Effect of *Senna occidentalis* Seed Extract and *Hyptis suaveolens* Essential Oil on Aflatoxin B1 Contamination of Stored Maize

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Abstract

Aflatoxin B1 contamination in stored maize, primarily caused by Aspergillus flavus, poses a significant threat to food safety and public health. This study aims to evaluate the antifungal efficacy of Senna occidentalis seed extract and Hyptis suaveolens essential oil as natural alternatives to synthetic fungicides. A. flavus was isolated from infected maize kernels and confirmed through DNA extraction, PCR amplification of the ITS region, and BLAST analysis, which revealed 100% sequence identity with GenBank accession OR646810.1. Plant materials were extracted using ethanol maceration (S. occidentalis) and steam distillation (H. suaveolens), followed by GC-MS profiling. Maize grains were treated with 5 mL, 10 mL, and 15 mL doses of each extract and stored for 30 days at 30 °C. Aflatoxin B1 levels were quantified using high-performance liquid chromatography (HPLC). The research findings showed that untreated maize contained 101 ± 1.0 µg/kg aflatoxin B1, while grains treated with synthetic fungicide (SAAF) had 5.0 ± 0.1 µg/kg (98% inhibition). S. occidentalis extract reduced aflatoxin levels to 15.3 ± 1.0, 10.3 ± 1.0 and 6.3 ± 1.0 µg/kg at 5 mL, 10 mL, and 15 mL doses, respectively (85–94% inhibition). H. suaveolens essential oil achieved reductions to 20.3 ± 1.0, 13.3 ± 1.0 and 8.3 ± 1.0 µg/kg (80–92% inhibition). The 15 mL dosage was the most effective for both treatments, approaching the efficacy of the synthetic fungicide. The antifungal activity is attributed to phytol, linoleic acid and esters in S. occidentalis, and terpenes such as eucalyptol and caryophyllene in H. suaveolens. These findings demonstrate the potential of plant-based treatments as eco-friendly and effective alternatives for aflatoxin mitigation in maize storage systems.

Keywords:
Bioactive compounds, Eco-friendly, Food Safety, GC-MS analysis, Natural Preservatives

Introduction

Aflatoxin contamination in maize, primarily caused by the toxigenic fungus *Aspergillus flavus*, remains a persistent challenge in global food security and public health (Imran et al., 2020; Haque et al., 2020). Aflatoxins, particularly aflatoxin B1, are classified among the most potent naturally occurring mycotoxins and are linked to numerous adverse health outcomes, including liver cancer, immunosuppression and stunted growth in children (Jallow et al., 2021; Shukla et al., 2021). Their contamination is heavily influenced by climatic conditions, poor postharvest practices and suboptimal storage environments, especially in tropical regions where maize is a dietary staple (Kamano et al., 2022). High humidity and warm temperatures favour fungal proliferation, amplifying the risk of aflatoxin accumulation and threatening food quality and human health.

Efforts to combat aflatoxin contamination have traditionally centred on synthetic fungicides and chemical preservatives, such as Carbendazim and Mancozeb. Although effective to some extent, these chemical agents are increasingly scrutinised for their environmental toxicity, potential bio-incompatibility and persistence in food systems (Maurya et al., 2021; Aremu et al., 2022). Their limitations have stimulated interest in

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exploring alternative strategies that combine efficacy with safety and sustainability. One such approach is the utilisation of plant-derived antimicrobials, particularly essential oils and botanical extracts known for their broad-spectrum antifungal properties and minimal ecological impact (García-Díaz et al., 2019; Pinto et al., 2023). Aromatic plants like *Origanum virens* and *Satureja montana* have shown remarkable antitoxigenic and antifungal potential at low dosages, with compounds such as carvacrol, (Z)-citral, and (E)-citral playing a dominant role in suppressing *A. flavus* growth and aflatoxin B1 biosynthesis (Xiang et al., 2020). In addition, studies on improved postharvest techniques, including hermetic storage and strategic drying, have demonstrated significant reductions in aflatoxin incidence by limiting fungal exposure and moisture accumulation (Bauchet et al., 2020; Marete et al., 2020).

Two promising botanical candidates gaining traction in recent research are *Senna occidentalis* and *Hyptis suaveolens*. *Senna occidentalis*, commonly known as the coffee senna, is rich in diverse bioactive compounds such as tannins, alkaloids, flavonoids and terpenes, and exhibits notable antioxidant, antimicrobial and anti-inflammatory properties (Nde et al., 2022; Kumar et al., 2024). Its medicinal attributes have been leveraged in treating conditions ranging from diabetes to chronic inflammation, making it a valuable plant for integrative pest management. *Hyptis suaveolens*, a member of the Lamiaceae family, also possesses potent antioxidant and antifungal capabilities, and its extracts contain xanthine oxidase inhibitors that underscore its pharmaceutical utility (Aye et al., 2020). The aerial parts of this plant are widely used in traditional medicine and show strong promise in eco-conscious pest control strategies.

Given the increasing demand for green technologies in agriculture and food preservation, this study aims to explore the antifungal efficacy of *Senna occidentalis* seed extract and *Hyptis suaveolens* essential oil against aflatoxin B1 contamination in stored maize. Their performance was compared to a commercial Sarbagya AgriTech Fungicide (SAAF) containing Carbendazim 12% and Mancozeb 63% WP, the conventional benchmark. By comparing natural agents with chemical alternatives, the study aims to identify safer, sustainable options that align with global goals for food security, human health and environmental protection.

