultural crops, thereby presenting substantial health hazards to both humans and animals. Its notable capacity for aflatoxin biosynthesis renders it a focal point of extensive scholarly inquiry, particularly in comparison to its phylogenetic counterpart, *Aspergillus parasiticus (*Zanon *et al*., 2013). The ecological habitats of both fungal species exhibit considerable overlap, and they favor analogous environmental conditions that facilitate both infection and the production of aflatoxins (Probst *et al*., 2010; Anaso and Alhassan 2025). From a morphological perspective, *A. flavus* is categorized into two discrete morphotypes: L (characterized by large sclerotia) and S (identified by small sclerotia), with the L morphotype being more prevalent, although it typically yields a lesser quantity of aflatoxin than does the S morphotype. This differentiation is of paramount importance, as the S morphotype was observed to predominate during the aflatoxicosis outbreak that occurred in Kenya in 2004, which raised substantial public health alarms (Probst *et al.,* 2010; Anaso *et al*., 2024a).

Aspergillus flavus flourishes as a competitive soil-dwelling fungal organism, frequently identified in agricultural environments, where it functions as a saprotroph on organic substrates, encompassing both plant and animal detritus (Payne and Yu, 2010). The optimal temperature range for the growth of *A. flavus* is between 25°C and 37°C; however, it exhibits the capacity to endure temperatures spanning from 12°C to 48°C (Klich, 2002; Alhassan and Anaso, 2024). This resilience to elevated temperatures facilitates its expansion predominantly in warmer climates, particularly within the latitudinal range of 26° to 36°, where the likelihood of mycotoxin contamination is considerably heightened.

The fungal species exhibits a remarkable capacity for the colonization of plant seeds and has the potential to induce aflatoxin contamination both prior to and subsequent to the harvest of various crops, including but not limited to peanuts, corn, cottonseed, and tree nuts (Amaike and Keller, 2011). Furthermore, *A. flavus* possesses the capability to aggressively infiltrate any food

products that have not been stored appropriately. Its predominant form within the soil is mycelium; however, it is also capable of producing resilient structures referred to as sclerotia, which play a significant role in its prolonged viability and can either regenerate into mycelium or generate conidiophores and conidia (Horn *et al*., 2014). Research has further demonstrated that sclerotia are capable of developing into ascocarps that harbor ascospores, although the involvement of these sexual spores in the epidemiology of diseases remains ambiguous (Olarte *et al*., 2012; Anaso 2023a; Anaso 2023b).

Strains within the *Aspergillus flavus* population exhibit diverse capacities for aflatoxin production, encompassing both toxigenic and nontoxigenic strains. Conversely, nontoxigenic strains are relatively rare in *Aspergillus parasiticus,* which typically synthesizes elevated concentrations of aflatoxins. The population dynamics of *A. flavus* are profoundly affected by environmental conditions, with warmer climates correlating with an increased prevalence of aflatoxin-producing strains (Moore *et al*., 2021).

The identification of sexual stages (teleomorphs) in both species implies the possibility of sexual recombination occurring under natural conditions. Both species exhibit heterothallism, characterized by the presence of two mating type loci (MAT 1–1 and MAT 1–2) (Horn *et al*., 2014). The prevalence of these mating types fluctuates across different geographic locales, with certain regions demonstrating nearly equal frequencies, which suggests a recombining population structure. Recent investigations have indicated that a balanced ratio of mating types is associated with enhanced recombination rates and diminished variability in toxin profiles among populations, highlighting the potential for species within Aspergillus section Flavi to engage in outcrossing and thereby enhance genetic diversity (Horn *et al*., 2014).

The production of aflatoxins by *Aspergillus flavus* represents a substantial public health issue, given that these mycotoxins result in the contamination of critical agricultural goods including maize, peanuts, cottonseed, and tree nuts. Gaining insight into the complex array of factors that affect aflatoxin biosynthesis and contamination levels across various crops is crucial for alleviating threats to food safety and human well-being (Klich, 2007; Anaso et al., 2021; Anaso et al., 2024b).

Climate change represents another pivotal element, as it modifies environmental parameters, including temperature and humidity, which can profoundly influence the distribution and incidence of A. flavus. Evaluating the ramifications of climate change on the ecological dynamics of A. flavus is imperative for predicting prospective aflatoxin contamination trends (Battilani *et al.,* 2016).

Furthermore, traditional chemical control strategies employed against *A. flavus* frequently engender environmental and health apprehensions, prompting the exploration of sustainable biological control alternatives. Nonetheless, the identification of effective biocontrol agents and the elucidation of their interactions with *A. flavus* continue to pose significant challenges (Moore, 2021).

Once agricultural products are compromised by aflatoxins, the detoxification options remain limited, constituting a significant hurdle, particularly for the food and feed sectors (Khlangwiset *et al*., 2011). Moreover, crops contaminated with aflatoxins present substantial threats to human health, resulting in prolonged exposure and an increased susceptibility to hepatocellular carcinoma (HCC). Examining the underlying mechanisms of aflatoxin toxicity and evaluating associated health risks constitutes a pivotal research endeavor (Gong *et al*., 2016).

Aspergillus flavus occupies a crucial role within the domain of agricultural economics due to its ability to contaminate crops with aflatoxins, which are extremely potent mycotoxins presenting serious risks to both agricultural yield and the health of humans and animals (Bennett and Klich, 2003). A comprehensive grasp of the ecological relationships, genetic variation, and complex biochemical pathways that control aflatoxin biosynthesis is crucial for adequately confronting the challenges related to *A. flavus* contamination.

This investigation is of paramount importance in elucidating the distribution of *A. flavus* in tomatoes, a predominant dietary component in Abuja. The identification of this fungus, coupled with an evaluation of antifungal susceptibility, will facilitate the formulation of efficacious control strategies aimed at alleviating aflatoxin contamination, thereby ensuring food safety and minimizing the health hazards associated with aflatoxin exposure.

The genetic heterogeneity present within A. flavus populations plays a significant role in determining its pathogenic capacity and mycotoxin synthesis. Exploring this genetic diversity is essential for the formulation of targeted interventions designed to reduce aflatoxin contamination (Drott *et al*., 2021; Anaso 2024d). The development of effective strategies for the management of aflatoxin contamination necessitates a comprehensive understanding of the regulatory frameworks that underpin aflatoxin biosynthesis. Future research endeavors should concentrate on innovative methodologies for curtailing aflatoxin production, which may include biological control mechanisms and enhanced agronomic practices (Iqbal *et al.,* 2019; Anaso 2024c).

