International Journal of Food Science

WILEY

RESEARCH ARTICLE OPEN ACCESS

Use of Yeasts for the Biological Control of Toxigenic *Aspergillus* sp. Associated With *Zea mays* L.

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Received: 7 April 2025 | **Revised:** 4 January 2026 | **Accepted:** 14 January 2026

Academic Editor: Poulami Jha

Keywords: biocontrol | maize | total aflatoxins | toxigenic *Aspergillus* sp. | yeasts

ABSTRACT

Zea mays "maize" is one of the most important staple foods for both humans and animals, but it is highly susceptible to pathogens such as toxigenic *Aspergillus flavus*. Yeasts, however, have the potential to control the growth and activity of toxigenic fungi, representing a viable and economical alternative for biocontrol. This study is aimed at evaluating the efficiency of yeasts in controlling toxigenic *A. flavus* associated with maize grains. To achieve this, toxigenic *A. flavus* and yeast isolates were obtained and identified, then coinoculated in vitro to assess their effect on mycelial growth inhibition and total aflatoxin production through yeast-derived volatile organic compounds (VOCs). We found that 147 of the isolated yeasts, when coinoculated with *A. flavus*, reduced mycelial growth up to 89%. VOCs from five yeast strains of the genera *Saccharomyces* and *Meyerozyma* inhibited colony growth up to 86% and induced changes at both the macro- and micromorphological levels in toxigenic *A. flavus*. Additionally, VOCs from four yeast strains reduced total aflatoxin levels up to 98%. In conclusion, one yeast strain, identified as *Meyerozyma caribbica*, effectively inhibited both mycelial growth and toxin production in toxigenic *Aspergillus* sp., demonstrating its potential as a biocontrol agent to reduce fungal contamination in maize grains.

1 | Introduction

Maize is one of the most important staple foods for both humans and animals, but it is highly susceptible to various pathogens, particularly filamentous fungi of the genera *Aspergillus* [1], *Fusarium* [2], and *Penicillium* [3], among others [4]. These fungi can significantly reduce crop yield and quality, leading to substantial agricultural losses [4, 5]. Moreover, many species of these filamentous fungi produce mycotoxins during crop cultivation, harvest, storage, and transportation, posing serious health risks to consumers [3, 6, 7]. Most mycotoxins are polyketide compounds, formed through condensation reactions that occur when the reduction of ketone groups in fatty acid synthesis pathways is disrupted under specific physical, chemical, and biological conditions. This process typically takes place at the end of the exponential phase or the beginning of the stationary phase of fungal growth [8, 9].

Mycotoxins are secondary metabolites that can be carcinogenic, hepatotoxic, and nephrotoxic, as well as cause immunosuppressive, dermatological, gastrointestinal, neurological, and reproductive disorders [10, 11]. Among mycotoxins, aflatoxins (AFs) are the most extensively studied and are considered the most significant food contaminants affecting both humans and animals

[12, 13]. AFs can exist in several forms, including AFs B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), G_2 (AFG_2), M_1 (AFM_1), and M_2 (AFM_2), among others [14, 15]. B-type AFs are so named because they fluoresce blue when exposed to long-wave (365-nm) ultraviolet (UV) light, whereas G-type AFs fluoresce green [16]. The M-type AFs, commonly found in the milk of animals, are designated "M" as an abbreviation for "milk" [17]. Among all AFs, AFB_1 exhibits the highest stability, toxicity, and carcinogenicity [14]. AFs are primarily produced by species from *Aspergillus* section *Flavi* and include *Aspergillus flavus* and *Aspergillus parasiticus* [18–20]. Not all *A. flavus* strains produce AFs (nonaflatoxigenic). Toxigenic strains of *A. flavus* predominantly produce B-type AFs, AFB_1 and AFB_2 [21], whereas the production of G-type AFs (AFG_1 and AFG_2) and M-type AFs (AFM_1 and AFM_2) has been reported only in rare, atypical strains and under specific conditions [22, 23] and should not be considered representative of the species as a whole [22].

Some *A. flavus* strains produce sclerotia, or fruiting bodies, composed of compacted mycelium with a dark outer layer. These structures represent a phenotypic adaptation for survival under adverse conditions and are used for classification into Group I and Group II, based on sclerotial size: small sclerotia ("S," $<400\mu$m) and large sclerotia ("L," $\geq 400\mu$m), respectively [24, 25].

Secondary metabolites produced by *Aspergillus* spp., including AFs, serve multiple functions. They are suggested to act as attractants, facilitating herbivorous insects' access to plant tissues and thereby promoting fungal entry and dispersal [26]; as repellents that deter competing pathogenic microorganisms [27]; or as protective agents against UV light and oxidative stress [28]. These compounds may also be involved in quorum sensing and act as stress-response mechanisms during plant infection or drought conditions [29]. Regardless of their ecological role, AFs pose a serious threat to both public health and the economy [16].

In developed countries, it is estimated that 40% of human productivity is lost due to diseases caused or exacerbated by AF exposure. Additionally, annual losses from affected crops, contaminated animal feed, and mitigation efforts amount to approximately $1.6 billion [10], representing 25% of global agricultural production [30]. To address this issue, regulatory agencies have established maximum permissible limits (MPLs) for AFs in food. The European Union has set limits ranging from 0.1 to 12μg/kg for B_1, 4 to 15μg/kg for total AFs, and 0.025 to 0.05μg/kg for M_1. In the United States, the maximum allowable concentration of B_1 in food is 20μg/kg [12]. In Latin America, regulations generally permit AF levels between 20 and 30μg/kg [31]. According to European Commission Regulation (EU) No. 2023/915, the maximum allowable level of total AFs in unprocessed maize intended for human consumption is 10μg/kg, while the limit for AFB_1 is 5μg/kg [32]. Peru does not have specific national regulations for AF limits in food. Instead, it follows the Codex Alimentarius maximum residue limit (MRL), which establishes a threshold of 20μg/kg for B_1 in food products [33]. AFs have been detected in various foods, with maize products being among the most affected, as well as the milk of animals that consume this grain. In the Ucayali region of Peru, a study reported that 50% of pig farms had at least one sample of feed ingredients or balanced feed contaminated with mycotoxins. Among these, 49.2% contained at least one AFB_1 at concentrations (26.2μg/kg) exceeding the MPLs. Additionally, 55.6% of the analyzed maize samples were deemed unacceptable due to the presence of AFs and toxigenic fungi [34].

The primary strategies for controlling mycotoxin-producing fungi in food are physical and chemical methods. However, these approaches have certain limitations, such as alteration of food quality, accumulation of residues above permissible limits [35], development of microbial resistance [36], and environmental contamination [37], compared to biocontrol [38], which utilizes microorganisms that are generally recognized as safe (GRAS) and do not pose risks to human or animal health. Biocontrol involves using microorganisms to regulate the growth and activity of pathogenic organisms through mechanisms such as competition, antibiosis, and parasitism [39, 40]. In the biocontrol of toxigenic *A. flavus*, nontoxigenic *A. flavus* strains [14, 41–43], bacteria [42, 43], and yeasts [44, 45] represent promising alternatives. However, aspects related to colonization stability, impact on food quality, and biosafety still require significant improvement. The application of atoxigenic *A. flavus* has proven effective in large-scale agriculture, particularly when endemic strains are used [46]. Nevertheless, some atoxigenic genotypes produce other metabolites, including cyclopiazonic acid (CPA), a specific inhibitor of calcium-dependent ATPase that can increase muscle contraction in animals and induce plant cell death [16, 47, 48]. Consequently, researchers have differing opinions on whether atoxigenic genotypes with an intact CPA biosynthesis gene cluster and CPA-producing potential should be used as biocontrol agents [41, 49].

Lactic acid bacteria are highly sensitive to environmental and nutritional conditions, which limits their colonization efficiency and antifungal activity [50]. In contrast, yeasts are more resilient, require minimal nutrients, efficiently utilize limited resources, rapidly colonize the host, parasitize fungal structures, and, through hydrolases, degrade fungal tissues while producing soluble and volatile organic compounds (VOCs) that inhibit pathogen growth [51, 52].

Competition, parasitism, and the secretion of enzymes and soluble organic compounds, among other mechanisms employed by antagonistic microorganisms, affect the mycelial growth of toxigenic *A. flavus* [53–55]. However, reductions in mycelial growth do not always correlate with decreased AF production [56], a phenomenon that can be explained by the production of compounds known as extrolites and VOCs by the antagonists [57]. Nonaflatoxigenic *A. flavus* strains producing both extrolites and VOCs may regulate the synthesis of toxic secondary metabolites through chemosensing. Extrolites and VOCs have been reported to significantly reduce AF production while completely inhibiting CPA synthesis [16, 57]. The major VOCs produced by antagonistic yeasts vary between species within the same genus [58], and not all are effective in controlling AF production [56]. VOCs are of particular interest because they act as long-distance chemical signals and modulate the physiology and secondary metabolism of target organisms, such as fungi or plants [59, 60]. Understanding the extrolites and VOCs produced by antagonists will help improve biocontrol strategies against *A. flavus* [61].