Materials and Methods

The plant materials, *Senna occidentalis* and *Hyptis suaveolens*, were collected from the lush vegetation in Gusau. Plant identification was carried out at the National Institute for Pharmaceutical Research and Development (NIPRD) Herbarium in Abuja. Voucher specimen numbers–NIPRD/H/7388 and NIPRD/H/7387 were allocated to *Senna occidentalis* and *Hyptis suaveolens*, respectively.

Maize grains: The maize grains (hybrid) utilised in this study were procured from local Maize Farmers within Gusau Metropolis.

Isolation of *Aspergillus flavus* from infected maize kernels

Sample processing

Infected maize kernels were selected and surface-sterilised with 0.1% ethanol for 1–2 minutes to eliminate non-target microorganisms. Following sterilisation, the kernels were rinsed thoroughly with sterile distilled water to remove residual ethanol.

Preparation of culture media

Potato Dextrose Agar (PDA) was prepared based on the manufacturer's instructions. Specifically, 15 g of agar, 20 g of dextrose, and 200 g of potato extract were dissolved in 1000 mL of distilled water (pH 5.6 ± 0.2). The medium was sterilised in an autoclave at 121 °C for 15–20 minutes. After sterilisation, the PDA was poured into pre-sterilised petri dishes and allowed to cool and solidify at room temperature.

Incubation and isolation of fungal colonies

Ten surface-sterilised maize kernels were evenly placed on each PDA plate. The plates were sealed using parafilm to maintain humidity and prevent external contamination. Incubation was carried out at 25 °C for 7 days in a Biochemical Oxygen Demand (BOD) incubator. Emerging fungal colonies were sub-cultured onto fresh PDA plates, and individual colonies were isolated based on morphological traits. Pure isolates were obtained through repeated sub-culturing and preserved on PDA slants at 4 °C.

Identification of Aspergillus flavus

Preliminary identification was based on colony morphology, which included yellow-green pigmentation, circular colony shape, and colony diameter ranging from 2–5 mm. Molecular identification techniques, including PCR amplification and sequencing, were employed to confirm the species as *Aspergillus flavus*.

Molecular identification *of Aspergillus flavus*

DNA extraction

Sub-cultured fungal isolates were incubated on PDA at 25 °C for 48 hours. Mycelia were harvested and placed on Whatman No. 2 filter paper, then stored at 4 °C overnight before freeze-drying for 48 hours. The freeze-dried mycelium was ground using liquid nitrogen, and genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen Sciences Inc.) following standardised protocols.

Polymerase chain reaction (PCR)

PCR amplification was performed using the Gene Amp® PCR System 9700 (Applied Biosystems). The internal transcribed spacer (ITS) region was targeted using primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATGATGC). Each 25 µL reaction contained:

- 4 µM of each primer (1 µL at 100 µM),
- 2 mM $MgCl_2$ in 2.5 µL of 10X buffer (FastStart Taq DNA Polymerase Kit, Roche),
- 200 µM dNTPs (0.5 µL at 10 mM),
- 0.04 U FastStart Taq DNA Polymerase (0.2 µL at 5 U/µL),
- 5 µL (20 ng) of genomic DNA (standardised to 4 ng/µL),
- DNase-free water to a final volume of 25 µL.

Thermal cycling conditions involved an initial denaturation at 92 °C for 4 minutes, followed by 35 cycles of denaturation at 92 °C for 40 seconds, annealing at 55 °C for 1 minute and 30 seconds, elongation at 55 °C for 2 minutes, and a final elongation at 72 °C for 5 minutes. PCR products were analysed using 1% agarose gel electrophoresis at 80 V, 400 mA for 60 minutes. Gels were stained with ethidium bromide, visualised under UV illumination, and photographed using the Molecular Imager® Gel Doc™ XR System (Bio-Rad). Band size was estimated using a 1.5 kbp DNA marker.

Sequencing and BLAST analysis

PCR products were sequenced using Sanger's method. Sequences were edited and analysed using BioEdit software to remove low-quality regions and assemble contigs if necessary. Final sequences were converted to FASTA format and submitted to the GenBank database. BLAST analysis was performed using the NCBI platform to determine homology with known sequences. Identification was confirmed by comparing the query sequence with reference entries and evaluating the alignment scores.

Extraction of *Senna occidentalis* seed extract

The seed extract was obtained using maceration with absolute ethanol at a 1:5 (w/v) ratio. Specifically, 4 kg of *S. occidentalis* seeds were immersed in ethanol and left to soak for 24 hours. After the soaking period, the mixture was filtered, and the resulting filtrate was concentrated using a water bath. The final extract was stored in appropriately labelled bottles and preserved in desiccators. This procedure was adapted from Aguele et al. (2023), with the solvent choice informed by Chaves et al. (2020).

Extraction of essential oil from *Hyptis suaveolens* leaves

Essential oil was extracted via steam distillation. A total of 10 kg of *H. suaveolens* leaves were arranged in a distillation setup, where steam was passed through the plant material to rupture cell structures and release the volatile oils. The vapour produced was then condensed into a liquid, and the essential oil was separated from the aqueous phase and collected. This method was based on the protocol described by Lubis et al. (2023).