Furthermore, there are concerns about the future scenarios of aflatoxin contamination due to the influence of climate change on the prevalence and distribution of *A. flavus*. There is a need for research to assess the potential impact of changing climatic conditions on *A. flavus* ecology and, subsequently, aflatoxin contamination (Pfliegler *et al*., 2020). The current research aimed to isolate and identify the *A. flavus* in spoilt tomatoes

The research objectives are;

1. To identify the *A. flavus* in spoilt tomatoes
2. To be able to run an antifungal sensitivity test on the isolates
3. To create awareness among local residents about the risks of infection by contamination in food products.

Materials and methodology

Study area

The data collection was conducted in various markets and retail establishments situated within the Federal Capital Territory (FCT), Abuja. The Federal Capital Territory, often referred to as FCT, constitutes a federal administrative region in the central part of Nigeria. Abuja, which serves as the capital city of Nigeria, is encompassed within this territory. Geographically, the Federal Capital Territory is located between latitude 9.000 North and longitude 6.000 East, extending to latitude 14.080 North and longitude 7.580 East. It encompasses a total land area of 713 square kilometers, housing an estimated populace of 3,464,000, reflecting a 5.67% increment since the year 2020 (Olla *et al*., 2020).

The area experiences a tropical savanna climate, characterized by distinct wet and dry seasons. The wet season extends from April to October, whereas the dry season is observed from November to March. The mean annual precipitation approximates 1,500 mm, with August recognized as the month with the highest rainfall. Temperature levels are relatively stable throughout the year, averaging around 30°C (Adams and Bamanga, 2020). The harmattan, a dry and dusty trade wind, influences the region during the dry season, predominantly from December to February.

Study design

The study is designed to be a cross-sectional study based on the availability of fresh & spoilt tomatoes at Gwagwalada in the FCT. A cross-sectional study is a type of observational research design that analyzes data from a population, or a representative subset, at a specific point in time. This study aims to identify and understand the prevalence and relationships of variables within a given population at one particular time.

A sample size of 120 was to be used and a total of (100) samples was collected at various stores and stands in Gwagwalada market and its environs.

List of materials

Equipment/ Glass wares

Beakers, Pasteur pipette, conical flask, test tubes, universal bottles, measuring Cylinders, cover slip, glass slides, glass petri dish, Universal bottles, distilled water, soap, water, test tube racks, conical flask, 70% ethanol, Bunsen burner, cylinder beaker, towels, glass slides. Weighing balance, hot Air over, Autoclave, incubator, water bath, wire loop, microscope, hot plate, Bunsen burner, incubator, conical flasks, gas oven, refrigerator, autoclave.

Agars

Malt extract agar or *Potato dextrose agar,* Czapek yeast extract agar, *Aspergillus flavus parasiticus agar (AFPA*) medium, sabouraud *dextrose agar*, *YME* broth, lactophenol cotton blue.

Sampling Technique

A simple random sampling technique will be employed to select tomatoes from Gwagwalada Local government Area (LGA) within the FCT.

Sample Size:

Sample size determination

A sample size is the number of observations or replicates included in a statistical sample. It is a subset of the population being studied, and it is selected to represent that population in a study. Sample size will be determined using the formula as described by Thrusfield (2005).

$N= Z^2pq/d^2$

Where:

N= Sample size

q=1-p

Z= 1.96 (normal distribution) from table

P= Prevalence rate from the average of previous studies

D=Desired absolute precision of 5% with 95% Confidence Interval

The sample size will be increased to 120 to increase precision.

Sample Collection

The Sample were gotten from stores and stands, samples of spoilt tomatoes were collected within Gwagwalada market.

Sample Transportation

All the samples were transported all in the same day to the Veterinary Microbiology laboratory University of Abuja for immediate and further processing.

Laboratory Isolation of *A. Flavus*

The purchased spoilt tomatoes (*Lycopersicon esculentum*) were transported to the Veterinary Microbiology laboratory, University of Abuja for fungal analysis which entails the isolation and identification.

In the process of isolating fungi, a total of 120 randomly selected spoiled tomatoes were collected and scrutinized, with a particular focus on the discolored regions. Small sections of the tomato samples, excised from the infected areas utilizing sterile forceps and a scalpel, were subjected to surface sterilization in a 1% hypochlorite solution for 2 minutes, followed by aseptic plating on Sabouraud dextrose agar (SDA) or potato dextrose agar (PDA). The inoculated plates were then incubated at temperatures ranging from 28°C to 30°C for a duration of 5 days, with daily observations for fungal growth (Mailafia *et al*., 2017).

The agar was prepared in accordance with the manufacturer's specifications, wherein 39 grams of PDA were dissolved in 1000 milliliters of sterile water and subsequently sterilized at 121°C for 15 minutes.

The immature fungal colonies were aseptically retrieved and transferred onto sterile PDA plates to establish pure cultures (Yusuf *et al*., 2022). The pure cultures were successfully obtained and preserved through sub-culturing each of the distinct colonies that developed on the PDA plates, followed by incubation at 28°C for a duration of 5 days.

Macroscopic Identification of Isolated Fungi

The petri dishes were meticulously examined utilizing cultural and morphological characteristics, including the growth pattern of colonies, the morphology of conidia, and the pigmentation characteristic of *Aspergillus flavus* (Tafinta *et al*., 2013).

Cultural Identification

Isolates derived from subculturing were systematically characterized and identified based on their colonial and morphological traits, which encompassed both macroscopic and microscopic examinations. Among the utilized characteristics were colonial traits such as size, surface texture, appearance, and the pigmentation observed on the reverse side of the colonies of sporing structures.

Morphological attributes, as delineated by Clayton in Thatana *et al*. (2017), were subsequently employed for the further verification of the isolates.

The characteristics such as colony growth, coloration, texture, and exudation, which can be classified as macroscopic features, were thoroughly investigated. And this was seen noting the initial growth phase frequently white to pale yellow, evolving to a vibrant yellow-green or olive-green as the conidia undergo maturation (Palumbo *et al*., 2023). The texture of the surface vary from fluffy to velvety, often contingent upon the substrate and environmental conditions.

The methodology established by Oyeleke and Manga was also employed for the identification of the isolated fungi utilizing cotton blue in lactophenol staining. The identification process involved the application of a droplet of the stain onto a clean microscopic slide with the assistance of a mounting needle, whereby a small fragment of the aerial mycelium from representative fungal cultures was extracted and placed in the droplet of lactophenol. The mycelium was evenly distributed on the slide using the needle. A cover slip was delicately positioned with minimal pressure to displace any air bubbles. The prepared slide was subsequently mounted and examined under a light microscope using ×10, ×40 and x 100 objective lenses.

