FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 16, June, 2026, pp 726 – 734
DOI: https://doi.org/10.33003/fjs-2026-1016-5732

Comparative Antifungal Activities of Clove (*Syzygium Aromaticum*), Mint (*Mentha Spicata*) and Scent Leaves (*Ocimum Gratissimum*) Extracts on Postharvest Moulds in Maize, Preliminary Aflatoxin and Amylase Screenings

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Received: 12 Aug. 2026
Accepted: 09 Sep. 2026
Published: 14 Sep. 2026

ABSTRACT

Postharvest moulds cause degradation in maize nutrients quality, and some produce aflatoxin. This study compares the potential of clove buds, mint and scent leave extracts on post-harvest moulds of maize, and preliminary aflatoxins and amylase screenings. Maize samples were collected from five cultivating communities in Ogun and Osun States. Grains were decontaminated for mould isolation, and characterized using morphological and microscopic features. Twenty gram each of botanical was extracted with n-Hexane using Soxhlet extractor, and 5, 25, 50, 75% dilutions was tested on six moulds isolates in agar well diffusion. n-Hexane, Tween 80, Ketoconazole were controls. Zone of inhibition (ZOI) was measure in millimeter. Amylase and ammonia vapour tests were done. Three-way ANOVA ($p<0.001$) and descriptive statistics carried out. Mould counts varied significantly (1.40 - 6.70 x 10^4 cfu/g) across locations. Thirteen mould species of *Aspergillus*, *Penicillium* and *Trichoderma* genera were found with occurrence of 69.08, 23.08, and 7.69% respectively. About 53.08% *Aspergillus* was positive for amylase, whereas ammonia vapour test was negative for all isolates. Clove differed significantly in antifungal potency to scent and mint leaves, whereas both were statistically similar. ZOI increased significantly with extract concentration, and highest (38±0.1 mm) was by clove at 75 and 50% dilution against *Penicillium* species and *A. niger*. A significant three-level interaction occurred between extract type, concentration, and mould species, indicating that antifungal activity was influenced by their combined effects. Therefore, plant extracts demonstrated concentration and mould species dependent efficacy, and could be useful in biocontrol of postharvest mould in maize.

Keywords: Postharvest Maize; Botanical Extracts; Antifungal Activity; Storage Moulds; Agar Well Diffusion; N-Hexane Extraction; Botanical Biocontrol

INTRODUCTION

Maize is an important staple food crop for the vast number of people in sub-Saharan Africa (SSA) where over 70% of it is produced through smallholder farmers. More than 60 million tonnes of maize is produced annually in this region, excluding South Africa (FAOSTAT, 2017). In Nigeria, the maize economy leads sub-Saharan Africa in gross production (12,948,920 million metric tons), and land area (6.5 million hectares) **(**FAOSTAT, 2022), and it's consumed as a staple food, contributing 43% of the calories need in common diet in Nigeria (Adiaha 2018**).** Compromise in the quality of stored maize due to postharvest fungi growth and multiplication, especially under tropical warm and humid conditions that supports fungi growth and production of their associated mycotoxins, especially aflatoxins (Ezekiel *et al*., 2021; Kolawole *et al*., 2020).

The most popular pathogens of agro-produce, including grains like maize, are *Aspergillus, Fusarium,* and *Penicillium* sp. *Aspergillus* species is favoured by warm (25 - 42 ^oC) and humidified weather (Liu et al., 2020). These general are important are crucial in safety of store produce due to their ability to produce mycotoxins**.** *Aspergillus* sp. produced aflatoxins, and the synthesis is linked to the production of spores (Klich, 2007; Tola *et al*., 2016). *Aspergillus* sp are also known to contribute to the production of ochratoxin A (OTA) in the temperate locations, while *Penicillium* species are known to synthesis ochratoxins at considerably low temperature like 5^oC (Tola *et al.,* 2016). *Trichoderma* species may also be found in small fractions as one of the mould contaminants, alongside the others fungal species, and in this situation they may be contaminants from environment (Atehnkeng *et al*. 2008; Akande *et al*., 2017**),** also causing to quality degradation in maize.

The negative impacts of moulds on grains include reduction in quality of grain evidence in decreased stored nutrients, change in colour and development of odour. Also, reduction in grain mass, density, germination of seed, quality of grain in storage, efficiency of feed derived from it, and characteristics of processed grains, and formation of mycotoxins, which is toxigenic to human and livestocks (Mohapatra *et al.*,2017; Yu & Pedroso, 2023).