Yeasts reported as antagonists of *A. flavus* growth and AF production include the genera *Saccharomyces* [62], *Aureobasidium*

[63], *Wickerhamomyces* [64], *Candida* [65], *Metschnikowia* [56], *Kluyveromyces* [53], *Pichia* [45], *Hanseniaspora* [58], and *Meyerozyma* [66]. The antagonistic mechanisms of these yeasts include competition for space and nutrients, parasitism, release of hydrolases, induction of host resistance, and production of soluble and volatile antifungal compounds [52, 53, 67], which inhibit *A. flavus* mycelial growth and reduce AF production, as reported for *Kluyveromyces marxianus* [53], *Hanseniaspora opuntiae* [58], *Pichia kudriavzevii* [45], *Wickerhamomyces anomalus* [64], and *Candida nivariensis* [65]. Yeast-mediated control of *A. flavus* has been demonstrated under high-humidity conditions and sugar-rich matrices [68]; however, efficacy under low-humidity conditions, such as in maize grains, impact on food quality during long-term storage, and dependence on the initial *A. flavus* inoculum require further investigation [69, 70]. Isolates from these yeast genera hold significant potential as biocontrol agents to ensure the safety of key food products for consumers, as well as the economic security of growers. Therefore, this study is aimed at evaluating the efficiency of yeasts in controlling the growth and total AF production of *A. flavus* associated with hard yellow maize grains.

2 | Material and Methods

2.1 | Isolation and Identification of Filamentous Fungi and Yeasts Associated With *Zea mays* L. Grains

A total of 42 samples (1 kg each) of hard yellow maize, commercially sold as animal feed, were obtained from 42 retail and wholesale distribution centers throughout Lambayeque, Peru, to isolate and characterize *Aspergillus* fungi with AF-producing potential, as well as yeasts with potential antagonistic activity. Isolated yeasts and filamentous fungi for future research interest were deposited in the culture collection at Centro de Investigación para el Fomento Sustentable (CIFOS).

Each maize sample was ground, and 10 representative grams were suspended in 90-mL saline solution (0.85% w/v NaCl) to achieve a 1:100 dilution. Each suspension was plated onto plates containing one of three possible media: potato dextrose agar (PDA) (Merck, Germany), Czapek Dox agar (Merck, Germany), or malt extract agar (MEA) (Merck, Germany). All media were supplemented with 25 μg/mL of chloramphenicol (Fisher, United Kingdom) to prevent bacterial growth [71] and incubated at 28°C for up to 7 days. Representative yeast and fungal colonies were harvested at 48 and 96 h, respectively, and subcultured in PDA tubes, which were subsequently stored at 4°C. Yeast morphotypes were differentiated based on colony morphology and asexual reproductive structures (yeast-like cells). Filamentous fungi were distinguished according to colony characteristics, including color, texture, consistency, appearance, margin, growth rate, reverse color, and pigmentation. For genus-level identification, monospore cultures were prepared. Filamentous fungal colonies were grown on PDA at 28°C for 5 days. Subsequently, a 5-mm agar disk containing the fungus was placed in an Eppendorf tube with 1 mL of 0.1% Tween 80 solution, vortexed, and serially diluted to obtain a suspension containing approximately 10^6 conidia/mL. An aliquot was plated on 2% water agar and incubated at 30°C until conidial germination was observed under 40x magnification. Germinated conidia were then transferred to PDA and incubated until the development of characteristic colonies was confirmed [72, 73].

Filamentous fungi were classified at the genus level using taxonomic keys by Barnett and Hunter [74] and Pitt and Hocking [75]. Specifically, *A. flavus* was identified using the keys of Samson [76] and the descriptions provided by Pitt and Hocking [75]. After 4–5 days of incubation on PDA at 28°C, the colony diameter (mm) was measured, and the daily growth rate (mm/day) was calculated. Additionally, after 5 days, the shape and diameter of the conidia were examined under a light microscope (Olympus BX41). After 21 days, the presence of sclerotia was evaluated, and their shape and average diameter were recorded [77]. Yeast-like fungi were identified using methylene blue staining, based on unicellular morphology [78] and asexual budding structures [79]. Genus-level identification was further supported by carbohydrate assimilation and fermentation profiles, along with distinctive characteristics such as germ tube and pseudohyphae formation, lactose fermentation, urease activity, and colony pigmentation [80, 81].

2.2 | Selection of Toxigenic *A. flavus* Associated With *Zea mays* L. Grains

The detection and quantification of total AFs produced by our isolated *A. flavus* were carried out using the enzyme-linked immunosorbent assay (ELISA) method (Bio-Shield Total for AFs B_1, B_2, G_1, and G_2, ProGnosis Biotech) [82]. The matrix used was Type I (maize), with a detection range of 0–50 μg/kg, a limit of detection (LOD) of 1.5 μg/kg, and a limit of quantification (LOQ) of 2.5 μg/kg. Manufacturer-provided quantification standards of 0, 0.5, 1.3, and 10 μg/kg were used, corresponding to 0, 2.5, 5, 15, and 50 μg/kg of total AFs, respectively. Samples with values exceeding the quantification range of the assay were diluted (1:2, 1:5, and 1:10) in 70% methanol, and the measured concentrations were multiplied by the corresponding dilution factor to determine the total AF concentration (μg/kg) in the samples [83].

For the AF assays, 10 g of maize kernels were placed in an Erlenmeyer flask, disinfected with 1% (v/v) sodium hypochlorite for 3 min, hydrated with 2.5 mL of distilled water, and sterilized in an autoclave at 121°C for 1 h [84]. Subsequently, 2 mL of sterilized distilled water and a 1-cm-diameter PDA disk, taken from the 7-day-old culture of one *A. flavus* isolate, were added. Noninoculated maize kernels served as controls, and three replicates were performed for each *Aspergillus* strain. The samples were incubated at 28°C for 15 days under constant conditions of 85% relative humidity.

After incubation, the maize kernels cultivated with the fungus were sterilized in an autoclave for 30 min, and sterilization was verified by plating the grains on PDA to ensure biosafety. The kernels were then pulverized and independently mixed with 25 mL of extraction solvent (70% methanol) for 2 min at room temperature. ELISA was performed according to the manufacturer's protocol. Finally, absorbance readings for each well were recorded at 450 nm using an ELISA plate reader [82] with Prognosis Data Reader v. 7.6 software (ProGnosis Biotech Ltd, Larissa, Greece). The *A. flavus* strain with the highest total AF

concentration was selected for the antagonism experiments. To prepare the conidial suspension, the selected toxigenic *A. flavus* strain was cultured on PDA at 28°C for 7 days. Conidia were harvested in a saline solution (0.85% NaCl, *w*/*v*) supplemented with 0.2% (*v*/*v*) Tween 80. The suspension was then adjusted to 10^4 conidia/mL using a Neubauer chamber [85, 86].

2.3 | Selection of Antagonistic Yeasts Against Toxigenic *A. flavus*

The antagonistic potential of the isolated yeasts was evaluated in vitro against the colony growth of toxigenic *A. flavus* using the dual culture method on PDA [87], with assays conducted in triplicate. One yeast isolate, from cultures grown on PDA at 28°C for 48 h, was streaked onto one side of a new PDA plate, and 5 μL of toxigenic *A. flavus* conidial suspension was placed on the opposite side. The control group consisted of *A. flavus* cultured without a yeast strain present. All plates were incubated at 28°C for 14 days, after which two perpendicular colony radii were measured [65] to determine the colony area:

$$\text{Colony area}\left(\text{cm}^2\right) = \text{R1} \times \text{R2} \times \pi,$$

where R1 is the longest radius and R2 is the shortest radius.

The percentage of inhibition was then calculated using the following formula [64]:

$$\text{Inhibition area}\,(\%) = \frac{C - T}{C} \times 100,$$

where C represents the area of the control colony and T represents the area of the treated colony.

In addition to growth, changes in macromorphology (e.g., colony color) and micromorphology (e.g., characteristics of hyphae and conidia) were observed and compared with the control. The six yeast isolates that caused the highest levels of inhibition were further tested in three independent experiments to assess the consistency of their antagonistic effects on *A. flavus* growth. A completely randomized experimental design was implemented, consisting of seven treatments: a control (T1) and six yeast isolates (T2–T7).

2.4 | In Vitro Assays Involving VOCs Produced by Antagonistic Yeasts Against *A. flavus*

Of the five yeast isolates assessed for consistent inhibition of *A. flavus*, the five most inhibitory isolates were further evaluated for the effectiveness of their VOCs at inhibiting toxigenic *A. flavus*. The impacts of their respective VOCs were assessed using a completely randomized design with six treatments, three replicates, and a total of 18 experimental units. The treatments consisted of a control (T1) and five antagonistic yeasts (T2–T6). The effects of yeast-produced VOCs were evaluated based on changes in toxigenic *A. flavus* colony growth, macro- and micromorphology, and AF production.