GC-MS analysis of extracts

Both the *Senna* seed extract (SSE) and *Hyptis* essential oil (HEO) were analysed using Gas Chromatography-Mass Spectrometry (GC-MS), employing a Shimadzu QP-2010 instrument with Electron Ionisation (EI) mode. The analysis utilised an Optima-5 MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a flow rate of 1.61 mL/min. The temperature program ranged from 60 to 300 °C with controlled ramping. A split injection mode was used with a 5:1 split ratio and an injection volume of 1.0 µL. The generated mass spectra were compared to entries in the NIST 11 database for compound identification, following the methodology described by Johnson et al. (2020).

Sample collection and preparation

Seeds of maize (*Zea mays* L.) were submerged for five hours in 100 mL of distilled water and sterilised for fifteen minutes at 121 °C in a laboratory autoclave (Yang & Chang, 2010). Thereafter, 50 g of the hybrid maize grains were measured and spread consistently in sterilised glass jars, treated with *H. suaveolens* essential oil and extract of *S. occidentalis* of 5mL, 10mL and 15 mL dosages, respectively. To minimise bacterial interference, maize grains (50 g per jar) were pre-treated with 5 mL of an amoxicillin solution (250–500 mg/L). Treated samples were held at ambient

temperature for 2–3 hours to ensure antibacterial efficacy before adding fungal inoculum. Three (3) discs of fungal mycelia cultured on potato dextrose agar were excised, incorporated into the maize grains, and placed at appropriate intervals to optimise the isolates' growth. The positive control was SAAF fungicide.

On the other hand, deionised water was meticulously mixed with Tween 80 and applied to maize grains (50 g), which served as the negative control. Afterwards, the treated maize samples were stored (incubated) at 30 °C for 30 days. The combination of media composition, chemical additives, environmental controls and fungal biology enabled the PDA to support fungal growth for a continuous 30-day period, aligning with the study's experimental design and evaluation timeframe.

Evaluation of aflatoxin levels in maize samples following treatment

The mycotoxin extraction was carried out by mixing the extraction mix with 5 g of ground samples and subjecting them to vortexing for about 30 seconds and 24-hour agitation via horizontal shaker. Thereafter, samples were subjected to 10-minute centrifugation at 3000 rpm, before filtration via a syringe filter of 0.20 µm. Aflatoxin content was analysed using High Performance Liquid Chromatography (HPLC), and the average of the three replicate values was evaluated for each respective sample (Pantano et al., 2021).

Data analysis

Statistical Package for Social Sciences (SPSS) version 23.0 was deployed to analyse the data. The means separation was conducted using the Duncan multiple range test, while data testing was carried out via analysis of variance (ANOVA).

Results and Discussion

Molecular identification of the fungus

Gel electrophoresis revealed distinct bands corresponding to the DNA samples with an estimated size of 600 bp (Plate 1). A molecular weight ladder was loaded alongside the samples, serving as a reference standard for fragment size verification. Although a positive control strain of *Aspergillus flavus* was not included, the molecular ladder provided a reliable guide for confirming the expected amplicon size. The amplified DNA was subsequently subjected to BLAST analysis using the NCBI platform. The resulting alignment showed a 100% sequence identity with GenBank accession number OR646810.1 and an E-value of 0.0, thereby confirming the identity of the isolate as *Aspergillus flavus* with high confidence.

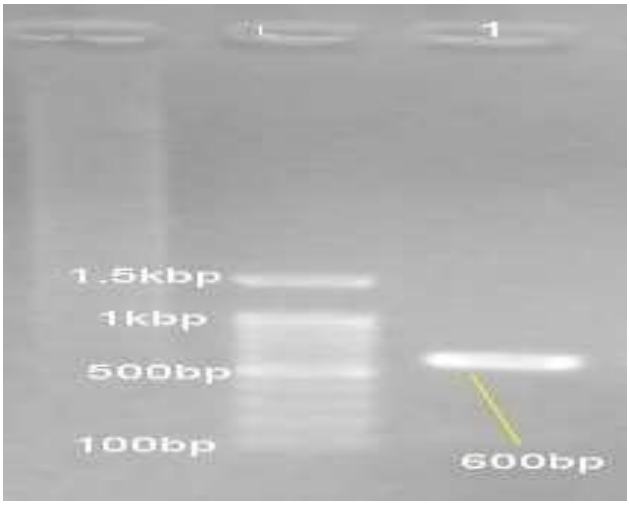

Plate 1: Gel Electrophoresis Pattern of *Aspergillus flavus* PCR Products

L = DNA molecular weight ladder

1= DNA Sample

Identification and composition of bioactive compounds in *Senna occidentalis* extract

Gas chromatographic analysis of *Senna occidentalis* seed extract revealed a diverse profile of 18 bioactive compounds. These compounds span multiple chemical classes, including fatty acids, esters, alcohols, and other organic molecules. Table 1 summarises the identified constituents, their molecular formulas, and relative compositions. Notably, substantial variations were observed in their percentage abundance, suggesting differential contributions to the extract's biological activity. The most prominent compounds, phytol l-(+)-Ascorbic acid 2,6-dihexadecanoate, Z-5,17-Octadecadien-1-ol acetate, ethyl 9,12,15-octadecatrienoate, and hexadecenoic acid, account for over 70% of the extract's total composition. Figure 1 presents the chromatographic peaks of these compounds, demonstrating their dominance in the extract matrix. Phytol, a diterpene alcohol, has been widely documented for its antioxidant, antimicrobial, and anticancer activities (Kumar et al., 2024). Its prevalence in the extract may inhibit oxidative stress and fungal growth. Similarly, unsaturated fatty acids such as linoleic acid and hexadecenoic acid possess membrane-disruptive properties and have shown inhibitory potential against *Aspergillus* species (Maurya et al., 2021). Fatty acid esters like ethyl 9-hexadecenoate and ethyl linolenate enhance lipophilic interactions with microbial membranes, thereby increasing permeability and cellular dysfunction

(Xiang et al., 2020). Alcohols such as phytol and 1-heptatriacotanol are structurally similar to bioactive long-chain alcohols reported for their antifungal and anti-inflammatory activities (Aremu et al., 2022). These findings corroborate earlier studies on *Senna* spp., which highlighted the plant's rich reservoir of terpenoids, flavonoids, glycosides, and alkaloids with therapeutic efficacy (Nde et al., 2022; Aguele et al., 2023).

