Assessment of *Pelargonium graveolens* oil as plant-based antimicrobial and aflatoxin suppressor in food preservation

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Abstract

BACKGROUND: Contamination of stored food commodities by moulds and mycotoxins results in qualitative as well as quantitative losses. Most of the synthetic antimicrobials used for preservation of stored food items produce side effects in the form of residual and mammalian toxicity. Recently some higher plant products have been recommended as safe alternatives of such synthetic antimicrobials. In the present investigation antifungal efficacy of some essential oils was evaluated against two toxigenic strains of *Aspergillus flavus* with special reference to the oil of *Pelargonium graveolens* to investigate its potential to inhibit aflatoxin B_1 secretion.

RESULTS: Essential oil of *P. graveolens* exhibited absolute fungitoxicity against both the toxigenic strains of *A. flavus*. The minimum inhibitory concentration of the oil was found to be 0.75 g L^{-1} and exhibited a fungistatic nature. It was found superior over the synthetic fungicides tested and exhibited a broad fungitoxic spectrum. The oil showed excellent anti-aflatoxigenic efficacy as it completely inhibited aflatoxin B_1 production even at 0.50 g L^{-1}.

CONCLUSION: This is the first report on the aflatoxin B_1 inhibitory nature of *P. graveolens* oil. It may be recommended as a novel plant-based antimicrobial as well as aflatoxin B_1 suppressor over synthetic preservatives in food protection.

Keywords: anti-aflatoxigenic; antifungal; *Aspergillus flavus*; *Pelargonium graveolens*

INTRODUCTION

Fungal contamination of food commodities is quite severe in tropical and subtropical countries. In addition to quantitative losses, fungal biodeterioration also causes qualitative changes in the food commodities, as most of the storage fungi are potential producers of toxic metabolites in the form of mycotoxins, which are hazardous to human and animal systems.[1] According to Food and Agriculture Organization estimates, one quarter of the world's food crops are affected by mycotoxins each year. Among the mycotoxins, aflatoxins produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus* on various food products raise the most concern, posing a great threat to human and livestock health as well as international trade. About five billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods[2] and aflatoxicosis has recently been recognized as sixth amongst the ten most important health risks identified by the World Health Organization for developing countries.[3] For the management of such losses, different synthetic chemicals as preservatives and fumigants have been recommended. However, the use of such chemicals in food protection has from time to time been cautioned against because of their residual toxicity, mammalian toxicity and adverse effects on food chain.[4] Recently, some higher plant products, viz. azadirachtin from *Azadirachta indica*,[5] carvone from *Carum carvi*[6] and allyl isothiocyanate from mustard and horseradish,[7] have shown their usefulness as safer alternatives to such synthetic antimicrobials in food preservation and plant protection. Such products of higher plant origin would be biodegradable, renewable in nature and safe to human health.[8] Plant products, especially essential oils, are recognized as one of the most promising groups of natural products for the formulation of safer antifungal agents.[9,10] In the context of agricultural pest management, botanical pesticides are well suited for use in organic food production in industrialized countries and can also play a significant role in postharvest protection of food commodities. In the present study an effort has been made to investigate antifungal activity of the essential oils of some higher plants against two toxigenic strains of *A. flavus* (Navjot 4NSt and Saktiman 3NSt), with special reference to the oil of the

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traditionally used plant *Pelargonium graveolens* Linn. ex Ait, in order to study its potential to inhibit aflatoxin B_1 secretion.

MATERIALS AND METHODS

Isolation of essential oils from plants

Fresh parts of 11 plant species, viz. *Ageratum haustonianum* Mill. (leaf), *Artemisia pallens* Wall. (leaf), *A. vulgaris* Linn. (leaf), *Boswellia serrata* Roxb. ex Colebr. (bark), *Caesulia axillaris* Roxb. (leaf), *Chrysanthemum indicum* Dc. (leaf), *Cinnamomum zeylanicum* L. (leaf), *Elettaria cardamomum* Maton. (leaf), *P. graveolens* Linn. ex Ait (leaf), *Salvia plebeia* R. Br. (leaf) and *Seseli indicum* Wight and Arn. (leaf), were collected locally from the campus of Banaras Hindu University, Varanasi, India. The plants were identified with the help of the *Flora of BHU Campus*.[11] Volatile fractions (essential oils) were isolated through hydro-distillation using Clevenger's apparatus and the oils were stored in clean glass vials at $6 \pm 2\,^{\circ}C$ after removing water traces with the help of capillary tubes and anhydrous sodium sulfate.[12]

Antifungal screening of essential oils from higher plants

The fungitoxic activity of essential oils against two toxigenic strains of *A. flavus*, namely Navjot 4NSt and Saktiman 3NSt, was tested at 1.0 g L^{-1} by the poison food technique[13] using Czapek's medium ($NaNO_3$, 2.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; sucrose, 30.0 g; agar, 15.0 g; distilled water, 1 L; pH 6.8 ± 0.2). The requisite amount of essential oil was dissolved separately in 0.5 mL acetone and then mixed with 9.5 mL of autoclaved Czapek's medium in Petri dishes (9.0 cm diameter). The prepared plates were inoculated aseptically with assay discs (5 mm diameter) cut from the periphery of 7-day-old cultures of the toxigenic strains of *A. flavus* grown on Czapek's medium. Control sets were prepared subsequently using sterilized distilled water in place of the oil. The plates were then incubated at $28 \pm 2\,^{\circ}C$ for 7 days. Percentage inhibition of the radial growth of the test fungus by the oils was calculated.[14]

$$\text{Percentage mycelial inhibition} = \frac{d_c - d_t}{d_c} \times 100$$

where d_c = mean colony diameter of control sets and d_t = mean colony diameter of treatment sets.