Microscopic Identification

After staining the isolate appears to have slender stalks or septate hyphae.The conidial heads are generally globose, and the conidia are produced in chains, characterized by their rough surface morphology. The arrangement and structural features of these conidia are vital for distinguishing *Aspergillus flavus* from other species within the *Aspergillus* genus. This was appreciated from x100 magnification oil immersion.

Antifungal Susceptibility Test

After sub-culturing on a fresh plate, the pure isolates are picked with a sterile swab stick and streaked evenly throughout the whole plate for each sample.

The antifungal discs are place appropriately on the plates and wait for 48hours to check for the zones of inhibition on the plates and the antifungal agents responsible for the clear zones of inhibition.

These zones of inhibition are carefully measured using a ruler, then the results are recorded

Results

Cultural isolation

Out of the 120 samples processed, 36(30%) were positive and 84(70%) were negative. From the 36 positive isolates, 34(28.3%) were positive for *Aspergillus flavus* giving the characteristic morphological and cultural features of white to pale yellow- green colonies having a velvety surface texture. The white appearance was noticed at day 3 post culture while discoloration was noticed at day 6 as seen in plate 1. 2 (1.6%) samples were identified as *Aspergillus niger* based on the cultural characteristics which was observed as black velvet colony on the agar plates as seen in plate 3.

The study resulted in the isolation of two organisms were isolated namely *A. flavus* and *A. niger.* Table 1 summarizes the prevalencre of these organisms with *A. flavus* detected in 28.3% of the samples and *A. niger* in 1.6% as seen in table 1. From table 1, the samples gotten from stand 1, there was only 2 isolates which was negative for *Aspergillus flavus* and positive for *Aspergillus niger* giving a prevalence of 1.6%.

From stand 2, there was 6(5%) isolates which was positive for *A. flavus* and negative for *A. niger*.

Out of the 30 samples gotten from stand 3, the 10(8.3%) isolates were *A. flavus* and this was the highest individual prevalence gotten which could be due to the increased sample size. In stand 4, there was a total of 8(6.6%) positive isolates and was only positive for *A. flavus an*d negative for *A. niger.* In stand 5, 20 samples were collected and there were 10 (8.3%) positive isolates, all for *A. flavus* and none for *A. niger.* Plate 1 shows the cultural morphology of *Aspergillus flavus* showing the characteristic whitish appearance at 24 hrs after inoculation on the agar plate. Plate 2 shows a characteristic greenish appearance of the *Aspergillus flavus* on the agar plate and this could be due to the synthesis of several secondary metabolites such as aflatoxins and having a fluffy velvety texture. The characteristic black appearance of *Aspergillus niger* on the agar plate was identified as shown in plate 3.

Table 1: Distribution of *A. flavus* isolates from various samples from Gwagwalada Area council Market.

SAMPLE LOCATION	NO. OF SAMPLES	NO. OF ISOLATES	*A. flavus*	*A.niger*	(PREVALENCE %) *A. flavus*	(PREVALENCE %) *A.niger*

STAND 1	25	2	0	2	0	1.6
STAND 1	25	6	6	0	5	0
STAND 1	30	10	10	0	8.3	0
STAND 1	20	8	8	0	6.6	0
STAND 1	20	10	10	0	8.3	0
RESULTS	120	36	34	2	28.2	11.6

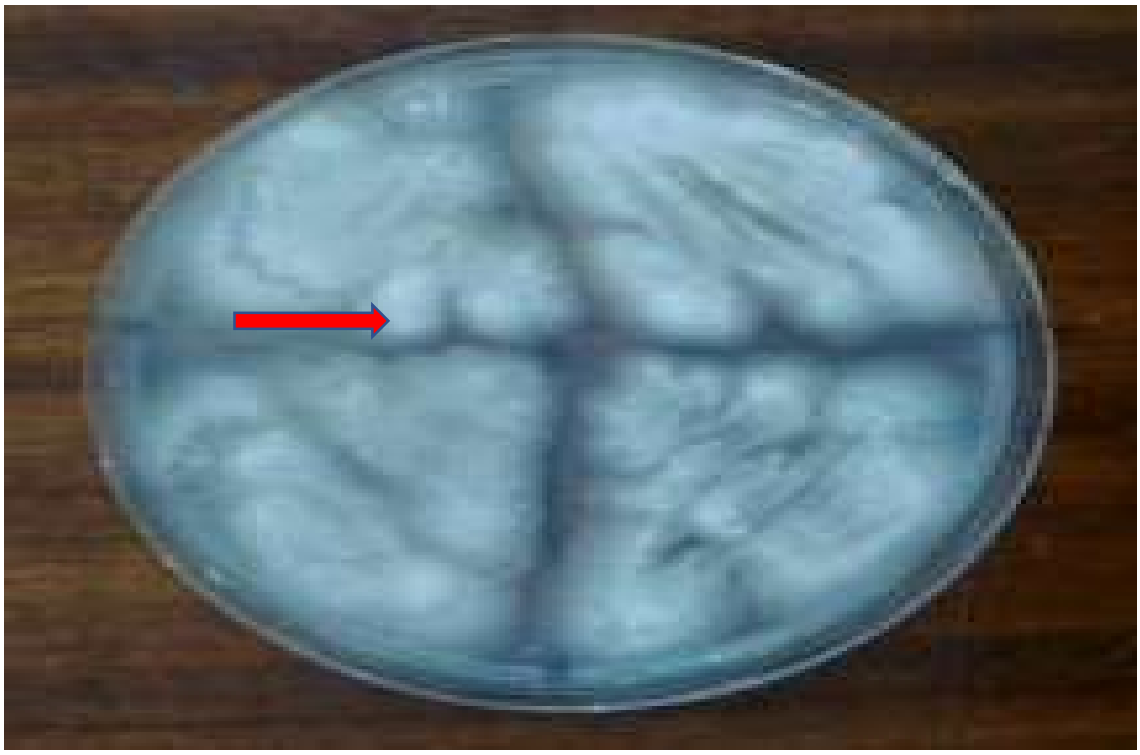

PLATE 1: Cultural morphology of *A. flavus* with red arrow showing the characteristic whitish appearance after 24 hrs