Conventional methods such as facilities sanitation and fumigation using approved pesticides and fungicides (e.g. USEPA approved in USA), constant maintenance of storage at 4^oC and at low moisture in the barn. Some disadvantages which ensued from these conventional methods, such as high cost, toxicity to humans and animals due to pesticide residues in the food chain, negative environmental impacts and biodiversity (Stack, 2000; Pimentel, 2005). The negative impacts listed are corroborated by close to 3 million reported pesticide and residues cases reported which were connected to agricultural and food storage (Kaur & Garg, 2014**)** aside associated chronic ailments such cancer, Parkinson syndrome, defects in birth, asthma and others (Yu, 2025) most small holder farmers who produced above 70 % annual maize outputs in the sSA lack drying and storage facilities

(FAOSTAT, 2017), and synthetic fungicides may not be affordable, thus necessitating need for effective alternatives. Also, in recent time consumers are shifting attention to consumption of agro-grains devoid of chemical and pesticides residues (Campolo *et al.*, 2018).

Essential oils (EOs), synthesized from plants parts, have demonstrated increased potency against storage moulds, bacteria (Stathas *et al*., 2023; Schmidt *et al*., 2018). They are attractive due to their status as Generally regarded as safe (GRAS) status, which make their application in food to be saved (Essential Oils Market Size, Share & Trends Analysis Report by Application; Jackson-Davis *et al*., 2023). EOs generally work directly or indirectly on fungal mycelia, by disrupting the cell wall and plasma membrane via formation of reactive oxygen species (ROS) on them, leading to leakage of its contents and cell death (Abdi-Mogbadun *et al.*, 2023; Yu, 2023**)**. EOs also decrease the expression of the mycotoxin synthesizing gene (Maurya *et al*., 2024) and impede expression of the molecular gene useful for the strengthening and synthesis of the cell membrane (Zhang *et al*., 2018)**.**

Clove (*Syzygium aromaticum*) EOs is among the plants sourced antimicrobials researched for the effectiveness of their chemical components such as Eugenol oil (78%), Beta-caryophyllene (13%), and terpenoid compounds. Clove is effective against postharvest moulds in grain such as OTA *Aspergillus niger*, *Aspergillus oryzae*, *A. ochraceus* (Hu *et al.*, 2019), *Fusarium* and *Penicillium*, *Trichoderma, Rhizopus*, and others (Karaca *et al.*, 2017**).** Mint (*mentha* spp.) is known for antimicrobial activities owning to the presence of bioactive compound such as menthol, menthone,1,8-cineole,limeone and other related volatiles (Dauqan & Abdullah, 2017; Diop *et al.*, 2016; Wu *et al.*, 2019). Mint EOs have also been found to be effective against most common postharvest mould genera and aflatoxin production (Karaca *et al*., 2017; Gwad *et al.*, 2024). Scent leaf (*Ocimum gratissimum*) is cultivated across the sub-Saharan African for its culinary and medicinal applications, It contained eugenol, thymol, carvacrol, p-cymene and other terpenoids known to have potent antifungal, antimycotoxigenic and antioxidant activities. It has been demonstrated to show inhibitory action on fungal pathogens of agro-food concerns (Ugbogu *et al*., 2021; Uchendu *et al.*, 2024)**.**

Several factors determine the chemical composition of the EOs available and in turns its biological efficacy among which are concentration of the extract, fungal species, method of extractions, locations, parts of plants extracted, extraction time, extraction solvent (Yu, 2025). However, regards to mould inhibition, mould species and concentration are most crucial. The response of the bioactive components in the EOs varies depending on concentration of extract, thus, more concentration more the inhibitory action (Zone of inhibition) produced. Fungi species differed in the cell wall architecture, response to stress and metabolic activity and cellular lipids composition (Ugbogu *et al.*, 2021). Many previous researchers rarely investigate these antifungal efficacy parameters together using many mould species in a single study. Therefore, it is necessary to evaluate these factors in combination for many multiple botanical extracts, In addition, combining antifungal evaluation with preliminary spoilage and aflatoxin screenings would provide broader assessment of the pragmatic valuation of the n-hexane extracts of the botanicals in postharvest grain protection. Therefore, the aim is to conduct comparative invitro antifungal activities of the botanical extracts of clove, mint leaf and scent leaf against selected postharvest moulds in maize, and determine the effect of concentration and mould species, and preliminary assessment of aflatoxin production and amylase production.

MATERIALS AND METHODS

Sampling and Sample Preparation

Dry maize samples were collected from farmers in Alabata village (Latitude 7.316667ºN, Longitude 3.500000 ºE) Agbetu village (Latitude 7.22748ºN, Longitude 3.42447ºE) Ogun state, and Ife-Odan (Latitude 7.83127ºN, Longitude 4.13040 ºE), Osun State. A total of 5 samples were collected for mycological, and labeled A-E accordingly. The maize samples collected were from the grains which are not less than 1momths postharvest, and it was collected from top, middle and bottom of the sack and mixed.

Sample Preparation

Fifty kernels of grain, average mass of 0.25-0.30 g each, was surface sterilized in three stages (Guarav *et al.* (2020). The weighed grains was blended in half concentration nutrient broth in sanitized blender for 5 minutes. The blend was incubated on rotary shaker for 4 h at room temperature following the modified methods of Rijavec *et al.* (2007) and Sotayo *et al.* (2026).