To assess yeast VOC impacts on *A. flavus*, Petri dishes containing yeast extract peptone dextrose agar (YPD) were inoculated with 100 μL of selected yeast cultures (10^7 cells/mL) and incubated at 25°C for 24 h. Meanwhile, separate Petri dishes containing PDA were center-point inoculated with 50 μL of a toxigenic *A. flavus* conidial suspension (10^4 conidia/mL) and allowed to drive for 1 h at 25°C. Each plate with the cultivated yeast was inverted over the fungal plate, sealed with Parafilm, and incubated at 28°C for 14 days. A control was included, consisting of the fungus grown on PDA without yeast [56]. After the incubation period, two perpendicular colony radii (R1 and R2) were measured, and the inhibition area was calculated as previously described [64, 65]. Macromorphological characters were assessed, noting differences between control and VOC-treated colonies. Additionally, mycelia were collected from the center of the toxigenic *A. flavus* colony using an adhesive tape strip. The samples were mounted on glass slides, stained with lactophenol blue, and observed under a light microscope to examine and compare micromorphological characters, VOC-treated colonies to the control.

Once the morphological assessments were completed, the plates containing *A. flavus* colonies were subjected to metabolite extractions in preparation for AF analysis. The toxin produced by *A. flavus* was quantified using the previously described ELISA method [82]. Briefly, a 10-g section of each *A. flavus* colony was excised and individually mixed with 25 mL of extraction solvent (70% methanol), then stirred for 2 min at room temperature. The ELISA method and absorbance readings were performed as previously described. A reference concentration of 51 μg/kg of total AFs was used to calculate the percentage reduction in toxin production, resulting from exposure to yeast VOCs. ELISA measurements were performed using three biological replicates per treatment, corresponding to three independent *A. flavus* plates exposed to the same VOC conditions.

2.5 | Molecular Confirmation of Species Identity for the Most Inhibitory Yeast Strain and for Toxigenic *Aspergillus* Strain Af4

The yeast strain exhibiting the strongest antagonistic effect on *A. flavus* growth and toxin production and the toxigenic *Aspergillus* strain Af4 were subjected to molecular identification. Fungal DNA extraction was performed using the Cenis method [88]. The internal transcribed spacer (ITS) gene was amplified via PCR using the universal primers ITS1F and ITS4. The PCR products were then sequenced using the Sanger method at the Mycology and Biotechnology Laboratory "Marcel Gutiérrez Correa" of the National Agrarian University La Molina (UNALM). The assembled sequences were preliminarily identified using BLASTN and aligned with related ITS gene sequences retrieved from the NCBI database [89]. Finally, phylogenetic analysis was conducted using MEGA11 [90], applying the neighbor-joining method with 1000 bootstrap replicates.

2.6 | Statistical Analyses

The percentage of colony growth inhibition and the total AF concentration produced by *A. flavus* in the presence of antagonistic yeasts were analyzed using parametric ANOVA. Prior to ANOVA, assumptions of normality and homogeneity of

variances were verified using the Shapiro–Wilk test and Levene's test, respectively. Both assumptions were satisfied ($p > 0.05$), allowing the use of ANOVA. Significant differences between treatments were determined using Tukey's multiple comparison test at a significance level of 0.05. All statistical analyses were performed using SPSS 27.0 for Windows.

3 | Results and Discussion

3.1 | Filamentous Fungi Associated With *Z. mays* L. Grains

A total of 205 filamentous fungi were isolated from the 42 maize grain samples. Based on the six colony characters mentioned previously, there were 14 possible groups that encompassed our isolates (Figure S1). Table S1 provides the specific colony characters for each group that were used to preliminarily identify these isolates to the genus level. The represented genera of filamentous fungi across all maize samples are shown in Figure 1.

The *Aspergillus* genus was identified in maize grains, consistent with previous studies [3, 91], which demonstrated that these fungi are common during cultivation, postharvest, and storage [91]. Maize grains often show high levels of contamination by the three toxigenic fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* [92], which are recognized as important contaminants both in the field and during storage. Several studies [3, 92, 93] have reported the predominance of *Aspergillus* species (40%–82%), followed by *Penicillium* (25%) and *Fusarium* (24%), or by *Fusarium* (14%) and *Penicillium* (9%). The dominance of *Aspergillus* species in maize grains, either at harvest or during storage, is attributed to their ability to adapt to diverse environmental conditions and to grow across a wide temperature range (18°C–48°C), even under low relative humidity in the field or storage environments [94]. Similar to *Aspergillus*, *Fusarium* species infect and contaminate agricultural products before and after harvest. They are among the dominant fungi associated with cereal grains, causing seedling blight as well as stem and root rot and producing mycotoxins such as fumonisins, among

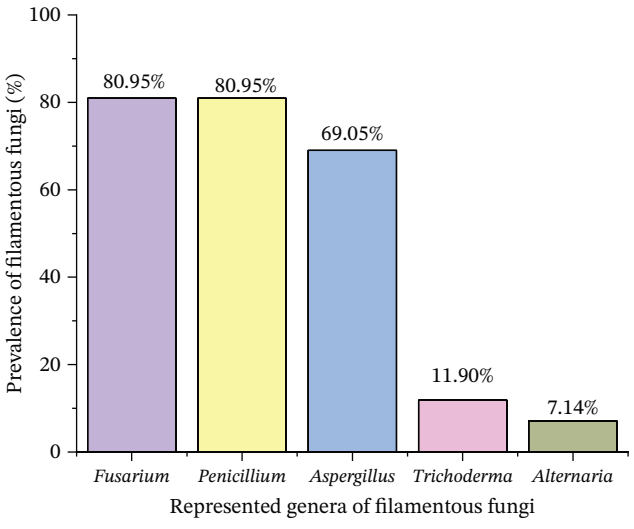


FIGURE 1 | Bar graph showing the prevalence of filamentous fungi, identified to the genus level, isolated across 42 maize samples.

others [30]. *Penicillium* species are known to be ubiquitous and nutritionally undemanding opportunistic saprophytes. Their predominance has been reported at 40%, compared to *Fusarium* (27%) and *Aspergillus* (22%) [95]. However, Jedidi et al. [96] concluded that *Fusarium* species are more active in the field than under storage conditions. In the present study, the maize grains analyzed were harvested by farmers and sold directly without prior storage, which may explain the predominance of *Fusarium* spp. [97, 98].

3.2 | Identification of Toxigenic *A. flavus* in Fungi Associated With *Z. mays* Grains

Across all 42 maize grain samples, 47 isolates were representatives of the genus *Aspergillus*. However, our goal was to find isolates of *A. flavus*. Based on their similar macro- and micromorphological characters (Figure 2), we identified 22 isolates of *A. flavus* across 42 maize grain samples (Tables S2 and S3). The average radial growth rate of *A. flavus* on PDA between Days 4 and 5 was 14–20 mm per day. The conidia were spherical to oval, with diameters ranging from 2.7 to 3.8 μm. Thirty-two percent of the isolates produced L-type sclerotia, which were globose to subglobose and brown in color, with an average diameter of 490–670 μm. Subjecting these *A. flavus* isolates to AF assays, only seven (32%) were found to be toxigenic, with total AF concentrations ranging from 75 to 310 μg/kg. We presume the upper limit was likely higher than 300 μg/kg because several samples reached the maximum detectable limit of ELISA. In contrast, the control samples showed concentrations below the detection limit ($< 1.5\,\mu$g/kg) after 15 days. Based on total AF production, isolate Af4 (Figure 2; Table S2) produced the greatest amount and was used for the antagonism experiments.

The concentrations of AFs found in our maize grain samples were consistent with the findings in other *A. flavus* studies [21, 83]. Coincidentally, the greatest AF concentration detected in this study, produced by *A. flavus* strain Af4, is comparable with another study [65] that quantified 310 μg/kg of AFs produced by *A. flavus* in maize grains over 14 days. Additionally, in vivo AF production by *A. flavus* in our maize grains correlates with a previously described in vivo infection model and results obtained under storage conditions [83].