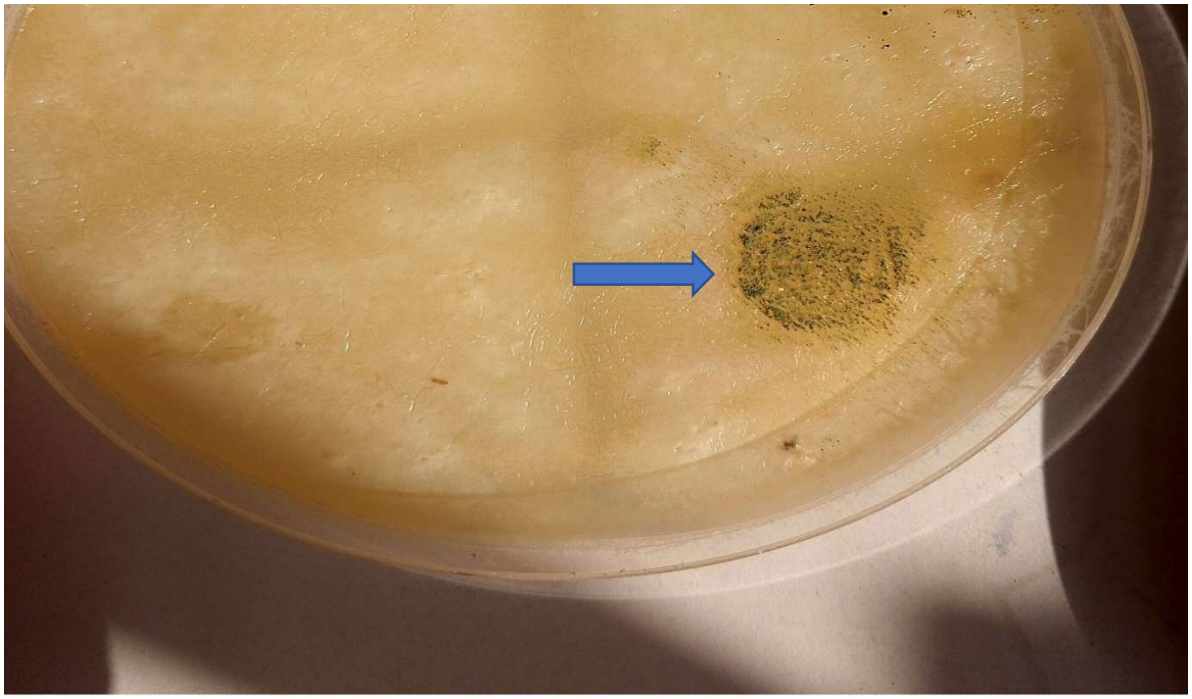

PLATE 2: Cultural morphology of *A. flavus* with blue arrow showing the characteristic greenish appearance

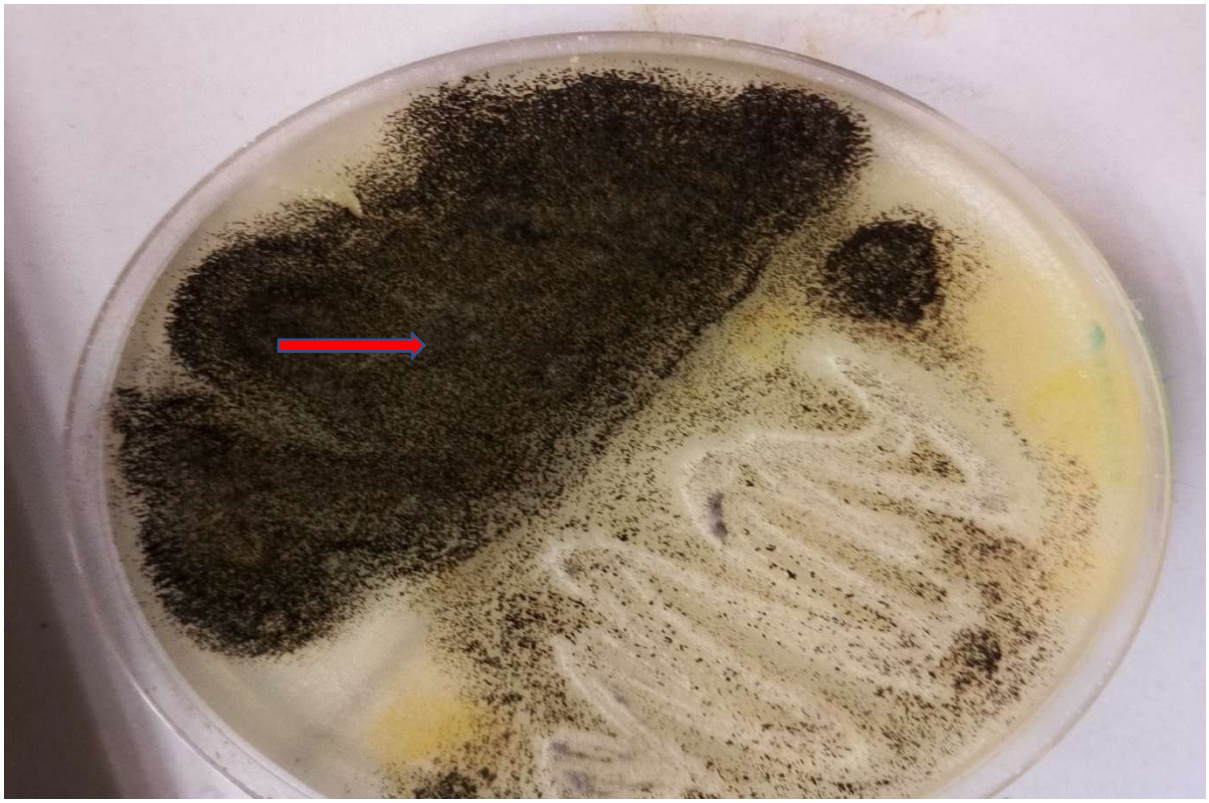

PLATE 3: Cultural morphology of *A. niger* on PDA with red arrow showing the characteristic black velvet appearance

Microscopic Identification

From the 36 positive isolates, 34(28.3%) were confirmed to be *A. flavus,* microscopy revealed a characteristic *A. flavus* as long slender septate hyphae with a globose conidial head, and the conidia are produced in chains, characterized by their rough edges under lactophenol cotton blue stain using x100 magnification as seen in Fig 3 and Fig 4.

Samples From stand 1, as shown in table 1, the 2(1.6%) isolates positive for *A.niger* was seen as black smooth conidial heads with a non-septate long hyphae as seen in Fig 5 with a x10 magnification. This low prevalence could be due to the sample size.

