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SHORT COMMUNICATION

Impact of spices and aromatic plants on the growth and mycotoxin production in fungi

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ABSTRACT

Spices and aromatic plants have antioxidant, and antibacterial qualities to extend food shelf life and quality. Thin-layer chromatography (TLC) identified mycotoxins in experimental fungus in this study. The mycotoxin analysis showed that *Aspergillus terreus, A. nidulans,* and *F. oxysporum* produced aflatoxin B1, G1, and ochratoxin with retention factors of 0.46, 0.48, and 0.56, respectively. The mycelial dry weight method assessed extract inhibition of experimental fungus. Clove extract inhibited fungal proliferation at higher concentrations, reducing mycotoxin generation. As clove extract concentration grew from 10mg/ml to 40mg/ml, *A. nidulans* mycelia multiplication decreased from 8% to 98%. As extract concentration increased from 10mg/ml to 50mg/ml, *F. oxysporum* decreased ranged from 53% to 100%. Celery and basil leaf extracts followed similar trends. As extract concentration grew from 10mg/ml to 50mg/ml, aflatoxin and ochratoxin generation were inhibited by 25% to 100% and 57% to 100%, respectively. These extracts may inhibit aflatoxin-producing fungi.

Keywords: antioxidant, aromatic plants, bioactive, chromatography, fungi, mycotoxins, preservative, spices

INTRODUCTION

The spices and aromatic plants have been utilized across centuries to elevate the flavour and nutritional profile of culinary creations. Beyond their gustatory advantages, spices offer health-protective attributes such as preservation, antioxidation, and antimicrobial properties, which profoundly influence the shelf-life and quality of food (Embuscado, 2015).

Clove (*Syzyum aromaticum*), derived from the aromatic flower bud of a tree, is known for its robust, warm, and sweet aroma. It finds extensive use in both culinary and medicinal domains, enhancing the taste of dishes like stews, curries and baked goods. Clove harbours various bioactive compounds including the potency of antimicrobials. (Hadidi et al., 2020). Extracted essential oil from clove buds demonstrates antibacterial efficacy against diverse food-borne pathogens (Chouhan et al., 2017; Batiha et al., 2020). Moreover, clove bud essential oil showcases antioxidant, antifungal, anticarcinogenic and anaesthetic properties (Shariti-Rad, 2017; Anacarso, et al., 2019).

Basil (*Ocimum basilicum*) belongs to the herb species, stands as an annual plant in the mint family (Lamiaceae) and boasts millennia of story in culinary practice (Agarwal et al., 2013). Basil entices with its distinctive aroma and flavour profile, characterized as sweet, peppery and faintly minty. Often added to dishes just before serving to retain its essence, basil serves as a cornerstone ingredient in various culinary endeavours. Basil leaves also infuse oil, vinegar, and teas with their aromatic essence (Dymock et al., 2015). Beyond olfactory allure, basil leaves house essential oils and compounds associated with antioxidant benefits, alongside being rich in vitamins and minerals (Hanifini et al., 2011; Hosseini-Parver et al., 2015).

Celery (*Apium graveolens* L) is a vegetative plant from the *Apiaceae* family and has an ancient cultivation history (Gauri et al., 2015). Spotting a long fibrous stalk tapering into leaves celery serves as a versatile vegetable, prized for its crispness and slightly salty taste. It commonly features salads, soups, and assorted dishes (Cho et al., 2019). Additionally, celery's high-water content and nutritional density make it a notable source of vitamins, minerals, and dietary fibre (Kooti et al., 2014). The plant is used around the world as a vegetable either for the crisp petiole (Leaf stalk) or the fleshy root. The inhibitory effects of spices are probably attributed to their major components. These effects are intriguing regarding the prevention of mycotoxin contamination in various foods, offering potential alternatives to current food additives. This study delves into exploring the impact of clove, basil and celery on growth and toxin production by toxigenic fungi. Through the examination of these spices, this study endeavours to unravel their potential influence on fungal behaviours and assess their viability as bioactive agents in curbing the generation of harmful toxins.