A. flavus exhibits several morphotypes, classified into Group I and Group II based on sclerotial size: small sclerotia ("S," $< 400\,\mu$m) and large sclerotia ("L," $\geq 400\,\mu$m). Group I includes both S and L strains, while Group II comprises only S strains. Both S- and L-type strains produce AFB_1 and AFB_2; however, they differ in their ability to produce AFG_1 and AFG_2, as well as in aggressiveness, AF yield, and number of sclerotia—all of which are typically higher in S strains. Conversely, L-type strains produce a greater number of conidia, which can be dispersed over long distances. Approximately 70% of *A. flavus* strains are capable of producing both AFB_1 and CPA [24, 25]. The majority of *A. flavus* strains investigated in previous studies produced L-type sclerotia [22, 47, 99]. Although S-type strains of *A. flavus* have been reported to produce higher levels of AFB_1 than L-type strains [100], the evaluation of 467 maize isolates showed that 64 were classified as N-type (nonsclerotial), 359 as L-type, and 44 as S-type. Sclerotial size exhibited no significant

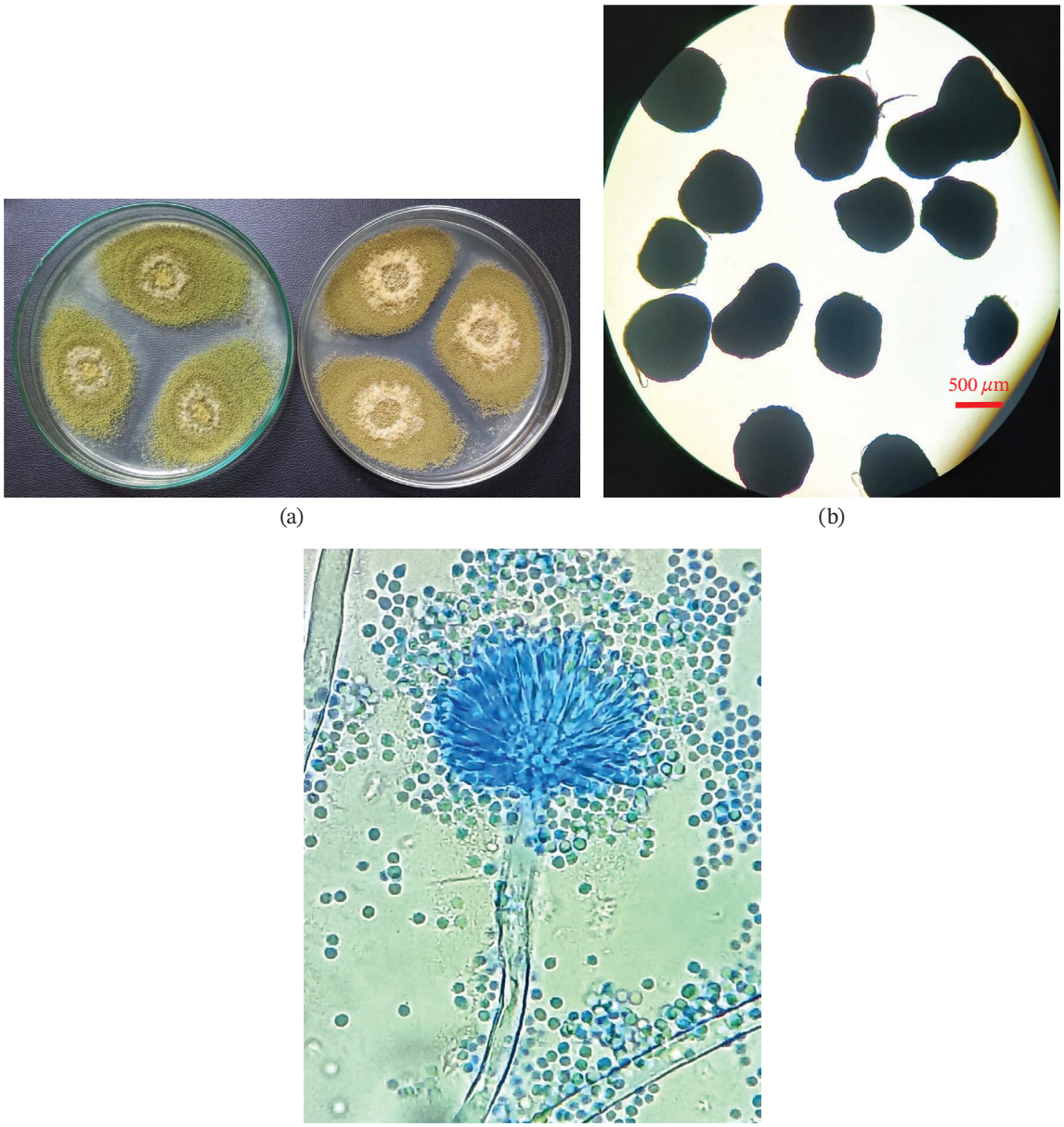


FIGURE 2 | The image corresponds to strain Af4. (a) Macroscale images of *A. flavus* colonies on PDA (left) and MEA (right) media and (b) L-type sclerotia. (c) Microscale image of conidiophore and conidia.

correlation with AFB_1 production ($R = 0.009$, $p = 0.891$). The dissociation between toxigenicity and sclerotial size indicates that these traits are under different genetic control and that sclerotial size cannot be used as a reliable indicator of AF risk [77].

The ELISA kit used in this study did not allow differentiation among the types of AFs (B, M, and G) produced by isolate Af4. Several techniques, including high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and PCR-based methods, have been employed for detecting AF production; however, these methods are relatively expensive [101]. *A. flavus* is the predominant producer of B-type AFs (AFB_1 and AFB_2), but it can also produce AFM_1, AFM_2, AFG_1, and AFG_2, along with other toxic metabolites belonging to the classes of indole-tetramates, nonribosomal peptides, and indole-diterpenoids. In a study involving 28 *A. flavus* strains, only four isolates produced all three types of AFs (B, G, and M), 13 isolates produced only B- and M-type AFs, one isolate produced only AFB_1 and AFB_2, and 10 strains produced B-type AFs alone. The researchers did not observe any strain capable of producing G- or M-type AFs without simultaneously producing B-type AFs [23].

3.3 | Identification of Yeast Strains From *Z. mays* L. Grains With Antagonistic Properties

There were 147 yeast strains isolated across 16 of the 42 maize grain samples, all producing cells that were round, elliptical, ovoid, or oval with characteristic budding, which is indicative of asexual reproduction [79]. There were four yeast genera represented across all maize grain samples. The most prevalent yeast genus was *Saccharomyces*, and the least prevalent was *Rhodotorula* (Figure 3; Tables S4 and S5). Each yeast isolate was

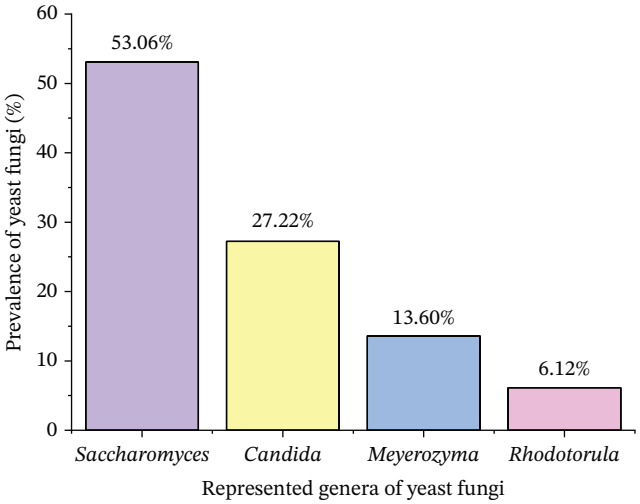


FIGURE 3 | Bar graph showing the prevalence of yeast fungi, identified to the genus level, isolated across 42 maize samples.

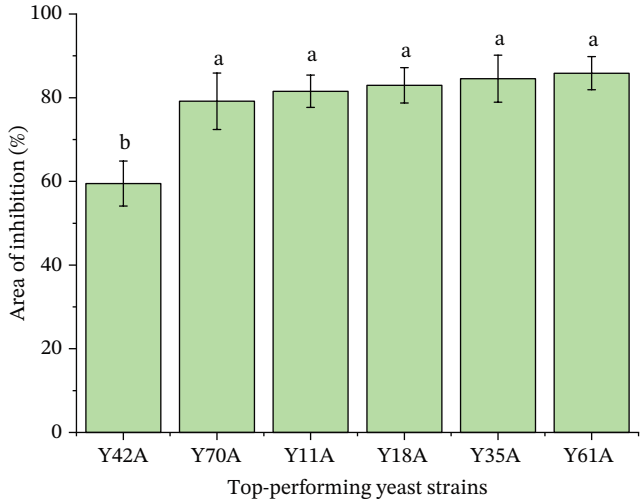


FIGURE 4 | Bar graph showing percent of inhibition for toxigenic *A. flavus* Af4 when coinoculated with each of six top-performing yeast strains, averaged across three replicates, with standard error bars and letters above each bar indicating significance of differences between treatments according to Tukey's multiple comparison test ($p < 0.05$).

TABLE 1 | Yeast strains causing at least 80% reduction in colony areas for highly toxigenic *A. flavus* strain Af4.