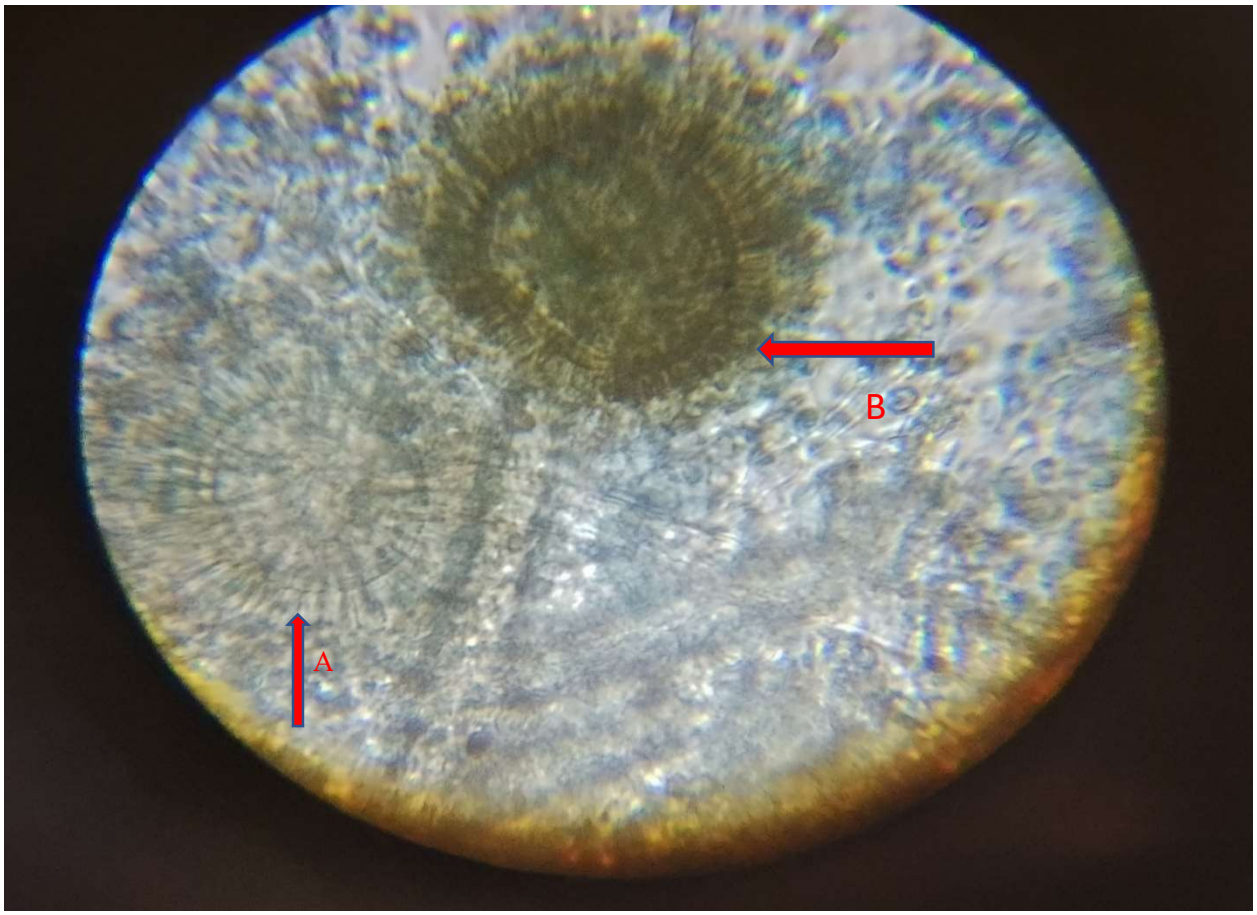

Fig 3: Microscpic appearance of *A. flavus* indicating the presence of the metulae arrow A and arrow B conidia Mg: X100

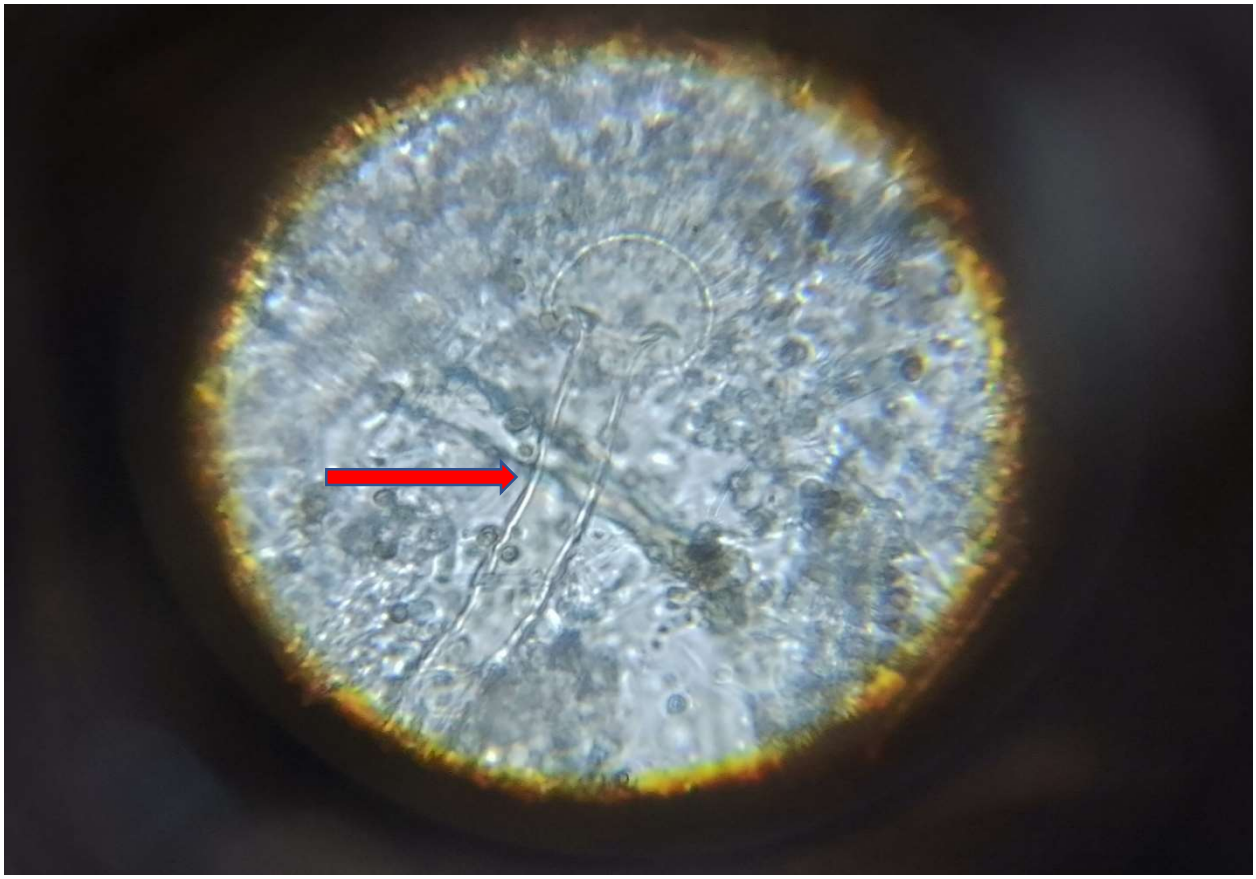

Fig 4: Red arrow shows the microscopic appearance of *A. flavus* indicating the Conidiophore of *A. flavus* at x 100