Comparatively, similar compound classes have been observed in other antifungal plant sources, such as *Origanum virens* and *Satureja montana*, where low-dose essential oils containing carvacrol and citral showed strong antifungal synergy against *A. flavus* (García-Díaz et al., 2019). These parallels suggest that *Senna occidentalis* holds equal promise in mitigating aflatoxin contamination in stored maize.

The chromatographic fingerprint (Figure 1) and compound diversity observed in this study affirm the potent antifungal potential of *Senna occidentalis* extract. The dominance of antioxidant-rich and antimicrobial constituents underscores its suitability as a plant-based alternative to synthetic fungicides like Carbendazim and Mancozeb. These findings validate the plant's therapeutic viability and support its integration into sustainable postharvest grain management strategies.

Table 2 presents the functional classification of compounds identified in the *Senna occidentalis* extract. The result reveals the presence of diverse phytochemicals, with esters being the most abundant group (59.65%), followed by alcohols (18.31%) and ascorbic acid derivatives (15.30%), indicating significant contributions to the extract's potential bioactive properties. Other notable components include fatty acids (2.34%) and ethers (2.17%). Minor constituents include aldehydes (0.51%), benzene derivatives (0.49%), silenes (0.70%), and steroids (0.53%).

Table 1: Phytochemical Profile of *Senna occidentalis* Extract

Name of Compound	Molecular Formula	% Composition
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	15.75
Phytol	$C_{20}H_{40}O$	15.57
l-(+)-Ascorbic acid 2,6, -dihexadecanoate	$C_{38}H_{68}O_8$	15.30
Ethyl 9, 12, 15 – octadecatrienoate	$C_{20}H_{34}O_2$	13.51
Z-5,17, - Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	13.16
Butyl 9, 12 – octadecadienoate	$C_{22}H_{40}O_2$	9.09
Methyl 17- methyl-octadecanoate	$C_{20}H_{40}O_2$	2.91
1-Heptatriacotanol	$C_{37}H_{76}O$	2.74
cis, cis- Linoleic acid	$C_{18}H_{32}O_2$	2.34
1,3-Dioxolane, 4-ethyl-5-octyl, -2,2-bis	$C_{15}H_{24}F_6O_2$	2.17
1,2-Benzenedicarboxylic acid, diisooctylester	$C_{24}H_{38}O_4$	2.12
Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	1.92
Silene, 1,4-phenylenebis (trimethyl-)	$C_{12}H_{22}Si_2$	0.70
Phthalic acid, cyclobutyl, tridecyl ester	$C_{25}H_{38}O_4$	0.67
5. alpha.-Ergost-8(14)-ene	$C_{28}H_{48}$	0.53
Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	0.52
16- Heptadecenal	$C_{17}H_{32}O$	0.51
1,2-Bis(tri-methylsilyl) benzene	$C_{12}H_{22}Si_2$	0.49

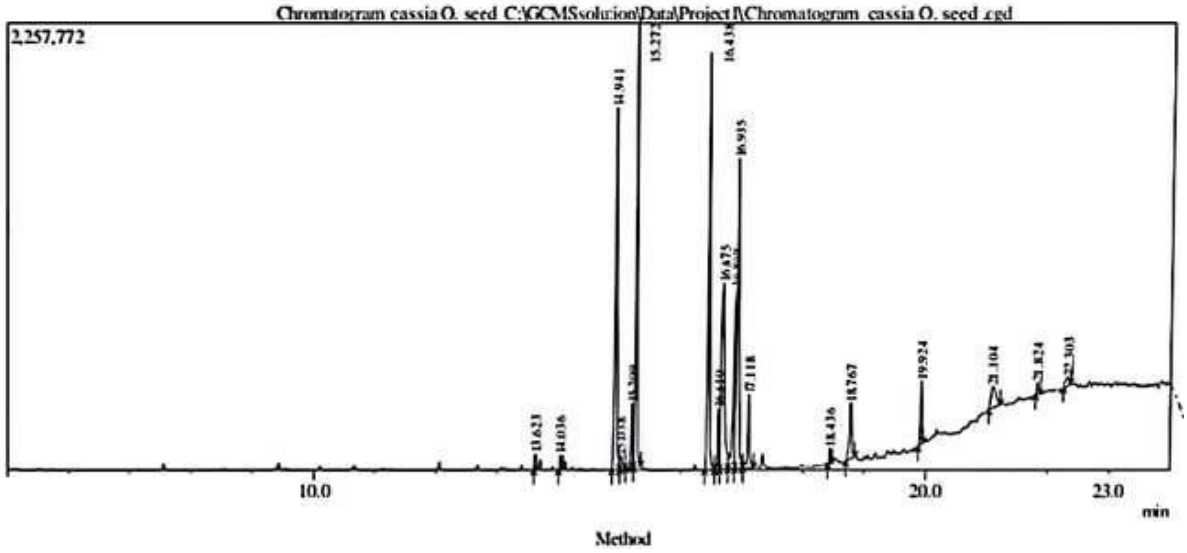

Figure 1: GC-MS Chromatogram for *Senna occidentalis*

Table 2: Functional Categorization of Bioactive Constituents in *Senna occidentalis* Extract