Yeast group (YG)	Strain code	Genus	Inhibition (%)
YG3	Y70A	*Meyerozyma*	92
YG6	Y18A	*Saccharomyces*	89
YG3	Y11A	*Meyerozyma*	88
YG7	Y35A	*Saccharomyces*	84
YG7	Y61A	*Saccharomyces*	83
YG7	Y42A	*Saccharomyces*	81

then placed into one of seven possible groups based on morphological characters (Table S3; Figure S2).

All 147 yeast isolates were screened for antagonistic properties against toxigenic *A. flavus* isolate Af4, and only 28% ($n = 41$) negatively impacted Af4, demonstrated by a reduction in fungal colony growth compared to the control. The range of reduced colony areas was between 31% and 89%. Additionally, changes in Af4 colony appearance were observed at both macro- and micromorphological scales.

As mentioned previously, the top-performing yeast strains (> 80% growth inhibition) were reevaluated to confirm the consistency of their inhibition against *A. flavus* isolate Af4. There were six yeast strains reexamined, representing two genera, which provided preliminary inhibition levels between 80% and 100%. Table 1 lists each yeast strain, along with its respective genus and level of inhibition against Af4. The average colony area for Af4 controls was $55.6 \pm 4.97\,cm^2$. In dual culture with each yeast strain, five maintained consistent growth inhibitions of at least 80% (Y70A) up to 86% (Y61A), resulting in average colony areas of 11.1 and $7.8 \pm 1.69\,cm^2$, respectively (Figure 4). The sixth yeast strain, Y42A, failed to consistently inhibit Af4 growth upon reexamination, resulting in only 60% inhibition. Tukey's multiple comparison test determined there were no significant differences among the five most inhibitory strains, but all five were significantly different compared to Y42A.

In addition to reduced colony growth, exposure of Af4 to the reexamined yeast strains also induced changes to colony color, which appeared more yellowish in color compared to the control (Figure S3). Moreover, microscopic examination revealed incidences of fragmented hyphae, collapsed vesicles and conidiophores, and a scarce number of conidia.

The antagonistic activity of yeasts belonging to the genera *Meyerozyma* and *Saccharomyces* against toxigenic Af4 has been demonstrated in other studies [56, 62]. The five yeast isolates from our study with the greatest antagonistic potential caused inhibition of Af4 growth and proved to be better inhibitors than most of the previously reported yeasts. The only comparable level of growth inhibition was obtained with *Saccharomyces* [62]. The maximum inhibition (86%) of the toxigenic *A. flavus* isolate Af4 achieved with *Meyerozyma* Y61A was comparable to the 85% inhibition reported for *Saccharomyces* sp. In addition, inhibition of sporulation and hyphal damage caused by the adhesion of yeast cells were also observed [62]. In contrast, the maximum inhibition obtained in this study was higher than the 38% reported for *Meyerozyma* sp., which in turn was surpassed by *Metschnikowia* sp. with 41% inhibition [56]. Other yeast genera with reported antagonistic activity against toxigenic *A. flavus* include *Aureobasidium* [44], *Hanseniaspora* [58], *Kluyveromyces* [102], *Lachancea* and *Pichia* [45], and *Wickerhamomyces* [64].

Yeasts primarily use antagonism as a mechanism in competition for nutrients and space. These microorganisms are typically more efficient than pathogens, rapidly multiplying with minimal nutrients and adapting more quickly to adverse conditions such as temperature fluctuations, salinity, pH variations, and the presence of fungicides. Previous studies have shown that cocultivation with yeasts results in reduced growth and reproductive

ability of their competitors [44, 45, 63]. In this study, coinoculation of highly toxigenic Af4 with each of five yeast strains resulted in reduced growth (up to 86%), as well as morphological changes that reduced the overall vigor of toxigenic Af4, primarily affecting structures necessary for asexual reproduction. The percentage of antagonistic yeasts obtained in this study was higher than the 23% reported by Natarajan et al. [102], which were isolated from the leaves, flowers, and green pods of *Cassia angustifolia*, but lower than the 35% reported by Jaibangyang et al. [65] that were from maize, rice, and sugarcane.

In nutrient-rich media, the antagonistic activity of yeasts against *A. flavus* may be attributed to the secretion of hydrolytic enzymes [103, 104] and the production of soluble compounds and VOCs, which have been shown to alter colony and hyphal morphology and inhibit sporulation [57, 64, 102].

The macroscopic and microscopic changes observed when Af4 grew in proximity to each of the antagonistic yeasts in dual culture were consistent with those previously reported by Abdel-Kareem et al. [62], who observed deteriorated hyphae at the contact site between *Saccharomyces cerevisiae* and toxigenic *A. flavus*. They described yeast cells adhering to and accumulating on *A. flavus* mycelia, as well as hyphae that appeared irregular, distorted, wrinkled, and/or swollen, with few to no conidia present. Similar phenotype changes in dual culture were reported by Parafati et al. [64], whereby the hyphae of *A. flavus* nearest to colonies of *Wickerhamomyces* and *Metschnikowia* yeasts appeared wrinkled and thinner and exhibited loss of intracellular content. The inhibition of mycelial growth of toxigenic *A. flavus* by yeasts suggests the involvement of VOCs, whose effects can be observed even in the absence of direct contact between the microorganisms. Uncharacterized compounds known as extrolites, together with VOCs produced by the antagonistic yeasts, have been associated with the reduction of both mycelial growth and AF production [57].

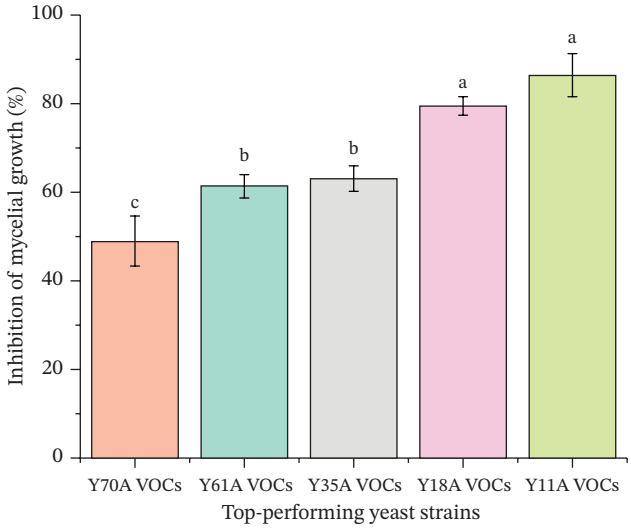
3.4 | Comparative Effect of Yeast VOCs on the Growth and Toxin Production of *A. flavus*

It was notable that five of our isolated yeast strains greatly inhibited the growth of toxigenic Af4 without physical contact. Therefore, we tested the potential for VOCs produced by these yeasts to inhibit Af4. Based solely on exposure to VOCs, we again observed reductions in colony growth ranging from 49% (Y70A) to 86% (Y11A) (Figure 5). Strains Y11A and Y18A caused about 80% inhibition of Af4, while Y35A and Y61A were comparable at around 60% inhibition of Af4. Tukey's test showed no significant difference between the two strains with 80% or more inhibitory potential, and both were significantly different from the percentages of inhibition caused by VOCs from the other three yeast strains. The colonies exposed to yeast VOCs exhibited reduced vegetative growth compared with the control. The first two colonies appeared white due to the absence of conidia, representing only dense mycelial mats without sporulation. In contrast, the third colony (Y35A) was white and less dense, with a larger diameter, similar to the fourth (Y61A) and fifth (Y70A) colonies, where sporulation was even observed at the center of the colony. Colonies that produced conidia showed limited conidiophore formation and altered conidial pigmentation, suggesting partial inhibition of sporulation. No sclerotia were observed in any of the treatments. The observed changes in micromorphology were supported by marked microscopic alterations, including thin, fragmented, and curled hyphae; absence of vesicles and conidia (Y11A and Y18A); thickened hyphae (Y35A); and scarce conidia (Y61A and Y70A).