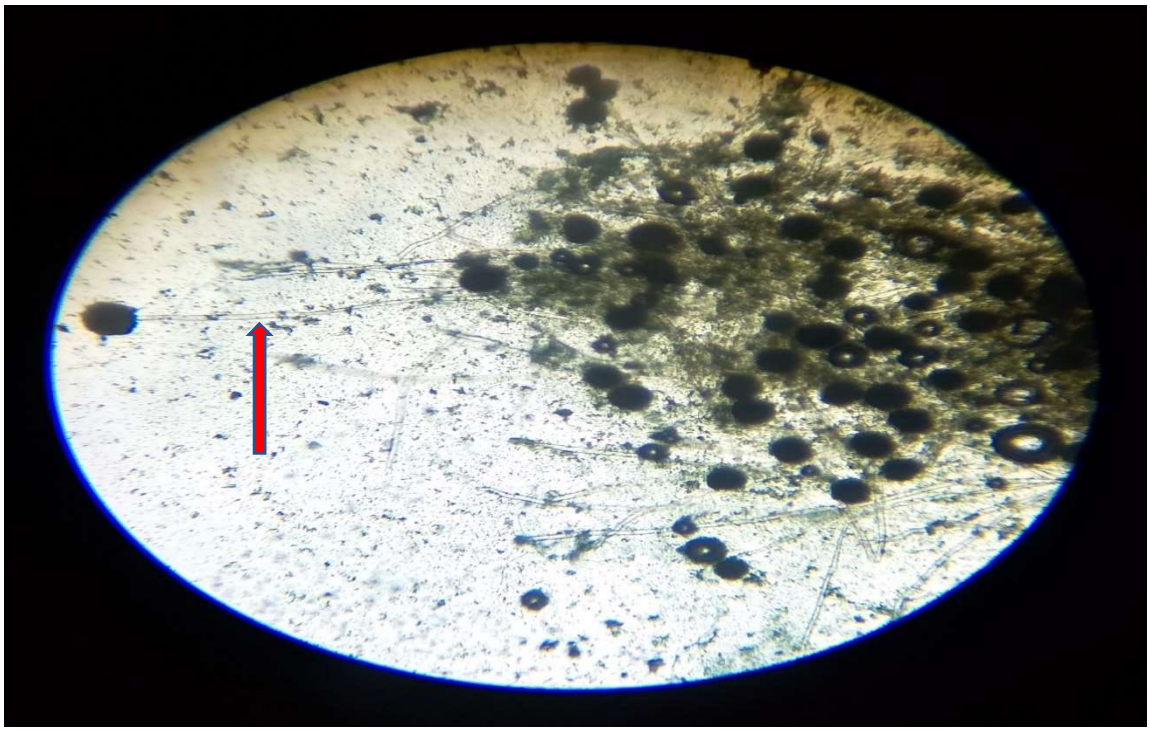

Fig 5: Microscopic appearance of *A. niger* with red arrow indicating the hyphae at x10

Antifungal susceptibility

Table 2 shows the antifungal susceptibility test result of *A. flavus* in millimeters (mm) after measuring with a metric ruler. The antifungal agents used includes Fluconazole(100µg), Griseofulvin(10µg), Metronidazole(50µg), Ketoconazole(15µg), Posaconazole(5µg), Caspofufungin(5µg), Voriconazole(1µg), Itraconazole(50µg), Amphotericin B(20µg) and Nystatin(100iu).

The highest zone of inhibition on the isolates was 30mm for Posaconazole, 23mm for Itraconazole and 20mm for ketoconazole, Nystatin, Caspofugin, voriconazole and Itraconazole. The lowest zone diameter of 0mm was observed for Itraconazole, Posaconazole, Caspofufungin, Voriconazole, Fluconazole, Griseofulvin, Metronidazole, Ketoconazole.

Table 3 and table 4 shows the various isolates susceptible to the antifungal agents, those intermediate and those resistant to the antifungal agents in their percentages. From the 6 isolates tested, Nystatin had 4(66.7%) susceptible and 2(33.3%) intermediates. Itraconazole had 1(16.6%) resistant, 1(16.6%) susceptible and 4(66.7%) intermediates. Posaconazole had 4(66.7%) intermediates and 2(33.3%) susceptible. There was 3(50%) resistant, 1(16.7%) intermediate and 2(33.3%) susceptible result for Caspofugin. For voriconazole there was 3(50%) intermediates, 2(33.3%) susceptible and 1(16.7%) resistant. For the 6 isolates Fluconazole, ketoconazole, Metronidazole and Griseofulvin showed (100%) resistance.Amphotericin B had 3(50%) intermediates and 3(50%) resistant.

Fig 6 shows the clear zones of inhibition which is due to the potency of the antifungal agents therefore inhibiting fungal growth around the disc. Fig 7 shows the zones of resistance on the agar plate therefore no clear zones of inhibition.

Table 2: Distribution of antifungal susceptibility test of *A. flavus* showing zones of inhibition to the nearest millimeters

Isolates	NY 100 iu	ITC 50 µg	POS 5 µg	CAS 5 µg	VO 1 µg	FLU 100 µg	AGF 10 µg	AMB 20 µg	MTZ 50 µg	KCA 15 µg

1	15	20	16	20	20	0	0	10	0	14
2	14	15	16	16	16	0	0	0	0	11
3	15	23	14	20	20	0	0	8	0	12
4	20	20	19	0	15	12	0	10	0	20
5	20	21	30	O	16	0	0	8	0	16
6	12	0	15	0	0	0	0	0	0	0

KEY- NY= Nystatin (100iu), ITC= Itraconazole (50µg), POS= posaconazole (5µg), CAS= Caspofufungin(5µg), VO= Voriconazole (1µg), FLU= Fluconazole (100µg) , AGF= Griseofulvin (10µg) , MTZ= Metronidazole (50µg), KCA= Ketoconazole (15µg)