MATERIALS AND METHODS

Clove, celery, and basil leaves utilized in this study were sourced from the Forestry Research Institute, Ibadan, Nigeria and authenticated following the criteria outlined by the International Committee for Botanical Nomenclature (ICBN). Fungal strains including *Aspergillus terreus*, *Aspergillus nidulans and Fusarium oxysporum* were employed in this study and maintained on Potato Dextrose Agar slants. The plant material was extracted following the method adopted by Banso et al (2023a).

The plant material (200g) was ground and mixed with ethanol (100ml) at room temperature, followed by agitation on a rotary shaker for 30 minutes. Filtration through Muslin cloth and a sterile Millipore filter (Banso and Ajayi, 2023) ensued. Fungal strains were cultivated on Potato Dextrose Agar slants at 25°C for 10 days until well sporulated. Spores, harvested in 1% Driwel, were adjusted to a final concentration of about 10^4 spores/ml. A portion of this suspension was inoculated into a culture medium containing a diluted extract and incubated at 25°C for 8 days, with visual monitoring of mold growth. After incubation, cultures were autoclaved, and the dry weight of the mycelia mat was determined by filtration and drying (Banso et al., 2020), comparing each test culture with an uninhibited control.

Thin-layer chromatography (TLC) detected mycotoxins by spotting both filtrates and standards on prepared TLC plates, using chloroform and ethanol solvents for development. Plates were examined under a UV lamp, and the retention factor (Rf) of compounds/toxins was calculated. The fluorescence intensity of separated mycotoxin spots was measured using a fluorodensitometer (model MPF-2A, Hitachi Ltd., Tokyo) (Banso et al., 2023b; Banso and Banso, 2023).

Results underwent analysis of variance (ANOVA), with means comparisons conducted via Tukey's multiple range tests in SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The assay for mycotoxin revealed that *Aspergillus terreus*, *Aspergillus nidulans* and *Fusarium oxysporum* produced Aflatoxin B_1 with a retention factor of 0.46, AflatoxinG1 with a retention factor of 0.48 and ochratoxin with a retention factor of 0.56 respectively (Table 1).

Table 1. Mycotoxin producing fungi

Fungi	Retention factor (R_f)	Mycotoxin
A. terruns	0.46	Aflatoxin B_1
A. nidulance	0.48	AflatoxinG_1
F. oxysporum	0.56	Ochratoxin

This study demonstrated that the inhibitory effect of clove extract on fungal growth increases with increased with higher concentrations of the extract, while the production of mycotoxin decreases (Table 2). For instance, mycelial reduction of *A. terreus* ranged from 6% to 100% across different concentrations of clove extract, while inhibition of aflatoxin B1 production increased from 30 % to 100 % as the concentration of the extract escalated. Similar trends were observed for *Aspergillus nidulance* and *F. oxysporum*. Likewise, the impact of celery leaf extract on the inhibition of fungal growth and toxin production was examined, and the outcomes are detailed in Table 3. It was observed that higher concentrations of the extracts led to an increased reduction in mycelial growth and inhibition of mycotoxin production across all tested fungi. The results of this study agree with the findings of Lokman, (2010) who reported that some plant extracts inhibited the growth and toxin production of toxigenic fungi. There is a relationship between mycelia dry weight and mycotoxin production; Khaoula et al. (2023) reported that every time, the concentration of plant extract was increased, the reduction of mycelia dry weight was accompanied by a decrease in secretion of aflatoxin B_1. This finding agrees with this present study. Exploring fungal inhibitors derived from local sources is imperative to address concerns surrounding the carcinogenic properties, environmental residue, and expense associated with synthetic fungicides (da Cruz Cabral et al., 2013). Moreover, there exists a critical need to devise alternative strategies to mitigate the detrimental effect of aflatoxin, produced by *Aspergillus terreus*, *Aspergillus nidulans* and *F. oxysporum.* on animal and public health (da Cruz Cabral et al., 2013). The susceptibility of these fungi to clove, celery, and basil extract underscores the potential of these natural compounds as inhibitors (Mmongoyo et al., 2017). Remarkably, while *A. nidulans* exhibited higher susceptibility to all extracts concerning mycelial growth inhibition, *A. terreus* and *F. oxysporum* displayed greater suppression of aflatoxin production. This discrepancy suggests that weak inhibition of fungal growth does not necessarily correlate with feeble inhibition of aflatoxin formation corroborating earlier research findings (Mmongoyo et al., 2017).