Functional group/class	% Constitution
Ester	59.65
Alcohol	18.31
Ascorbic acid derivative	15.30
Fatty Acid	2.34
Ether	2.17
Silenes	0.70
Steroids	0.53
Aldehyde	0.51
Benzene derivative	0.49
Total	**100**

Bioactive composition of *Hyptis suaveolens* essential oil

The gas chromatographic profiling of *Hyptis suaveolens* essential oil revealed the presence of about 30 distinct bioactive compounds, highlighting the plant's biochemical complexity and therapeutic potential. Among these, Eucalyptol (13.37%) and 4-methylene-1-(1-methylethyl) Bicyclo [3.1.0] hexane (11.85%) were the most abundant constituents, followed by Fenchone (8.94%), Caryophyllene (5.30%) and Gamma-Terpinene (5.21%). These findings confirm a rich mixture of monoterpenes and sesquiterpenes, which are well-documented for their antimicrobial, anti-inflammatory and antioxidant properties (Li et al., 2020; Aye et al., 2020).

Eucalyptol (also known as 1,8-cineole) is widely recognised for its broad-spectrum antimicrobial activity and ability to inhibit fungal growth, including species of *Aspergillus* (Raut & Karuppayil, 2014). Caryophyllene, a sesquiterpene, has shown promising anti-inflammatory and antifungal potential, often working synergistically with other terpenes to enhance bioactivity (García-Díaz et al., 2019). The detection of oxygenated compounds such as Caryophyllene oxide (1.00%) and (-)-Spathulenol (0.46%) further supports the extract's pharmacological significance, as oxygenated terpenoids are considered more bioactive due to their enhanced polarity and reactivity (Maurya et al., 2021).

Though present in small amounts, less abundant compounds such as Cholest-14-en-3-ol (1.88%) and Kaurene (1.09%) contribute to the oil's overall functional profile. Cholesterol-type molecules have been linked to membrane-stabilising effects in lipid bilayers, while Kaurene, a diterpene hydrocarbon, is often noted for its phytotoxic and antimicrobial properties (Nde et al., 2022; Kumar et al., 2024).

These compositional attributes of *Hyptis suaveolens* are consistent with previous studies that underscore its medicinal relevance. For instance, Aye et al. (2020) demonstrated potent antioxidant activity in *Hyptis suaveolens* extracts due to its xanthine oxidase inhibitory constituents. Additionally, Aguele et al. (2023) reported antifungal potency of this plant's

essential oil against storage fungi, reinforcing its potential application in postharvest management systems.

Compared with *Senna occidentalis* extract, which showed dominance of fatty acids, esters and alcohols, the essential oil of *Hyptis suaveolens* leans more heavily into terpene chemistry. This divergence in phytochemical classes may suggest complementary antifungal modes of action. *Senna* exhibits membrane-disruptive effects via lipophilic fatty acids. *Hyptis suaveolens* offers a terpene-rich profile conducive to oxidative stress regulation and microbial inhibition (Shukla et al., 2021).

The chromatographic peaks (Figure 2) confirm the abundance and resolution of these components, providing a strong biochemical foundation for *Hyptis suaveolens* as a sustainable alternative to synthetic fungicides. Its varied composition enhances the potential for synergistic interactions between compounds, which can result in higher bioefficacy in suppressing aflatoxin production by *Aspergillus flavus* (Xiang et al., 2020).

Table 4 presents the functional classification of the compounds found in *Hyptis suaveolens* essential oil, revealing a diverse chemical composition, with Terpenes being the most abundant class, constituting 43.31% of the total composition followed by Alcohols (23.01%), Ketones (11.93%), Ethers (13.37%) and Aromatic Hydrocarbons (3.42%) with the remaining classes, Oxides, Esters, Polyenes, and Sterols, are present in smaller proportions.

Evaluation of aflatoxin B1 suppression across treatments

The experimental data outlined in Table 5 demonstrate significant variation in aflatoxin B1 levels across treatments, confirming the differential antifungal efficacy of both synthetic and natural agents. The untreated negative control (N) recorded the highest aflatoxin B1 level at $101 \pm 1.0\ \mu g/kg$, providing the contamination baseline for evaluating inhibition percentages. All other treatments showed statistically significant reductions in aflatoxin concentration ($p < 0.001$), validating the effectiveness of applied interventions.