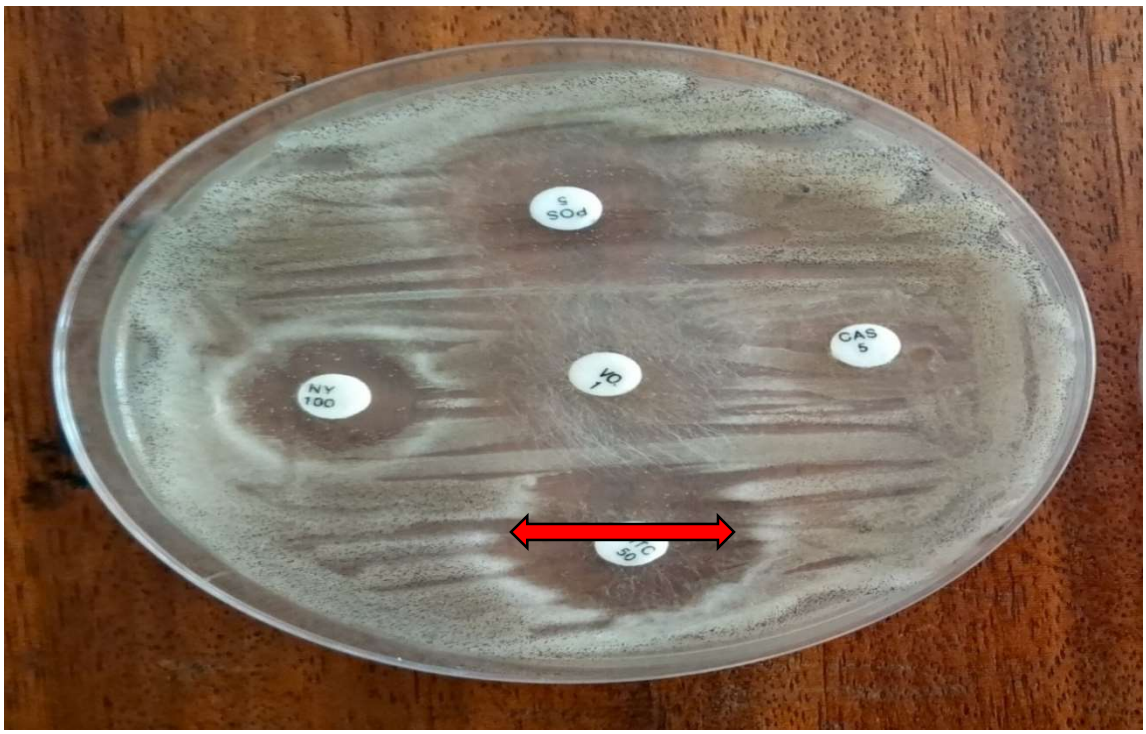

Fig 6: Red arrow shows the clear zones of inhibition indicating susceptibility of the isolates to the antifungal agents tested.

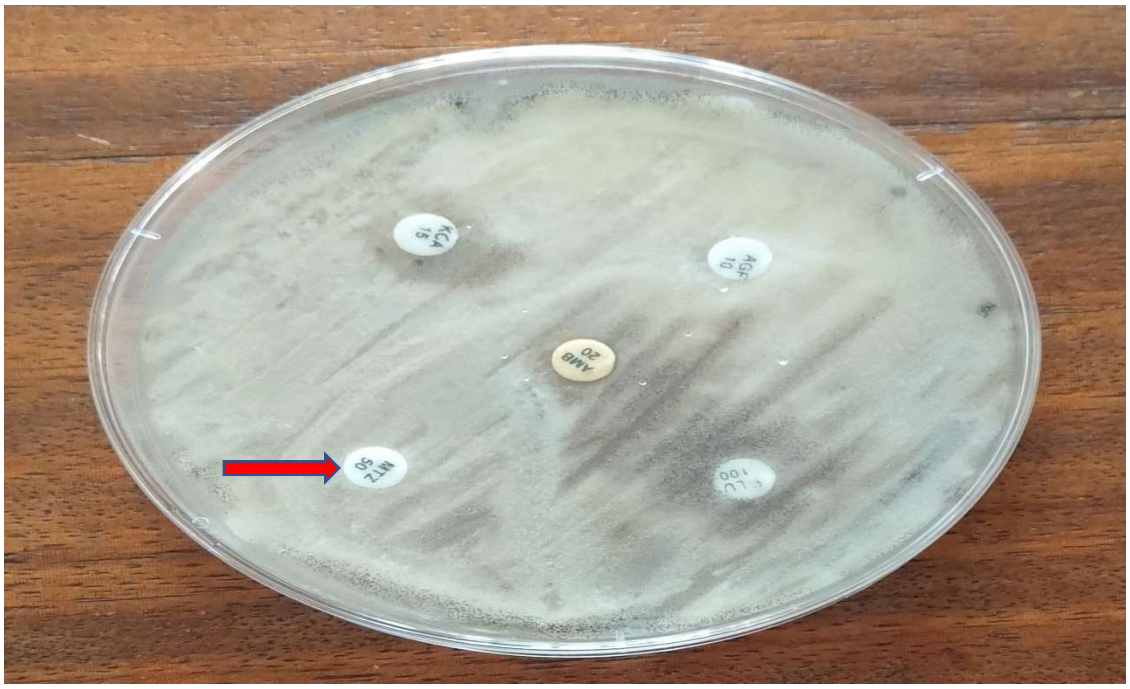

Fig 7: Red arrow shows the zones of resistance of the isolates to the antifungal agents tested.

Table 3: Antifungal susceptibility pattern of *A. flavus* isolates tested

Isolates	NY 100 iu	ITC 50 µg	POS 5 µg	CAS 5 µg	VO 1 µg	FLU 100 µg	AGF 10 µg	AMB 20 µg	MTZ 50 µg	KCA 15 µg
1	S	I	I	S	S	R	R	I	R	R
2	I	I	I	I	I	R	R	I	R	R
3	S	S	I	S	S	R	R	R	R	R
4	S	I	S	R	I	R	R	I	R	R
5	S	I	S	R	I	R	R	R	R	R
6	I	R	I	R	R	R	R	R	R	R

Table 4: Antigungal susceptibility test of *A. flavus* showing the percentages of the sensitive, Intermediate and resistant

Isolates	NY 100 iu	ITC 50 µg	POS 5 µg	CAS 5 µg	VO 1 µg	FLU 100 µg	AGF 10 µg	AMB 20 µg	MTZ 50 µg	KCA 15 µg
Sensitive (%)	66.7	16.6	33.3	33.3	33.3	0	0	0	0	0
Intermediate (%)	33.3	66.7	66.7	16.7	50	0	0	50	0	0
Resistant (%)x	0	16.6	0	50	16.7	100	100	50	100	100

Key- NY= Nystatin (100iu), ITC= Itraconazole (50µg), POS= posaconazole (5µg), CAS= Caspofufungin (5µg), VO= Voriconazole (1µg), FLU= Fluconazole (100µg), AGF= Griseofulvin (10µg), MTZ= Metronidazole (50µg), KCA= Ketoconazole (15µg), AMB= Amphotericin B (20 µg)
S= Susceptible, I= intermediate, R=Resistance

Discussion

Aspergillus flavus grows on PDA agar with a distinctive characteristic yellow-green colonies as a result of synthesis of several secondary metabolites such as aflatoxins, aflavinin, kojic acid e.t.c (Jackson and Dobson 2016). The initial growth phase is frequently white to pale yellow, evolving to a vibrant yellow-green or olive-green as the conidia undergo maturation. The texture of the surface may vary from fluffy to velvet (Palumbo *et al*., 2023).