Table 2. Inhibitory effect of clove extract on growth and toxin production by toxigenic fungi

Concentration (mg/ml)	*A.terreus*		*A. Nidulans*		*F. oxysporum*	
	Mycelium (mg)	Aflatoxin B_1 (µg/ml)	Mycelium (mg)	AflatoxinG_1 (µg/ml)	Mycelium (mg)	Ochratoxin (µg/ml)
Control	140	50	360	4.0	500	15
10	132(6)	3.5(30)	330((8)	3.4(23)	410(18)	70(53)
20	110(21)	2.5(50)	250(42)	2.0(50)	315(37)	3.5(76)
30	90(36)	0(100)	210(31)	0.6(85)	209(58)	0.6(96)
40	0(100)	0(100)	6(98)	0.2(90)	0(100)	0(100)
50	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)

Note: Numbers in parentheses indicate percent inhibition

Table 3. Inhibitory effect of celery leaf extract on growth and toxin production by toxigenic fungi

Concentration (mg/ml)	*terreus*		*Nidulans*		*F. oxysporum*	
	Mycelium (mg)	Aflatoxin B_1 (µg/ml)	Mycelium (mg)	AflatoxinG_1 (µg/ml)	Mycelium (mg)	Ochratoxin (µg/ml)
Control	140	5.0	360	4.0	500	15.0
10	130	2.5(50)	330(8)	3.0(25)	400(20)	6.5(57)
20	100	1.5(70)	320(11)	2.0(50)	310(38)	3.0(80)
30	80	0(100)	200(44)	0.4(90)	200(60)	0.4(97)
40	0(100)	0(100)	5.0(99)	0.2(95)	0(100)	0(100)
50	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)

Note: Numbers in parentheses indicate percent inhibition

The inhibitory effect of basil leaf extract on fungal growth and toxin production followed a similar pattern, as shown in Table 4. Higher concentrations of the extract led to increased inhibition of mycelial growth and mycotoxin production for all tested fungi. Considering that aflatoxigenic fungi synthesize aflatoxin through a multifaceted biosynthetic process, the capability of these extracts to impede aflatoxin production hints at their potential to destroy this biosynthesis pathway effectively (Holmes et al., 2008). Consequently, these extracts could serve as valuable resources to safeguard food crops, particularly during storage, against both fungal decay and aflatoxin contamination. For small-scale farmers, utilizing clove, celery and basil extracts in chips, powders or leaf form could offer an economical preservation method for cereal crops. Such an approach could potentially alleviate concerns regarding fungal resistance to synthetic fungicides, as the intricate composition of these extracts may overcome such resistance (da Cruz Cabral et al., 2013). Furthermore, the identification of inhibitors capable of completely halting biosynthesis remains crucial.

Table 4. Inhibitory effect of basil leaf extract on growth and toxin production by toxigenic fungi

Concentration (mg/ml)	*A.terreus*		*Nidulans*		*F. oxysporum*	
	Mycelium (mg)	Aflatoxin B_1 (µg/ml)	Mycelium (mg)	AflatoxinG_1 (µg/ml)	Mycelium (mg)	Ochratoxin (µg/ml)
Control	140	5.0	360	4.0	500	15.0
10	90(36)	2.0(60)	200(44)	1.5(63)	305(39)	12.5(17)
20	60(57)	1.5(70)	110(69)	1.2(70)	206(59)	9.0(40)
30	45(68)	1.3(74)	80(78)	1.0(75)	90.5(82)	4.5(70)
40	30(79)	0.5(90)	40(89)	0.4(90)	60.0(88)	2.0(87)
50	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)

Note: Numbers in parentheses indicate per cent inhibition

CONCLUSION

This research affirms that extracts of clove, celery and basil leaves possess potential bioactivity against aflatoxin-producing organisms, inhibiting both mycelia growth and aflatoxin production. The demonstrated efficacy of these extracts suggests their potential application as natural inhibitors to safeguard stored crops from fungal spoilage and aflatoxin contamination particularly to economically disadvantaged framers in Nigeria, who could benefit from incorporating clove, celery and basil leave chips and powders into cereal crops to mitigate aflatoxin producers during storage. Given the historical use of these herbs as food additives and herbal remedies, their integration into cereal crops for post-harvest fungal control is likely to be both safe and beneficial for enhancing food security.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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