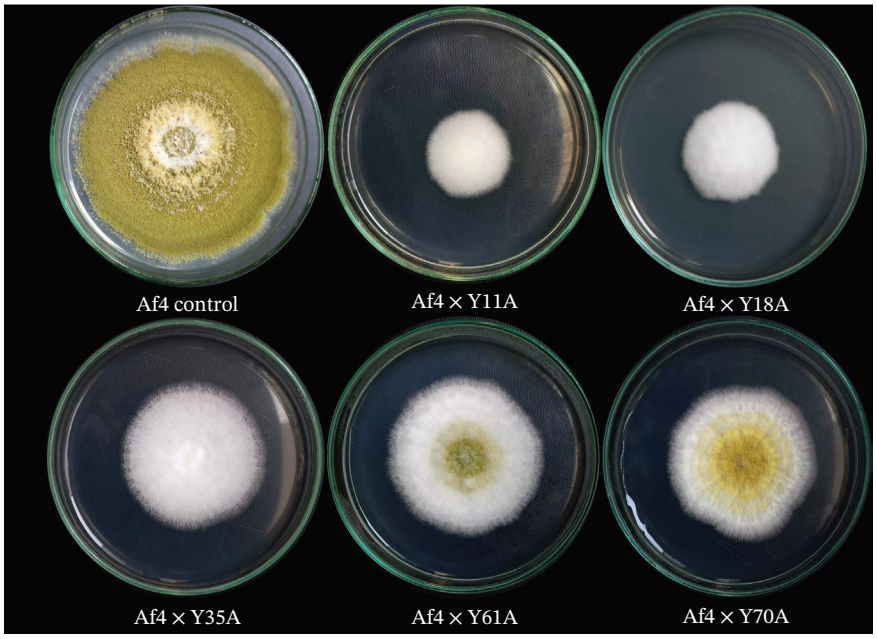
Unlike the changes in growth, each yeast VOC treatment yielded a significant difference in AF production. With yeast strains Y11A and Y18A, reductions of 98% and 93%, respectively, were achieved. In contrast, two of the remaining yeasts showed lower reductions in AF levels, reaching 76% (Y35A) and 41% (Y61A). The only yeast strain whose VOCs exhibited no inhibitory effect on AF levels was Y70A. The inhibition of sporulation of toxigenic *A. flavus* by antagonistic yeasts is highly significant, as AF biosynthesis is closely linked to fungal growth and development [14]. In most cases, the production of secondary metabolites by fungi occurs simultaneously with sporulation, and it has been shown that *A. flavus* mutants with altered sporulation do not produce AFs [105]. Conversely, it has also been reported that the colony growth of *A. flavus* and the levels of AFB_1 and AFB_2 were significantly reduced in dual culture with *Alternaria alstroemeria*; however, sporulation of the toxigenic fungus was not affected [14].

The Af4 strain produced 310 μg/kg of total AFs in our corn kernel assay. However, our VOC experiments used synthetic PDA medium. On PDA control plates, the Af4 strain produced 51 μg/kg of total AFs. Exposure of this strain to uncharacterized VOCs produced by the five yeast strains resulted in AF levels as low as 1 μg/kg (98% reduction), which was caused by VOCs from yeast strain Y11A (Figure 6). The discrepancy observed in total AF production by *A. flavus* in culture medium (51 μg/kg) and in maize grains (310 μg/kg) can be explained by the type of substrate used and its availability. In future studies, it is recommended to evaluate VOC-mediated inhibition directly on maize grains (in situ) to determine whether inhibitory efficacy varies at higher AF concentrations. AF production also depends on inherent characteristics of the crop, such as genotype, nutritional composition, and water activity (AW), as well as extrinsic factors including temperature, humidity, environmental stress, and geographical location [16, 24, 106]. One challenge in AF detection is the inconsistency of production across different substrates. A strain of *A. flavus* that produces low AF concentrations in one substrate may produce high levels in another [16]. For example, *A. flavus* CCM-AS02 did not produce detectable total AFs in synthetic yeast extract sucrose (YES) medium; however, when inoculated on maize grains, 70.5 ± 446.1 μg/kg were quantified [83]. Similarly, *A. flavus* GP 15-4 produced AFB_1 reaching 111.86 mg/kg in maize and 38.63 mg/kg in peanuts [41]. In vitro, VOCs from *Meyerozyma caribbica* and *Meyerozyma guilliermondii* inhibited the growth of *Lasiodiplodia theobromae* by 16% and had no effect on *Rhizopus stolonifer*. However, in vivo assays showed that the severity of *L. theobromae* was reduced by 44% with *M. caribbica* and 64% with *M. guilliermondii*. Regarding *R. stolonifer*, reductions in severity of 92% and 71% were achieved when treated with *M. caribbica* and *M. guilliermondii*, respectively [107].

The antifungal effect of VOCs is consistent with the findings of Dikmetas et al. [56], who reported a reduction in colony



(a)



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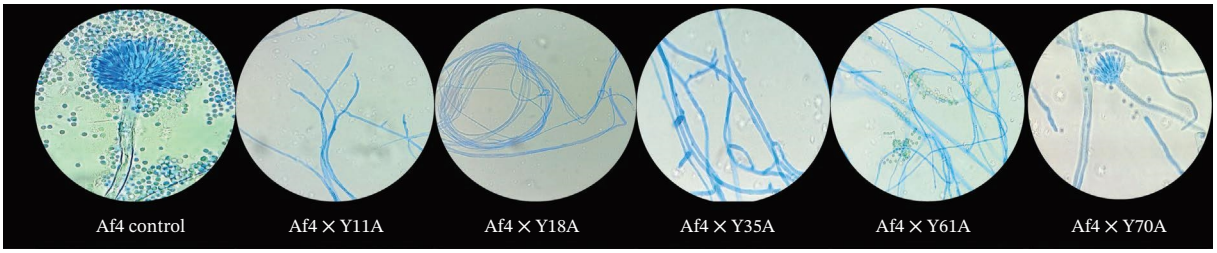

(c)

FIGURE 5 | Changes in growth of toxigenic Af4 upon exposure to VOCs from each of five yeast strains. (a) Bar graph showing percent of inhibition for each yeast strain, averaged across three replicates, with standard deviation bars and letters above each bar indicating significance of differences between treatments according to Tukey's multiple comparison test ($p < 0.05$). (b) Macroscale changes in colony appearance. (c) Microscale changes in colony appearance.

growth (11%–12%), white mycelium coloration, and a significant decrease in sporulation and AFB_1 toxin production due to *Moesziomyces bullatus*, *Metschnikowia* aff. *pulcherrima* DN-MP, and *Metschnikowia* aff. *fructicola* 1-UDM. Similarly, Jaibangyang et al. [65] found that VOCs from *C. nivariensis* inhibited colony growth (65%), reduced conidial germination (49%), and lowered AF production (75%) in maize grains contaminated with *A. flavus*. In the same context, known VOCs identified from yeast strains have been tested against *A. flavus* [58, 103, 108]. Many of which inhibited the growth of *A. flavus*, as well as caused

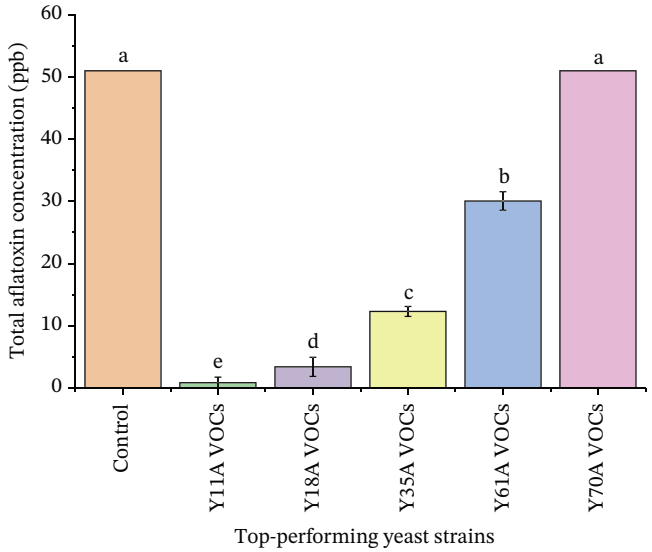

FIGURE 6 | Bar graph showing total aflatoxin levels for toxigenic Af4 after exposure to uncharacterized VOCs produced by each of five yeast strains, averaged across three replicates, with standard deviation bars and letters above each bar indicating significance of differences between treatments according to Tukey's multiple comparison test ($p < 0.05$).

discoloration of conidia, reduced sporulation, and decreased AF production. Again, most of these known VOCs were not as effective as the uncharacterized VOCs from our study. The reduction (86%) in mycelial growth of *A. flavus* by VOCs from the yeast Y11A over 14 days was similar to the 86% inhibition reported for VOCs from *K. marxianus* over the same period [53] and exceeded the inhibition observed with VOCs from *M. caribbica* (41% over 7 days) [66], *P. kudriavzevii* and *Lachancea thermotolerans* (24% after 7 days) [45], *Hanseniaspora uvarum* (34%) and *H. opuntiae* (17% after 10 days) [58], and *M. bullatus* (22%) and *M. pulcherrima* (13% after 3 days) [56]. Similarly, the reduction (98%) in total AFs produced by the toxigenic Af4 isolate due to VOCs from Y11A was comparable to the 99% and 93% reductions reported for VOCs from *K. marxianus* after 7 and 14 days, respectively [53], and exceeded the effects of VOCs from *M. fructicola*, which reduced AF levels by 83% and 40% after 3 and 7 days of incubation, respectively [56].