Moulds Isolation

After seven-fold serial dilution, 0.1 ml blend was plated on potato dextrose agar (PDA) prior seeded with 0.01% chloramphenicol, and incubated for 5-7 d at 28ºC. The mean mould counts was done for each samples and recorded in colony forming unit per gram (cfu/g).

Moulds Identification

Pure isolates ware identified using morphological and microscopic characteristics, and features obtained were compared with the description in the keys of Samson *et al.* (2019) and Atlas of Soil and Seed fungi (Watanabe, 2010).

Qualitative Amylase Screening

The pure mould isolates selected streaked on starch agar plate, and incubated for 5 days for 28ºC. Following incubation, the plates were flooded with iodine solution and read after 30 seconds. Amylase positive plates was indicated by the presence of a clear zone (of hydrolysis) around the streaks while blue-black colour around the streaks indicated a negative result.

Qualitative Screening for Aflatoxigenic Potential of Mould Isolates

Seven (7) days spot inoculated PDA plates of each pure isolates was inverted on cover of petri-plate containing 2 mL of concentrated Ammonia solution, and covered for 15 mins for interaction of the vapour with the colony. Colour change that ranged from yellow/dark yellow/pink/reddish brown was a positive indicator of aflatoxin synthesis, otherwise, it was negative (Yazdani *et al.* 2010).

Preparation of Plant Samples for Botanical Extracts

Fresh leaves of both mint (*Mentha spicata*) and scent (*Ocimum gratissimum*) and buds of clove were obtained, washed with sterile distilled water, and allowed to air dried for 7 d in a well ventilated enclosure. The leaves and buds were blended to increase the surface area for ease of extraction.

n-Hexane Extraction of the Botanicals with Soxhlet Extractor

Each of the blend botanical (20 g) was dispensed into a thimble and fixed into the extraction chamber of the Soxhlet extractor. About 150 ml of n-Hexane was filled into the affixed round-bottom flask for the extraction of the botanical for 120 mins. The extraction procedure was repeated till the blend was exhausted. Post-extraction recovery of the n-Hexane for subsequent extraction was done using rotary evaporator to leave behind concentrated botanical extract. The percentage extract yield of each botanical was also estimated.

Antifungal Susceptibility Testing with Botanical Extracts

Preparation and Adjustment of Spore Suspension

Spore suspension of each of the test mould was prepared from freshly prepared sub-cultured PDA plates. The colonies were flooded with 5 ml of sterile distilled water containing 5% Tween 80. The mycelium masses were scrapped gently with sterile inoculating loop. The plate was vortex for 15-30 second, and broth filtered (Whatman filter paper 1) into sterile tube. Then, then 0.01 ml of the filtrate was loaded into the cavity of a hemocytometer, and counted. The concentrated spore suspension of each of the test mould were diluted (1:1000) in sterile distilled water, and 0.01 ml loaded, and counted as previously done. The spores/ml was obtained using:

$$\frac{\text{spore counts per square x dilution factor x } 10000}{\text{number of square counted}}$$

Where 10,000 is a scale up constant for the volume to mL, and number of squares counted was 4.

Agar Well Diffusion Method

Then, 0.1 ml of the adjusted spore suspension was spread plated onto PDA plate, and inoculum was allowed 15 mins to percolate the agar. Then, a sterile cork borer was used bore four wells into the agar, and labelled 5 %, 25%, 50%, and 75% denoted with dilution of the plant extract, prepared by diluting each plant extract with varying volume on Tween 80. Thereafter, 0.1 ml of each of the extract dilution was pipetted into the labelled wells. Exactly 0.1 ml each of Ketoconazole (1 mg/ml), Tween 80, and mix of n-Hexane in Tween, were set up as antifungal, emulsifying, and solvent controls respectively. The experiment was done in triplicates. And incubated in an upright position at 25^{o}C for 3 d. Zone of inhibition (millimeter) was measured with ruler.

Statistical Analysis

The mean of the fungal load of the maize samples, zone of inhibition was separated into mean and standard deviation with MS Excel 2013 (Microsoft Corporation). The test of significance was done using the three-way ANOVA to ascertain the influence of three parameters - two and three levels interactions, and Tukey post-hoc test. Levene's test for normality and homogeneity of variance was done (using SPSS version 21, IBM Corporation). Descriptive statistics was used to express fungal distribution and amylase test. All experiments were done in triplicates.

RESULTS AND DISCUSSION

Results

The mean count of the mould in the maize sample differed significantly amongst the maize samples. Sample E was significantly higher than all other samples. Generally, the fungal load of the maize ranged 1.40 x 10 ^{4} – 6.70 10 ^{4} cfu/g. The lowest counts was found in sample A (Figure 1).