Table 3: Chemical Composition of Essential Oil derived from *Hyptis suaveolens*

Name of Compound	Molecular Compound	% Composition
Eucalyptol	$C_{10}H_{18}O$	13.37
Bicyclo [3.1.0], hexane, 4- methylene-1- (1-methylethyl)-	$C_{10}H_{16}$	11.85
Fenchone	$C_{10}H_{16}O$	8.94
Bicyclo[2.2.1], heptan-2-ol, 1,3,3- trimethyl-	$C_{10}H_{18}O$	8.74
Caryophyllene	$C_{15}H_{24}$	5.30
Gamma- Terpinen	$C_{10}H_{16}$	5.21
Cyclohexane, 1-ethenyl-1-methyl- 2- (1-methylethenyl), - 4 -	$C_{15}H_{24}$	3.05
Phenanthrene, 7-ethenyl- 1, 2, 3, 4, 4 a,4.	$C_{16}H_{14}$	2.50
	$C_{15}H_{24}O$	2.45
Bergamotol, Z-. alpha. -trans-	$C_{15}H_{26}O$	2.45
Bicyclo [2.2.1], heptan-2-ol, 1,7,7- trimethyl-, (1S-endo) -.	$C_{10}H_{18}O$	2.17
1H-Cyclopenta [1,3] cyclopropa, [1,2] benzene, octahydro- 7,	$C_{15}H_{24}$	2.02
(-)-Terpinen -4 -ol	$C_{10}H_{18}O$	1.91
Cholest – 14 – en - 3 -ol, (3. beta.5. Alpha.)	$C_{27}H_{46}O$	1.88
Cyclohexane, 1-ethenyl-1- methyl-2-, (1-methylethenyl) – 4	$C_{15}H_{24}$	1.77
Camphor	$C_{10}H_{16}O$	1.73
Bicyclo, [5.3.0] decane, 2- methylene-5-, (1-methylvinyl) -8-.	$C_{15}H_{24}$	1.65
(+)- 4-Carene	$C_{10}H_{16}$	1.64
H-Cycloprop[e]azulen-7-ol, decahydro-1, 1, 7- trimethyl, -...	$C_{15}H_{24}O$	1.62
Cyclohexanol, 2-methyl-5-, (1- methylethenyl)-, (1. alpha.	$C_{10}H_{18}O$	1.46
alpha- Phellandrene	$C_{10}H_{16}$	1.48
1,3,6,10, - Cyclotetradecatetraene ,3, 7, 1.	$C_{14}H_{18}$	1.34
1,4,7, -Cycloundecatriene, 1,5,9,9- tetramethyl-, Z, Z, Z-	$C_{15}H_{24}$	1.28

Ethanone, 1- (1-methyl-2- cyclopenten-1-yl) -	$C_{10}H_{14}O$	1.26
Kaurene	$C_{20}H_{32}$	1.09
Bicyclo[3.1.0], hexan-2-ol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{18}O$	1.02
Caryophyllene oxide.	$C_{15}H_{24}O$	1.00
Naphthalene, decahydro-4a-methyl, - 1-methylene-7- (1-met...	$C_{15}H_{24}$	0.97
1-Naphthalenol, decahydro-1, and 4a-dimethyl-7-(1-methyleth...	$C_{15}H_{24}O$	0.94
Kaur-16-ene	$C_{20}H_{32}$	0.93
Isocaryophillene	$C_{15}H_{24}$	0.88
7 - Isopropyl, -1,1,4 a - trimethyl - 1,2,3,4,4a	$C_{15}H_{26}$	0.85
Lanceol, cis	$C_{15}H_{24}O$	0.75
17 - Norkaur, -15 - ene, 13- methyl- , (8.be	$C_{19}H_{30}$	0.59
2-Norpinanol, 3, 6,6-trimethyl-	$C_{10}H_{20}O$	0.56
Benzene, 1, 2, 3, 4, -tetramethyl-.	$C_{10}H_{14}$	0.50
Cedren-13-ol, 8-	$C_{15}H_{26}O$	0.48
Fenchyl acetate	$C_{12}H_{20}O_2$	0.47
(-), - Spathulenol	$C_{15}H_{24}O$	0.46
Kaur-15-ene, (5. alpha., 9. alpha., 10.be	$C_{20}H_{32}$	0.43
(3,7-Dimethyl-octa- 2,6-dienyl), benze	$C_{16}H_{22}$	0.42
Naphthalene, 1, 2, 3, 4, 4 a, 5, 6,8 a - octahydro-7-methyl, -4- m...	$C_{15}H_{24}$	0.36
Selina-6, - en - 4 – ol	$C_{15}H_{26}O$	0.36
Gamma – Elemene	$C_{15}H_{24}$	0.34
Fenchol, exo-	$C_{10}H_{18}O$	0.33
Ledol	$C_{15}H_{26}O$	0.30
1-Phenanthrenemethan ol, 1,2,3,4,4a,9	$C_{15}H_{12}O$	0.29
Kaur-16-ene	$C_{20}H_{32}$	0.27
1H - Cycloprop[e]azulene, decahydro- 1,1, 7-trimethyl- 4- me...	$C_{15}H_{24}$	0.25
1,1,4a-Trimethyl-5,6-dimethylenede	$C_{15}H_{22}$	0.25