The effect of VOCs on mycelial growth and colony morphology of *A. flavus* was demonstrated using eight epiphytic yeasts, achieving inhibition ranging from 22% (*M. bullatus*) to 13% (*M. pulcherrima*) after 3 days. The inhibitory effect decreased by Day 7, reaching 13% with *M. bullatus*, while no inhibition was observed with *M. pulcherrima*. Based on the AF analysis results, only VOCs from *M. fructicola* were effective at controlling AFB_1 production by *A. flavus* (83% and 40% after 3 and 7 days of incubation, respectively), whereas VOCs from other isolates were either ineffective or promoted AFB_1 production. As can be seen from the results, there was no link between mycelium growth inhibition and AFB_1 reduction [56].

The VOCs of *K. marxianus* YSP12 showed the maximum mycelial growth inhibition of 90%–93% at 7 days after exposure and 55%–86% at 14 days. Deformed hyphae and the absence of conidia and conidiophores were observed, indicating inhibition of sporulation in *A. flavus*. Yeast volatile exposure on *A. flavus* showed a significant inhibitory effect on AFB_1 biosynthesis (98%–99% at 7 days and 61%–93% at 14 days) with respect to the untreated control. Gas chromatography–mass spectrometry analysis confirmed the presence of antimicrobial compounds such as dimethyl trisulfide, ethyl acetate, ethanol, 3-methylbutanal, 2-methyl-1-butanol, and 3-methyl-1-butanol in the volatiles [53]. VOCs from *H. uvarum* achieved the maximum reduction in mycelial growth of *A. flavus* (34%) after 3 days, while VOCs from *H. opuntiae* reached 17% inhibition after 10 days. AFB_1 levels at 3 days were 47 and 3 μg/kg in the presence of *H. opuntiae* and *H. uvarum*, respectively. In the presence of *H. opuntiae*, AFB_1 remained relatively stable (5000–6000 μg/kg) from Days 7 to 21, whereas with *H. uvarum*, it reached a maximum of 11,669 μg/kg at Day 12, compared to 451 μg/kg at 3 days and 19,813 μg/kg from Days 3 to 15 in the control. Both yeasts inhibited growth and AF production; however, the predominant VOCs identified differed. In *H. opuntiae*, acids were the main compounds (acetic acid, 2-methylbutanoic acid, and isobutyric acid), whereas in *H. uvarum*, esters (ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate) and alcohols (isoamyl alcohol, 2-methyl-1-butanol, and phenethyl alcohol) predominated [58]. Similarly, VOCs from *Aureobasidium* spp. PAL144 and PAL120 reduced *A. flavus* growth by 55% and 52%, respectively, with the major compounds identified being methyl benzeneacetate for PAL144 and 2-phenylethanol for PAL120 [66].

The VOCs produced by antagonistic yeasts against *A. flavus* typically include esters, amines, alcohols, aldehydes, acids, alkanes, alkenes, ketones, aromatics, ethers, furans, cyclic hydrocarbons, sulfides, and terpenes [58, 102]. Known VOCs, identified from yeasts that have been tested against *A. flavus*, include 1-pentanol [65], as well as 2-phenylethyl acetate and furfuryl acetate [108]. The latter two VOCs were shown to impact *A. flavus* at the gene level through suppression of the *aflR* gene, which regulates AF production. Only the study by Natarajan et al. [102] observed reductions comparable to what we observed in this study. Therefore, it is important that future studies capture and characterize the specific VOCs produced by our top-performing yeast strains for testing as pure compounds.

In the case of nontoxigenic *A. flavus*, competitive exclusion explains the reduction in mycelial growth of toxigenic *A. flavus*; however, it has been observed that decreases in AF production are not always correlated with reductions in the growth of the toxigenic fungi [61]. The presence of uncharacterized extrolites, together with VOCs, is suggested to significantly reduce AF and CPA levels, even in the absence of physical contact between toxigenic and nontoxigenic fungal strains. On solid medium, inhibition of mycelial growth was demonstrated, along with 61%–78% reduction in AFB_1 and 55%–66% reduction in CPA produced by three *A. flavus* strains cultivated in the presence of extrolites secreted by a nontoxigenic *A. flavus* strain. In liquid medium, reductions of 3%–64% in AFB_1 and 92% in CPA were observed for *A. flavus* LA3; however, CPA production increased by 279% in the presence of *A. flavus* LA4 [57].

Interestingly, not all yeast strains inhibited AF production. For example, strain Y70A moderately inhibited the vegetative growth of *A. flavus* but did not significantly affect AF levels. The reason for this differential response is unclear, suggesting that some

VOCs may act specifically on fungal growth pathways and/or AF biosynthesis. It is possible that Y70A lacks the ability to produce the metabolite responsible for this suppression. Future studies will be necessary to confirm or refute this hypothesis.

3.5 | Molecular Identification of the Yeast With the Highest Efficiency in Controlling *A. flavus*

The Y11A yeast strain demonstrated the highest efficiency in controlling toxigenic strain Af4. Therefore, we conducted a molecular investigation to identify this strain to the species level. Comparison of its ITS sequence (GenBank accession number: PQ631869.1) via BLASTN query, and subsequent phylogenetic inference with other yeast fungi, indicated Y11A is an isolate of *Meyerozyma caribbica* (Figure 7).

Previous studies have shown that *Meyerozyma* species exhibit antagonistic activity against the growth and toxin production of *Aspergillus carbonarius* [109] and *Aspergillus ochraceus* [103]. Moreover, exposure of fruits such as grapes to *M. caribbica* VOCs has been associated with increased antioxidant activity, reduced expression of genes involved in toxin synthesis, and endogenous accumulation of gamma-aminobutyric acid (GABA), a molecule that plays a crucial role in plant adaptation to adverse conditions [103]. Additionally, *M. caribbica* has been reported to degrade toxins produced by *A. carbonarius* within 24 h, highlighting its detoxification potential [110]. *M. caribbica* Y11A, which survives under adverse conditions such as the low water content of maize grains from which it was isolated, demonstrated an inhibitory effect (86%) on the mycelial growth of toxigenic *A. flavus* and produced VOCs that reduced AF production by 98%. These VOCs are active at low concentrations, can diffuse throughout the surrounding atmosphere, and exert inhibitory effects against target pathogens without contaminating food commodities [66]. Therefore, *M. caribbica* Y11A has potential for managing toxigenic *A. flavus* in cereals such as maize, as well as other pathogens responsible for postharvest and storage diseases in fruits and vegetables.

The strain Af4 was confirmed as *Aspergillus* sp. through BLASTN analysis and phylogenetic inference. However, due to the high similarity of the ITS gene, it was not possible to determine the species with precision (GenBank accession number: PX285676.1) (Figure 8).

Populations of *A. flavus* are highly diverse in terms of morphological, functional, and genetic features. This species harbors functional genes for mating, revealing sexuality as a potential contributor to its diversification [23]. The diversity within *Aspergillus* section *Flavi* highlights the need for polyphasic