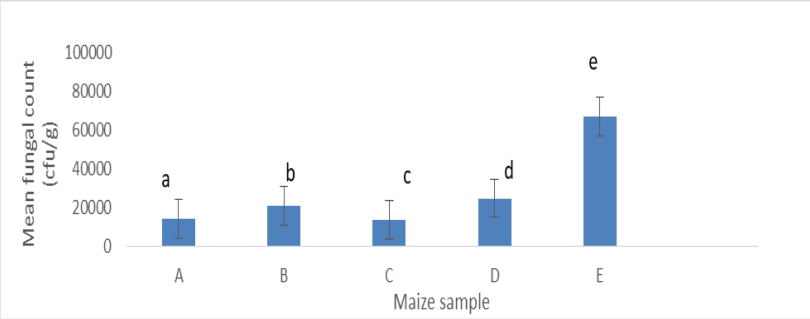

Figure 1: Mean fungal counts of the moulds in the maize samples

Bar with different alphabet were significant at p<0.001 (ANOVA Tukey posthoc test). Values are mean of triplicate observations

The morphological characteristics of the 13 fungal species isolated indicated that they were mainly of the genera *Aspergillus, Penicillium,* and *Trichoderma*. The morphological properties of the isolates revealed that they are filamentous moulds. The mycelia colour of the isolates ranged from varying green colours to black, brown, and greenish-blue. The reverse plate colours varied from yellow to chocolate brown. The microscopic examination revealed mostly round smooth conidia with septate hyphae (Table 1).

The members of the genus *Aspergillus* isolated were *A. flavus, A. niger, A. oryzae*, and *Aspergillus* species, and they amounted to 68.23% of the mould population. The balance was shared by *Penicillium* and *Trichoderma* species at 23.03% and 7.69% respectively (Figure 2). Among the *Aspergillus* members, *A. flavus* species was 44%; *A. niger* was next with 22.22%; *A. oryzea* was 11.11%, and *Aspergillus* species also occurred with 22.22%. The *Aspergillus* members were found co-occurring with other moulds in all the maize samples A–E.

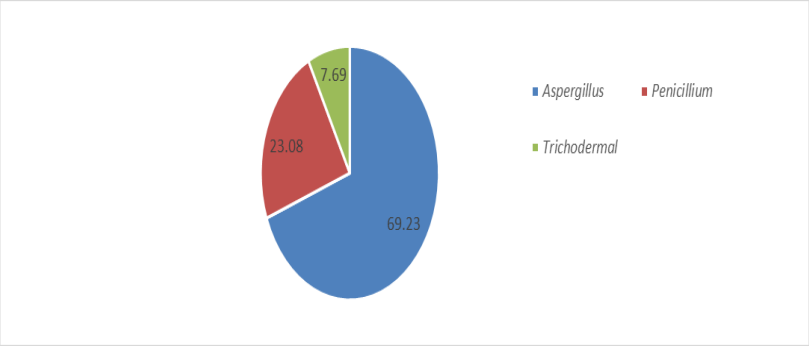

Figure 2: Frequency distribution of the post-harvest moulds isolated from maize samples

The result of the qualitative amylase screening of the moulds indicated that 53% of the *Aspergillus* isolated were amylase positive, while 15.38% were amylase negative. All the *Penicillium* species and *Trichoderma* species isolates constituted 23.08% and 7.69% were amylase negative (Figure 3)

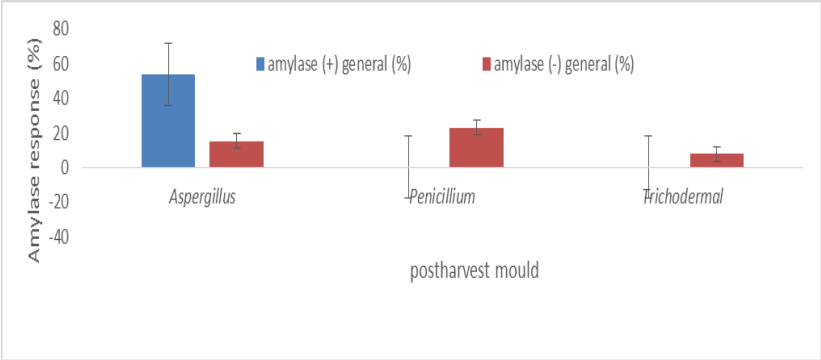

Figure 3: Distribution of the amylase test response by post-harvest mould general

Ammonia Vapour Test

The ammonia vapour test for all the mould isolates was negative.