From this study microscopy shows, septate hyphae with length of about 200 µm and their diameters typically range from 8 to 12.8 µm and the conidial heads appeared globose, which were in chains, characterized by their rough edges morphology, which corroborate with the findings of Nyongesa *et al.,* (2015). Microscopy therefore is an important tool utilized in the identification of *Aspergillus* species prevalent in the study area.

The overall prevalence of *A. flavus* for this study was 28.2%. Notably the prevalence varied across different tomato stands in the market. This findings differs significantly from the 5% prevalence reported by Mailafia *et al*., (2017). This may be attributed to the differences in sample size/ types used in the studies.

Overall prevalence of *A. flavus* isolated from purchased spoilt tomatoes was 28.2%. Our findings which is lower than the report of Tafinta *et al.,* (2014) who reported 25% prevalence from sweet oranges in Jos North Central Nigeria and this could be attributed to the number or the type of fruits examined in both researches. Some reports have shown a higher prevalence of 89.3% obtained from a work done by Danasaki *et al*., (2022) in Maiduguri this higher prevalence than our findings on *A. flavus* this could be due to the difference in the sample size.

A. niger was also isolated from the samples with a prevalence of 1.6% which was lower than the 38% prevalence reported by Mailafia *et al*., (2017) and this could be due to the difference in sample types. According to the report from Samuel and Orji (2015) in Awka, Nigeria, *A. niger* proved to have a higher prevalence of 47.27% which differs from the prevalence in our research.

The Antifungal susceptibility test involved the use of the agents such as Nystatin (100iu), Itraconazole (50µg), Posaconazole (5µg), Caspofufungin (5µg), Voriconazole (1µg), Fluconazole (100µg), Griseofulvin (10µg), Metronidazole (50µg), Ketoconazole (15µg) and Amphotericin B (20 µg). From this study, the isolates were sensitive to 5(50%) of the antifungal agents, this includes Nystatin (100iu), Itraconazole (50µg), posaconazole (5µg), Caspofufungin (5µg), Voriconazole (1µg) and showed resistance to 8(80%) antifungal agents which are Itraconazole (50µg), Caspofufungin (5µg), Voriconazole (1µg), Fluconazole (100µg), Griseofulvin (10µg), Metronidazole (50µg), Ketoconazole (15µg) an d Amphotericin B(20 µg) . Intermediate results was gotten in 6(60%) antifungal agents.

The isolates from this study shows high susceptibility to Nystatin (100iu), Itraconazole (50µg), posaconazole (5µg), Caspofufungin (5µg), Voriconazole (1µg) and this could be due to the fact that they are not readily available and frequently used here while Fluconazole (100µg), Griseofulvin (10µg), Metronidazole (50µg), Ketoconazole (15µg), Amphotericin B (20µg) showed resistance which could probably be caused by the frequent use and easy accessibility of these antifungal agents.

From this research, Nystatin had 4 (66.7%) sensitive, and 2(33.3%) intermediates. The high susceptibility shows that it can be an effective antifungal medication therefore aid in disease control.

Itraconazole had 4 (66.7%) intermediates, 1(16.6%) sensitive, and 1 (16.6%) Resistants and this showed lower resistance than that of Yang *et al*., (2021) who had resistance 3(50%) and this could be as a result of a higher sample size. Gharaghani *et al*., (2020) and Denardi *et al.,* (2017), had a lower resistance of 3.3% and 7.3% which does not agree with our research.

From this study, there was a frequency of 66.7% intermediates in Posaconazole and 33.3% sensitive.

Caspofufungin had 50% resistant which was in contrast to the report of 1% gotten from Yang *et al.*, (2021), 33.3% sensitive and 16.7% intermediate. From research done by Gharaghani *et al*., (2020), they had a 76.7% resistant which is higher than the result from our research and this could be attributed to a higher sample size of 77. Voriconazole had 50% intermediate. 33.3% sensitive, 16.7% resistant. This does not agree to the result of 70% sensitivity gotten from the research done by Diekema *et al*., (2003) and this could be attributed to the higher number of test isolates which is 30.

Fluconazole, Griseofulvin, Metronidazole, and Ketoconazole had 100% resistance which could be due to their easy accessibility and indiscriminate use since they're the frequently used antifungal agents. The resistance could also be attributed to the naturally occurring amino acids substitution (Leonardelli *et al*., 2016).

Amphotericin B had 50 % resistance and 50% intermediates. From this report, shows a resistance higher than the report gotten from Yang *et al*., (2021) having a resistance of 23%. However, Denardi *et al*., (2017) reported a higher resistance of 72.5% and this could be attributed to the higher number of isolates used which is 40. The high resistance make it challenging to treat fungal infections effectively and this can facilitate the spread of fungal diseases.

Conclusion

This study provides information of food borne pathogens such as *A. flavus* and *A. niger*. We were able to properly isolate and identify the *A. flavus* isolates from spoilt tomatoes in the FCT. The isolates grew as yellow-green velvety colonies on *Potato dextrose agar* and as result a long slender hypha with globose conidia radiating from the vacuole was seen under the Microscope when stained with Lactophenol in cotton blue stain which is a typical characteristic of *A. flavus*.

The overall prevalence of *A. flavus* for this study is 28.2%. The prevalence was distributed in the various tomato stands in the market. The antifungal susceptibility test revealed that the isolates were sensitive to Posaconazole, Itraconazole, Caspofufungin, Voriconazole, and Nystatin as such are still potent drugs. Conversely the isolates exhibited resistance to Fluconazole, Griseofulvin, Metronidazole, Ketoconazole and Amphotericin B suggesting that these antifungal agents may not be reliable treatment options. From this research, the high prevalence of *A. flavus* isolates from spoilt tomatoes is a potential threat to man and animals.

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