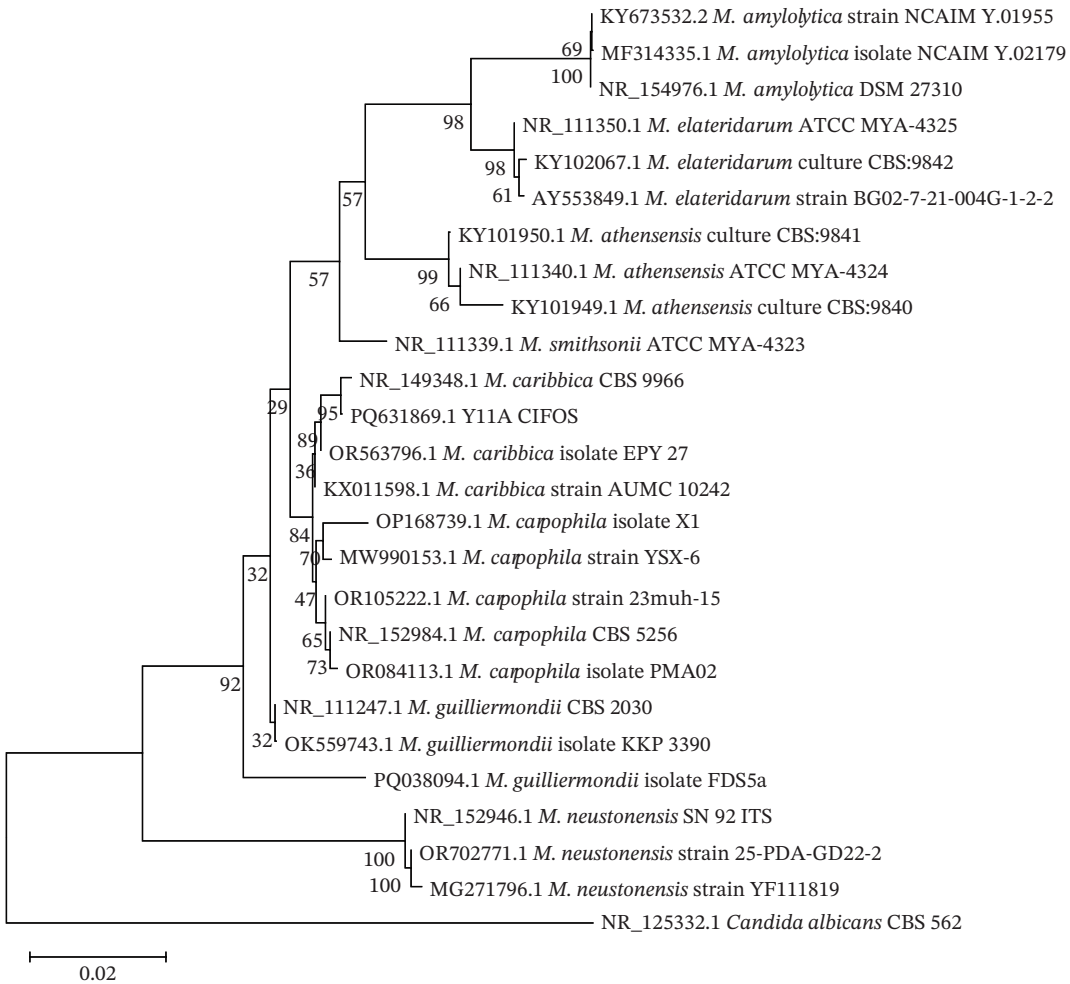

FIGURE 7 | Phylogenetic tree inferred for the ITS region of multiple *Meyerozyma* species, including strain Y11A, with bootstrap values at the branch nodes and *Candida albicans* as the outgroup.

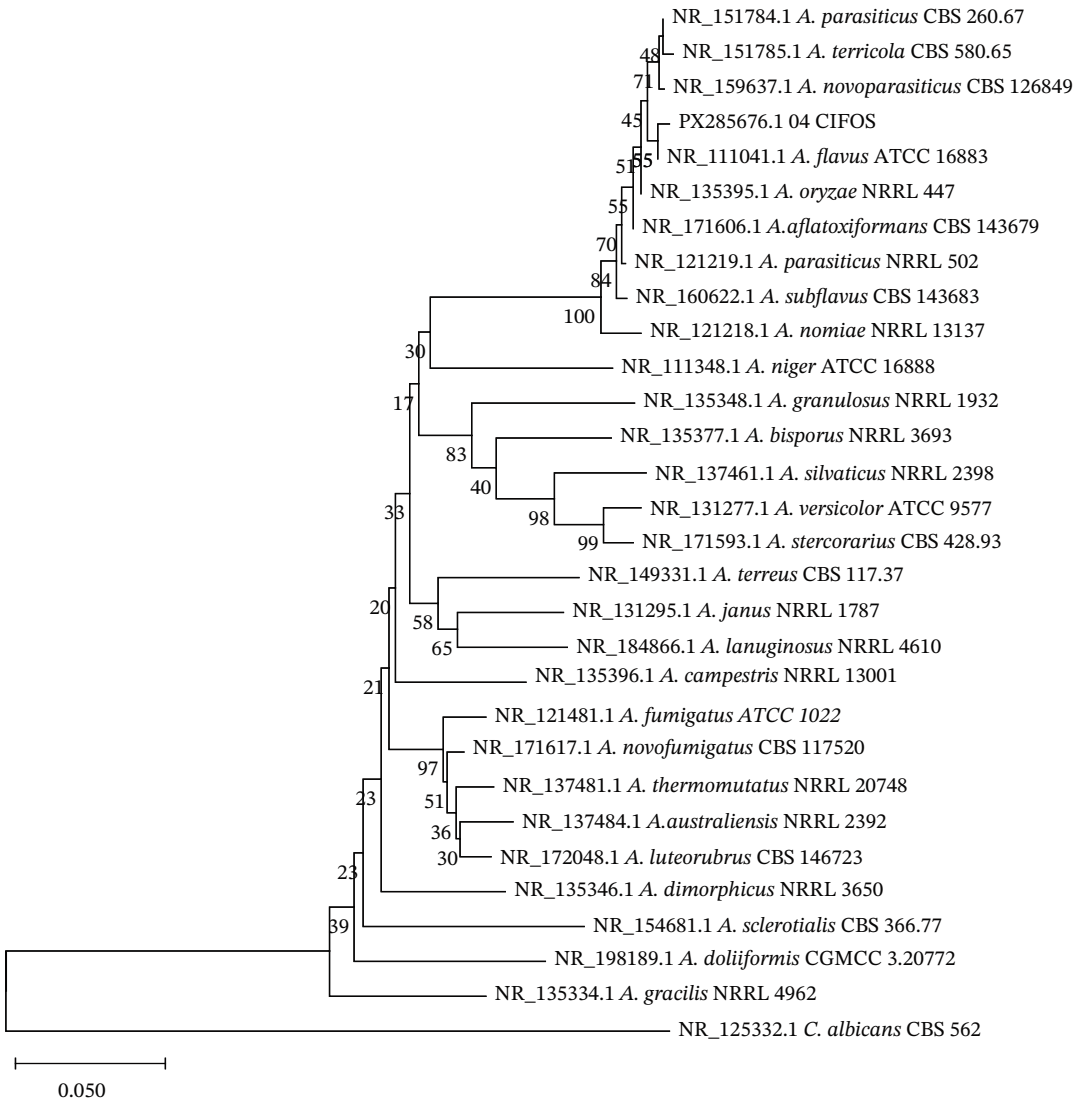

FIGURE 8 | Phylogenetic tree inferred for the ITS region of multiple *Aspergillus* species, including strain Af4, with bootstrap values at the branch nodes and *Candida albicans* as the outgroup.

approaches—combining morphological, metabolic, and genetic analyses—when working with this complex group of species [23]. Typically, *A. flavus* is identified based on morphological characteristics, along with sequencing of β-tubulin, calmodulin, and RNA polymerase II (second largest subunit) genes, in addition to the ITS region [77, 99]. In the present study, only the ITS region was sequenced, which does not provide sufficient resolution for species-level discrimination within this section, limiting the identification to the genus *Aspergillus*.

Therefore, future work should focus on confirming *A. flavus* identification using molecular markers such as β-tubulin and calmodulin, characterizing the VOCs produced by *M. caribbica* Y11A, and determining the optimal yeast concentration for controlling toxigenic *A. flavus* in maize grains under controlled laboratory conditions and in fast-growing animals fed maize contaminated with AFs and inoculated with antagonistic yeasts. Additionally, the antifungal activity of *M. caribbica* and *Saccharomyces* sp. should be evaluated in both mono- and mixed-culture systems, and the underlying biocontrol mechanisms should be investigated.

4 | Conclusion

Of the 147 yeast isolates tested for antagonistic potential against toxigenic *Aspergillus* sp. strain Af4, a yeast strain of *M. caribbica* (Y11A) caused significant inhibition of not only vegetative growth and asexual reproduction by the toxigenic Af4 but also the production of AF. The mechanism of action supporting its antagonistic success appears to be its production of one or more VOCs. These characteristics make it a promising candidate for biological control strategies aimed at reducing pesticide use and mitigating AF contamination. The biological control of pathogens using yeasts like *M. caribbica* supports the goal of sustainable agriculture, which is crucial for export-oriented crops.

Author Contributions

Marilín Sánchez-Purihuamán: investigation, methodology, formal analysis, writing – original draft, writing – review and editing. **L. M. Córdova-Rojas:** investigation, methodology. **Junior Caro-Castro:** formal analysis, writing – original draft, writing – review and editing. **Ada Barturen-Quispe:** writing – original draft, conceptualization. **Segundo Vásquez-Llanos:** formal analysis, methodology. **Heber**

Robles-Castillo: conceptualization, investigation. **Carmen Carreño-Farfán:** conceptualization, investigation, methodology, writing – original draft, writing – review and editing.

Acknowledgments

The authors thank the Centro de Investigación para el Fomento Sustentable (CIFOS) for providing the laboratory facilities for the development of this research work.

Funding

No funding was received for this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All the data are available in the manuscript file. However, if supporting information is needed, it will be made available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting information)* Table S1: Colony characteristics used to identify to the genus level and group isolates of filamentous fungi. Table S2: Morphological characteristics of *Aspergillus* spp. isolated from maize grains. Table S3: Characteristics of *Aspergillus* spp. isolates, including sclerotial type and aflatoxin concentration. Table S4: Macroscopic and microscopic features used for genus-level identification and grouping of yeast isolates. Table S5: Differential characteristics of yeast genera isolated from maize grains. Figure S1: Plate images showing colony characters for each filamentous group (FG) used in this study, along with its fungal genus name. Figure S2: Plate images showing the colony morphology of each yeast group (YG) analyzed in this study, along with the corresponding yeast genus identification. Figure S3: Macro- and microscale images illustrating the interaction between the toxigenic *A. flavus* strain Af4 and the six most effective yeast strains used in this study.