Table 1: Morphological Characteristics of Fungal Isolates and Qualitative Amylase Test

Isolates/Sample	Macroscopic characteristics	Microscopic characteristics	Amylase	Probable organism
A1	Green filamentous mycelium, raised in the middle, and dull white colour at the tip. Reverse plate colour was yellow	Round, smooth long chain conidia with septate hyphae	+	Aspergillus flavus
A2	Green filamentous mycelium, raised in the middle, and dull white colour at the tip. Reverse plate colour was yellow	Round, smooth long chain conidia with septate hyphae	+	Aspergillus flavus
A5	Teal-green spots produced embedded into the agar	Septate hyphae, conidiophores, phialides, and conidia	-	Trichoderma sp
B3	Greenish blue sponge mycelium with white pigmented edge	Mycelium is branched, multinucleated with septate hyphae	-	Penicillum species
B1	At beginning, white mycelia mass network turned black powdery filamentous mycelium after few days. Reverse plate colour was brown chocolate.	Septate hyphae, smooth colored globose conidiophores and conidia.	-	Aspergillus niger
B4	Powdery dust like rough surface with light green pigmentation	Round, smooth long chain conidia with septate hyphae	¬+	Aspergillus species
C3	Greenish blue sponge mycelium with white pigmented edge	Mycelium is branched, multinucleated with septate hyphae	-	Penicillium species
C2	Greenish blue sponge mycelium with white pigmented edge	Mycelium is branched, multinucleated with septate hyphae	-	Penicillium species
C1	At beginning, white mycelia mass network turned black powdery filamentous mycelium after few days. Reverse plate colour was chocolate brown.	Septate hyphae, smooth colored globose conidiophores and conidia.	-	Aspergillus niger
D2	Powdery, brown pigments with punctiform growth,	Spherical to oval shaped, smooth conidia in clusters	+	Aspergillus species
D3	Cotton like filaments on the outside with olive green pigments on the inside and a flat elevation.	conidiophores with uniserate heads, elongated vesicle with sterigmata located on the top producing smooth conidia	+	Aspergillus flavus.
E1	Cottony/fluffy texture. Colony appeared white initially, but developed into a greenish yellow mycelium mass	Aseptate conidiophore is unbranched and arose from the mycelium.	+	Aspergillus oryzea
E3	Rough, filamentous growth with pale-dull green pigment	Rough conidiophores with uniserate heads.as smoth conidia, elongated vesicle with sterigmata on the top producing smooth conidia.	+	Aspergillus flavus

The percentage yield of scent leaves, mint leaves, and clove bud extracts was 24.50%, 23.00%, and 13.15% respectively. The colour of the extracts varied from thick greenish black for mint, clove buds gave a pale yellow colour, and scent leaves resulting extract was green and watery (Table 2)

The results of the antifungal susceptibility test indicated that the plant extracts differed significantly ($p < 0.001$) from one another in response, with clove being the most responsive, followed by mint leaves and scent leaves (Table 3). In addition, the antifungal efficacy of the extract differed significantly ($p < 0.001$) from one another. More so, the test moulds differed significantly ($p < 0.001$) in susceptibility. The highest susceptibility value was recorded by *Aspergillus niger* and *Penicillium* sp., both being 38.00 mm, while the least susceptibility recorded was 13.50 mm by sp. The antifungal effects at certain concentrations depend on the extract. The antifungal efficacy of the *Trichoderma* botanical extracts depends significantly ($p < 0.001$) on the mould species. All six mould species varied significantly ($p < 0.001$) from one another in terms of susceptibility. The mould species susceptibility to all three extracts followed the same pattern, with *Aspergillus niger* being the most susceptible and *Penicillium* species being the least. The trend observed was *Aspergillus niger* < *Aspergillus flavus* < *Aspergillus oryzae* < *Trichoderma* species < *Penicillium* species < *Penicillium* species. In addition, the susceptibility of the test moulds depends significantly ($p < 0.001$) on the concentration of the extract used. The concentration of the extract varied significantly ($p < 0.001$) among them. The higher the concentration, the more susceptible the test moulds were. The trend observed in the susceptibility in all three extract was 5% < 25% < 50% < 75% dilution. All the test moulds were susceptible based on the trend *Aspergillus niger* < *Aspergillus flavus* < *Aspergillus oryzea* < *Trichoderma species* < *Penicillium sp.* < *Penicillium sp.* Significant ($p < 0.001$) interactions occurred between the antifungal parameters — extract, concentrations, and test moulds. At 5%, 25%, 50%, and 75% concentrations of clove extract, *A. niger*, *Trichoderma* species, *Penicillium* species, and *A. niger* were the most susceptible moulds, respectively. For scent leaves, *Trichoderma* was most susceptible at 75% dilution. Lastly, for the mint leaves at 5%, 25%, 50%, and 75% extract concentrations, *Trichoderma* species, *Trichoderma* species, *A. flavus*, and *Penicillium* species were the most susceptible. Ketoconazole (antibiotic control) recorded highest zone of inhibition (ZOI) for *Penicillium* species with value of 22.50±0.60 mm, and the lowest zone of inhibition was observed for *A. niger* with the value of 16.00±0.40 mm, while both *Trichoderma* species and *A. oryzae* recorded same ZOI with the value of 20.00±0.30 mm and 20.00±0.30 mm respectively. There was no zone of inhibition recorded on n-Hexane and Tween-80 plates.