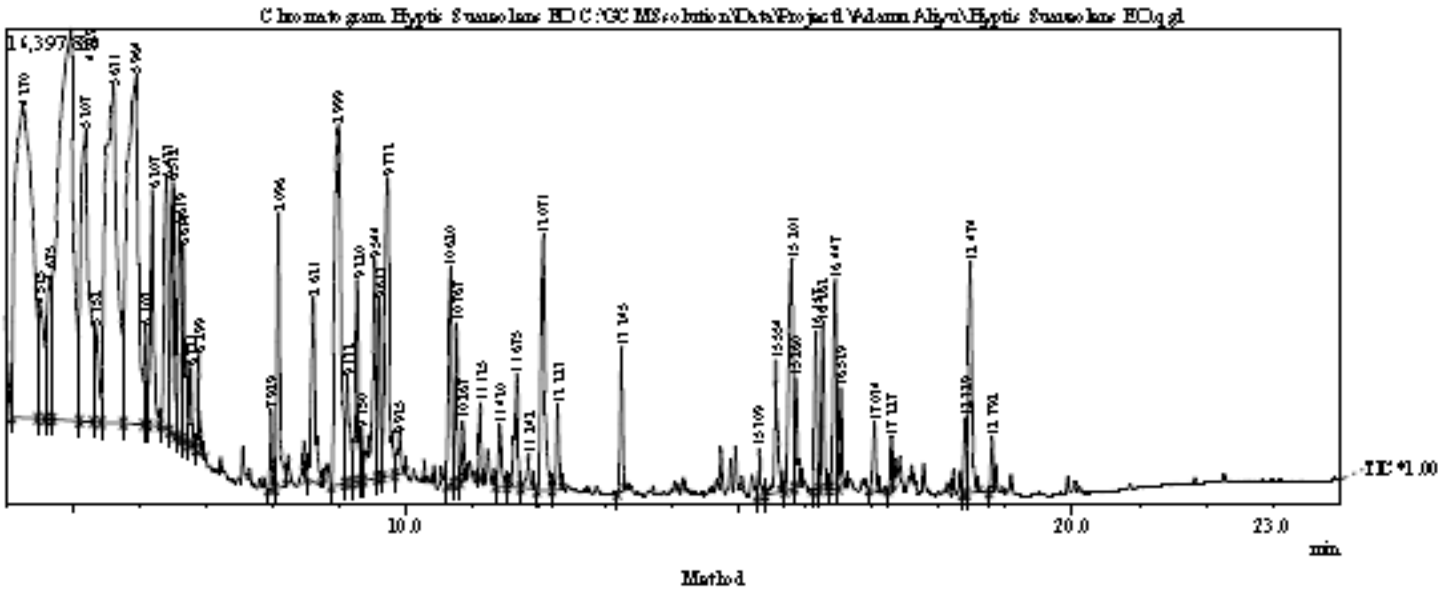

Figure 2: GC-MS Chromatogram for *Hyptis suaveolens*

Table 4: Functional Categorization of Bioactive Components in *Hyptis suaveolens*

Functional group	% composition
Terpenes	43.31
Alcohols	23.01
Ketones	11.93
Oxides	1.27
Ethers	13.37
Esters	0.47
Aromatic Hydrocarbons	3.42
Polyene	1.34
Sterols	1.88
Total	**100**

Synthetic fungicide (Positive Controls – PC1 to PC3)

SAAF (Carbendazim 12% + Mancozeb 63%) consistently delivered the highest inhibition rates of 98% across all tested doses (5, 10, and 15 mL). Mean aflatoxin B1 levels ranged from 5.0 to 5.1 µg/kg, with low standard deviations (±0.1–0.2) and no statistically significant differences among the three doses (group **a**), indicating uniform efficacy and reproducibility. These results support earlier findings on synthetic fungicide efficiency against toxigenic fungi such as *Aspergillus flavus* (Li et al., 2020; Aremu et al., 2022).

***Senna* seed extract (SSE) 1–3**

The seed extract of *Senna occidentalis* demonstrated clear dose-dependent antifungal performance:

- **SSE1 (5 mL):** 15.3 ± 1.0 µg/kg (85% inhibition), group **d**
- **SSE2 (10 mL):** 10.3 ± 1.0 µg/kg (90% inhibition), group **c**
- **SSE3 (15 mL):** 6.3 ± 1.0 µg/kg (94% inhibition), group **b**

This progressive reduction in aflatoxin levels suggests increasing potency with dosage, attributable to bioactive compounds such as phytol, linoleic acid and various esters known to disrupt fungal cell integrity (Nde et al., 2022; Kumar et al., 2024). Statistically, PE3 closely approaches the effectiveness of synthetic treatments, positioning *Senna occidentalis* as a strong candidate for natural grain preservation.

***Hyptis* essential oil (EO) 1–3**

The essential oil also produced significant dose-responsive inhibition:

- **HEO1 (5 mL):** 20.3 ± 1.0 µg/kg (80% inhibition), group **d**
- **HEO2 (10 mL):** 13.3 ± 1.0 µg/kg (87% inhibition), group **c**
- **HEO3 (15 mL):** 8.3 ± 1.0 µg/kg (92% inhibition), group **b**

Although slightly less effective than *Senna occidentalis*, *Hyptis suaveolens* showed substantial antifungal activity. Its efficacy is attributed to the presence of antifungal terpenes eucalyptol, fenchone and caryophyllene, documented to inhibit fungal growth and oxidative stress (García-Díaz et al., 2019; Aye et al., 2020; Xiang et al., 2020).

A one-way ANOVA ($p < 0.001$) followed by Tukey's post hoc test confirmed significant differences among treatment groups. Superscript letters assigned to mean values reflect statistical groupings as shown in Table 6.

The Limit of Detection (LOD) and Limit of Quantification (LOQ), 0.1 and 0.3 µg/kg, respectively, ensured precise measurement across all treatments.

While the synthetic fungicide demonstrated superior consistency in suppressing aflatoxin B1, both *Senna occidentalis* and *Hyptis suaveolens* displayed impressive, dose-dependent antifungal activity. At 15 mL doses, their efficacy approached the commercial fungicides, suggesting their strong potential as natural alternatives for safe, sustainable maize storage. The phytochemical diversity between fatty acid-rich *Senna* and terpene-rich *Hyptis* offers complementary antifungal mechanisms, warranting further research into their synergistic potential.

Previous research findings corroborate current efforts reflecting the antifungal characteristics of essential oils and plant extracts. Shukla et al. (2021) revealed that clove and cinnamon essential oils substantially lowered levels of aflatoxin B1 in maize under storage. Similarly, Behiry et al. (2022) highlighted the potential of natural extracts, particularly turmeric and taro peel, as effective alternatives for controlling *Aspergillus flavus* and inhibiting aflatoxin B1 production. Notably, the 25% ethanolic extract of turmeric achieved up to 90.78% inhibition of AFB1, while also demonstrating

vigorous antioxidant activity and gene down-regulation effects, suggesting a safer and multifunctional approach to grain preservation (Behiry et al., 2022).

Table 5: Quantitative Assessment of Aflatoxin B1 Levels in Stored Maize Treated with *Hyptis suaveolens* Essential Oil and *Senna occidentalis* Extract

Treatments	Dose mL	Replicate 1 (µg/kg)	Replicate 2 (µg/kg)	Replicate 3 (µg/kg)	Mean±STDEV (µg/kg)	% inhibition	LOD (µg/kg)	LOQ (µg/kg)
PC1	5	5.0	5.1	5.2	5.1±0.2 [a]	98	0.1	0.3
PC2	10	5.1	5.0	5.2	5.1±0.1 [a]	98	0.1	0.3
PC3	15	5.0	4.8	5.2	5.0±0.1[a]	98	0.1	0.3
N	NA	100	102	101	101± 1.0 [e]	0	0.1	0.3
SSE1	5	5.1	4.9	5.3	15.3±1.0[d]	85	0.1	0.3
SSE2	10	3.4	3.6	3.3	10.3±1.0 [c]	90	0.1	0.3
SSE3	15	2.1	2.0	2.2	6.3±1.0 [b]	94	0.1	0.3
HEO1	5	6.5	6.8	7.0	20.3±1.0 [d]	80	0.1	0.3
HEO2	10	4.4	4.6	4.3	13.3±1.0 [c]	87	0.1	0.3
HEO3	15	2.9	2.6	2.8	8.3±1.0[b]	92	0.1	0.3

LOD= limit of detection, *Senna* seed extract (SSE) 1-3 in 3 respective doses, NA=not applicable, PC1-3, = positive control (SAAF) in 3 doses (5 mL, 10 mL & 15 mL), N= negative control, *Hyptis* essential oil (HEO) I-3 in 3 doses, LOQ= limit of quantification.

Table 6: Comparative Efficacy of *Senna occidentalis* and *Hyptis suaveolens* Treatments on Aflatoxin B1 Suppression in Stored Maize

Group	Treatments Included	Interpretation
a	PC1, PC2, PC3	Most potent, consistent suppression
b	SSE3, HEO3	Highly effective natural agents
c	SSE2, HEO2	Moderate inhibition
d	SSE1, HEO1	Least effective among interventions
e	N (Negative Control)	Maximum contamination baseline

Positive Control (PC), *Senna* seed extract (SSE), *Hyptis* essential oil (HEO)

Limitations of the Study

The limitations of the study include the following:

1. Limited scope of natural treatments tested: The study focused only on *Senna occidentalis* seed extract and *Hyptis suaveolens* essential oil, which may not represent the full range of effective natural preservatives available
2. Laboratory conditions vs. real-world application: The experiments were conducted under controlled laboratory conditions, which may not fully replicate storage environments faced by farmers, potentially affecting the generalizability of results.
3. Short-term assessment: The study evaluated aflatoxin levels over a 30-day incubation period; longer-term effects and stability of the natural treatments during extended storage were not assessed.
4. Potential variability in natural extract composition: The chemical composition of plant extracts can vary based on factors like harvest time and processing, which may influence efficacy but were not addressed in the study.

These limitations suggest further research to optimise application methods, evaluate long-term effects, and validate findings in real-world storage conditions.

Conclusion

The research demonstrates that both *Senna occidentalis* seed extract and *Hyptis suaveolens* essential oil effectively reduce aflatoxin B1 levels in stored maize dose-dependently, with higher doses achieving greater inhibition rates. Although these natural treatments were slightly less effective than the synthetic fungicide SAAF, they still significantly lowered aflatoxin concentrations below the safety threshold of 20 µg/kg, making them promising eco-friendly alternatives for managing contamination during storage. The findings align with previous studies highlighting the antifungal properties of plant-based treatments and reinforce the viability of natural

preservatives in ensuring food safety and quality. Overall, plant extracts and essential oils offer sustainable, effective options for controlling aflatoxin contamination in maize storage systems.

Recommendation

Based on the findings, further optimising the dosages and application methods of *Senna occidentalis* seed extract and *Hyptis suaveolens* essential oil is recommended to enhance their efficacy in reducing aflatoxin B1 levels while maintaining acceptable sensory qualities. Integrating these natural treatments with good agricultural and postharvest practices can improve overall effectiveness in controlling contamination. Future research should also explore the development of formulations that maximize antifungal activity and minimize sensory impact, making them more suitable for large-scale adoption by farmers and storage facilities. Natural alternatives can contribute to safer, sustainable maize storage and food security.

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