Table 2: The Percentage Yield and Characteristics of the Extract of Clove Buds, Mint and Scent Leaves

Botanical	Total dry leaves (g)	Leaves extracted (g)	Extract recovered (g)	*Extraction yield (%)	Colour of extract	Consistency
Mint leaves	120	20	4.6	23.00	Greenish black	Thick
Clove buds	180	20	2.63	13.15	Pale yellow	Watery
Scent leaves	65	20	4.9	24.50	Green	Watery

*Extraction yield (%) is the ratio of the mass of the crude extract obtained to mass of plant materials extracted

Table 3 Antifungal Susceptibility Testing Of Plant Extract against Mould Isolates from Maize

	5%			25%			50%			75%					
Test mould	**C**	**S**	**M**	**C**	**S**	**M**	**C**	**S**	**M**	**C**	**S**	**M**	**K**	**N**	**T**
	Mean zone of Inhibition in millimeters (mm)														
Aspergillus nigera	30.50±0.17a	-	15.00±0.10c	25.5±0.40a	-	15.50±0.20c	33.50±0.40a	-	17.50±0.20c	38.00±0.10a	20.50±0.20b	22.00±1.00b	16.00±0.40	-	-
A. flavusb	25.00±0.20a	-	16.50±0.20c	27.00±0.20a	-	18.00±0.70c	31.50±0.30a	-	23.00±0.20c	27.50±0.30a	17.50±0.30b	18.00±0.10b	-	-	-
A. oryzaec	15.00±0.20a	-	17.00±0.20c	28.50±0.60a	-	19.50±0.40c	24.00±0.20a	-	16.50±0.20c	37.00±0.20a	-	16.50±0.40c	20.00±0.30	-	-
Trichoderma speciesd	13.50±0.11a	-	19.50±0.50c	32.50±0.40a	-	21.00±0.20c	37.50±0.40a	14.00±0.10b	18.00±0.20b	32.00±0.66a	21.00±0.20b	18.50±0.60b	20.00±0.60	-	-
Penicillium speciese	19.00±0.50a	-	17.50±0.20c	27.00±0.40a	-	19.50±0.30c	38.00±0.10a	-	18.50±0.31c	37.50±0.40a	-	20.00±0.50c	-	-	-
Penicillium speciesa	29.00±0.72a	-	16.00±0.40c	27.00±0.40a	-	16.50±0.20c	27.00±0.20a	-	19.50±0.10c	26.00±0.20a	-	33.00±0.30c	22.50±0.60	-	-

Key: C = clove oil, S = scent leaf oil, M = mint oil, K= ketoconazole (1mg/ml), N = N-hexane, T= Tween 80. Values are mean of triplicate determinations with three ways ANOVA and Tukey posthoc test. For concentration of extracts, values followed with alphabet on the across horizontal groups differed significantly ($p < 0.001$). For moulds species, responses differed significantly ($p < 0.001$) among mould species followed with different alphabets. For botanicals comparison within concentration group, values across horizontal determinants followed with different alphabet differed significantly ($p < 0.001$).

Discussion

The results obtained in this study indicated the occurrence of moulds of the general *Aspergillus*, *Penicillium* and *Trichoderma* with counts significantly difference according to locations.

There was significant (high mean counts) of moulds in the samples obtained from Ife-Odan, Osun State (mean values E = 6.70×10^4, and D = 2.50×10^4 cfu/g) compared to those obtained from Abeokuta (mean B = 2.10×10^4, A = 1.45×10^4). Variance in fungal load could have resulted from various factors such as the rainfall patterns, source of sample,

time of sampling, temperature, agronomic and storage practices differences in the sampling locations considered. The combination of these factors could influence the composition and frequency of occurrence of mould species in a particular location. Akande *et al.* (2017) obtained fungal counts in maize in the open markets in Osun state, and found 1.50×10^5 - 2.1×10^5 cfu/g, and the toxigenic mould species were *Aspergillus* with 85.70% followed by *Fusarium* and *Penicillium* with 14.3 %, the comparable low mould counts obtained in our study could be due to sampling season and sample source differences. Our sampling was done in the dry seasons and obtained directly from the farmers and not open markets, where possible of cross-contamination with atmospheric spores could have resulted in the increase in the mould counts. Ezekiel *et al.* (2020) reported on contamination and fungal load in openly vended ready-to-eat dried products in Ogun and Lagos States, including maize-based products; the mean fungal load count ranged $7.00 \times 10^2 - 2.50 \times 10^3$ cfu/g. Also, Sipasi (2021) detected high fungal counts in openly vended maize based products (aadun) in Akure and Ondo metropolis; mean counts ranged $1x10^5$ - $9x10^5$ cfu/g. *Edzili et al.* (2025), in their current review on maize contamination in agroecological zones in Nigeria, supported our results obtained in both locations in Ife-Odan (Osun State) and Odeda (Ogun State), located within the derived savannah belt of the agroecological zone (AGZ), in which we reported *Aspergillus* species was 69.23%, followed by *Penicillium* for 23.03% and *Trichoderma* 7.69%. *Edzili et al.* (2025) also reported that *Aspergillus* (37.13%), *Fusarium* (23.13%), and *Penicillium* (13.76%) were the main toxigenic genera found in warm environments like Nigeria. The *Trichoderma* species found have a low percentage of abundance compared to both *Aspergillus* and *Penicillium* general. Atehnkeng *et al.* (2008) identified *Trichoderma* as one of the least identified (0.4%) species in maize in his work that span 3 AGZs in Nigeria. Conventionally, *Trichoderma* are known for their role in soil fertility and nutrients; thus, their occurrences may be contamination from the environment or postharvest activities**.**

It is noteworthy that members of the *Aspergillus* species co-contaminate all the maize samples examined in Agbetu and Alabata (Ogun State) and Ife-Odan (Osun State). This should call for concern due to the possibility of the synthesis of the respective mycotoxin types, which can co-occur in the grains, thereby constituting public health risks to consumers who use maize as a staple. However, the presence of toxins does not guarantee toxin production. Moreover, within the *Aspergillus* species, *A. flavus* and *A. niger* constituted 44.44% and 22.22% respectively, and were found contaminating more than one-fourth of the grains examined. *A flavus* specifically could constitute a risk, being a common producer of aflatoxins, and *A. niger* could produce ochratoxin A (OTA). Owoo & Oladipo (2025), in their study on the mycoflora of stored maize across 5 farms in Oshogbo, Osun State found *A. flavus* as the leading contaminant, and recommended the need for more food safety campaigns in the area.

Sequel to the foregoing, the ammonium vapour test was done, and it indicated that no aflatoxin was produced under the culture conditions and level of sensitivity of the test. The Ammonium vapour test is a preliminary, qualitative, and less sensitive test. Therefore, the results obtained should be interpreted cautiously, and taken as preliminary, given the report of Yazdani *et al.* (2010), who compared different methods of aflatoxin quantification and their sensitivity. Their results indicated that the Ammonium hydroxide vapour test gave a false negative test, and the same result (negative) was later confirmed positive using thin layer chromatography (TLC), and Abd El-Aziz *et al*. (2021) affirmed that the preliminary screening vapour test was 63.6% in accuracy relative to HPLC, 100%. Thus, application of more sensitive and quantitative methods such as HPLC, TLC, and LCMS is recommended.

The result of the preliminary amylase test indicated that only the members of the genus *Aspergillus* were positive, and from this, *A. flavus* amounted to 66.67%, *Aspergillus* species constituted 33.33%, and surprisingly, the two *A. niger* isolates recorded negative results for starch hydrolysis. The positive amylase test indicated amylolytic and starch hydrolyzing capacity in the isolates, and this could lead to nutrient depletion and storage degradation associated with mould growth and multiplicity on grains. Regardless, only an amylase test is insufficient to establish spoilage activities because the expression or otherwise may depend on factors such as microbial strain (Harwood *et al*., 2018), conditions, and period of storage. Both Yu & Pedroso, 2023; Mohapatra *et al*. (2017) reported that mould attack on grains leads to reduction of grain quality and nutrients, grain mass, aside mycotoxin formation. Also fungal infection could lead to degradation of starch molecules, and connected physical properties (Wang *et al*., 2022**).**

The occurrence of amylase-positive *Aspergillus* isolates, especially *A. flavus* and other contaminating mould genera in postharvest maize, emphasizes the need to mitigate the growth and multiplication of spoilage moulds.

The mitigation of spoilage moulds is important to ensure grains maintains quality without the mycotoxin contamination; therefore this study also focuses on botanical biocontrol of postharvest moulds in maize using extracts of clove buds, mint leaves, and scent leaves.

In our study, the n-hexane extract of the three botanicals differed significantly ($p < 0.001$) from one another, and the antifungal efficacy of the extracts also differed significantly ($p < 0.001$). These two observations indicated that the antifungal power of the three extracts varied from each other. From our results, the comparative response of the botanicals indicated that clove bud extract was significant in efficacy (zone of inhibition (ZOI) estimated mean values - 28.71 mm) compared to both scent leaves and mint leaves extracts, while the efficacy of both scent leaves (ZOI estimated mean values - 19.00 mm) and mint leaves (ZOI estimated mean values - 18.86 mm) were not statistically different in terms of their antifungal inhibition as decided by their respective ZOIs. Also, the trends in terms of (using the estimated mean values), the decreasing order of potency of extracts under the experimental conditions was clove buds > scent leaves > mint leaves. This is in tandem with the findings of Edzili *et al.* (2024), who reported that three aromatic botanicals he examined showed variance in their inhibitory activity against moulds isolated from maize grains, and this was due to their variation in active phytochemical.

The trend in the response of the test moulds (using the estimated mean values) to all the three botanical extracts in the decreasing order was *A. niger* > *A flavus* > *A. oryzea* > *Trichoderma* species > *Penicillium* species. Clove extract, contained eugenol oil (78%) and beta-caryophyllene (13%) as the leading compounds; mint contained mainly menthol, menthone, and limeone, while scent leaves also contained mainly eugenol, thymol, carvacrol, and p-cymene. All three botanicals have been found to be effective against the common moulds found in grains such as *Aspergillus niger, A. oryzae, A. ochraceus, Penicillium, Trichoderma*, and other general (Karaca *et al*., 2017, Hu *et al*., 2019; Gwad *et al.,* 2024), as well as suppression of the aflatoxin expression gene in toxigenic moulds (Ugbogu *et al*., 2021; Uchendu *et al.,* 2024). Main while, it is possible for the same indicator

mould(s) to present varying antifungal susceptibility to botanical extracts containing somewhat similar phytochemical compounds and extracted using similar methods. In this scenario, other factors such as part of the botanicals extracted, age of plants, concentration, and other factors would count. This is in tandem with the findings of Awojide *et al*. (2024), who applied crude essential oil of *S. aromaticum* (clove) against *A. niger*.

Our study also indicated that the antifungal effects at certain concentrations varied significantly ($p < 0.001$) with the dose or concentration of the extracts used. Specifically, it indicated that the antifungal efficacy of the three botanical extracts against the six test moulds increased with concentration, that is, 75% (estimated mean ZOI, 25.67 mm) was the highest while 5% (estimated mean ZOI, 19.45 mm) was the lowest responses observed. Similarly, Gwad *et al.* (2024), in his study on the fungicidal and anti-aflatoxigenic potential of cinnamon essential oils on *A. flavus* in postharvest wheat grains, found out that the oils expressed a concentration-dependent antifungal action against *A. flavus.* Also, Grillo *et al*. (2026) observed concentration dependent inhibitory activities, just like our study, in the inhibition of dermatophytes by ethanolic extracts of two botanical leaves - *Azadirachta indica* and *Ocimum gratissimum.*

Our results indicated a significant ($p < 0.001$) three-way interaction existed between antifungal parameters-botanical extract, concentration, and fungal species. This implied that the influence of concentration was not the same across the botanical extracts or test moulds. Specifically, from statistical inferences: Clove bud extract interacted at different concentrations against different test moulds indicating that *A. niger*, *Trichoderma* species, *Penicillium* species (1), and *A. niger* were most susceptible at 5%, 25%, 50%, and 75% respectively. For scent leaves, *Trichoderma* species was the most susceptible test mould at 75%. Lastly, mint leaf extract inhibited *Trichoderma* species, *A. flavus*, and *Penicillium* species (2) at both 5% and 25%, 50% and 75% respectively. In terms of the concentration interactions among the botanicals, the findings of both Atnafu *et al*. (2024) and Gwad *et al.* (2024) made our findings feasible within the prevailing experimental conditions because they both reported that the two factors-fungi tested and botanical extract-determined the level of the antifungal potency, and also, concentration of the extract does modify the response further.

The extract yields differed amongst the three botanicals used in this study; mint leaves were the highest, followed closely by scent leaves and lastly clove buds. However, the amount of the extract yield may just depict the fraction of the constituents that is available for extraction as phytochemicals, and does not necessarily imply antifungal efficacy, which largely depends on the type of phytochemicals and their corresponding concentration in the extract (Atnafu *et al.*, 2024; Gwad *et al*. (2024).

In this study, Ketoconazole is a standard antifungal drug control. According to Eloff *et al*. (2019), who reviewed natural antimicrobial compound against *Furasium* species stated that the inclusion of suitable positive antimicrobial (antifungal) control for purpose of assay validation, rather than basis for comparison. Unlike most natural antifungal studies, which did not include either antifungal control or unreported cases (Seepe *et al*., 2021), our study included standard antifungal drug, solvent control (n-hexane), and emulsifier control (Tween-80). The result of the controls indicated that the Zone of inhibitions obtained for the botanicals were not due to either the extraction solvent or emulsifier, but a reflection of their antifungal efficacies.

CONCLUSION

In this study, n-hexane extracts of the three botanicals demonstrated antifungal effects against the selected moulds from postharvest maize. The significant interaction of the antifungal parameters–botanical extract, concentration, – and mould species–and as a standalone parameter, implied that the efficiency of the antifungal properties hinged on these factors. The results further corroborate the potential of the botanicals tested as proven plant-based source options for the mitigation of contamination in postharvest maize.

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