During antifungal screening of the essential oils of the 11 plant species, only the oil of *P. graveolens* showed 100% inhibition. Hence the oil of *P. graveolens* was subjected to further experiments in the present investigation.

Minimum inhibitory concentration and nature of toxicity of essential oil of *P. graveolens*

The essential oil of *P. graveolens*, which showed absolute fungitoxicity against both the toxigenic strains of *A. flavus* during screening programme, was selected for further investigation. To discover the minimum inhibitory concentration (MIC) at which the *Pelargonium* oil showed absolute fungitoxicity, experiments were carried out using the above-mentioned poisoned food technique with graded concentrations of the oil in Czapek's medium, i.e., 0.15, 0.25, 0.50, 0.75 and 1.0 g L^{-1}. The nature of toxicity (fungistatic/fungicidal) of the oil was determined by the method of Thompson.[15] Inhibited fungal discs of the oil-treated sets were reinoculated into the fresh medium and revival of their growth was observed.

Fungitoxic spectrum and comparison of fungitoxicity of the essential oil of *P. graveolens* with some prevalent synthetic fungicides

The mycotoxic spectrum of *P. graveolens* oil was evaluated by the poisoned food technique at 0.75 g L^{-1} against nine fungi, viz. *A. fumigatus, A. niger, A. terreus, Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, Helminthosporium oryzae* and *Trichoderma viride* (procured from the Division of Mycology and Plant Pathology, IARI, New Delhi, India). The efficacy of the oil was compared with some synthetic fungicides procured from Sigma (St Louis, MO, USA), viz. benzimidazole (benomyl), diphenylamine (DPA), phenylmercuric acetate (Ceresan) and zinc dimethyl dithiocarbamate (ziram).

Efficacy of *Pelargonium* oil in arresting aflatoxin elaboration

Testing of the essential oil of *P. graveolens* in checking the synthesis of aflatoxin B_1 by the toxigenic strain (Saktimam 3NSt) of *A. flavus* was done in the present investigation using SMKY medium (sucrose, 200 g; $MgSO_4.7H_2O$, 0.5 g; KNO_3, 0.3 g; yeast extract, 7.0 g; distilled water, 1.0 L; pH 5.6 ± 0.2).[16] The method following Sinha *et al*.[17] was adapted for the estimation of aflatoxin B_1. Different concentrations of the oil, viz. 0.25, 0.50, 0.75 and 1.0 g L^{-1}, were prepared separately by dissolving the requisite amount in 0.5 mL 5% Tween-20 and then mixing with 25 mL SMKY medium in 100 mL Erlenmeyer flasks. Control sets were kept parallel to the treatment sets without essential oil. The flasks were then inoculated aseptically with 1 mL spore suspension ($\sim 10^9$ spores L^{-1}) prepared in 0.1% Tween-80[18] and incubated at $28 \pm 2\,^{\circ}C$ for 10 days. The content of each flask was filtered (Whatman filter paper no. 1) and mycelium was oven dried at $100\,^{\circ}C$ to constant weight for biomass determination. The filtrate was extracted with 20 mL chloroform in a separating funnel. After separation, chloroform extract was passed through anhydrous sodium sulfate kept in Whatman filter paper no. 42. The extract was evaporated to dryness in a water bath at $70\,^{\circ}C$. Dry residue was dissolved in 1 mL chloroform and 50 µL chloroform extract was spotted on a thin-layer chromatography (TLC) plate (20 × 20 cm silica gel-G), then developed in

a toluene–isoamyl alcohol–methanol (90:32:2, v/v/v) solvent system.[19] The intensity of aflatoxin B_1 was observed in an ultraviolet fluorescence analysis cabinet at an excitation wavelength of 360 nm.[20] The presence of aflatoxin B_1 was confirmed chemically by spraying trifluoroacetic acid[21] and by spraying the developed plates with an aqueous solution of 50% sulphuric acid.[22,23]

For quantitative estimation, spots of aflatoxin B_1 on the TLC plate were scraped and dissolved in 5 mL cold methanol and centrifuged at 3000 × *g* for 5 min. The optical density of the supernatant was recorded at a wavelength of 360 nm (Systronics India Ltd, Mumbai, India) and the quantity of aflatoxin B_1 in control and treatment sets was calculated.[24]

$$\text{Aflatoxin } B_1 \text{ content } (\mu g\ kg^{-1}) = \frac{D \times M}{E \times L} \times 1000$$

where D = optical density, M = molecular weight of aflatoxin B_1 (312), E = constant (21 800) and L = path length (a 1 cm cell was used).

Statistical treatment of results

Analysis of data was performed using the SPSS program, version 11.0. Mean and standard error of data were calculated using SPSS software.[25]

RESULTS AND DISCUSSION

During antifungal screening of the 11 essential oils at 1.0 g L^{-1} against both aflatoxigenic strains of *A. flavus*, a considerable variation in fungal growth inhibition was recorded. However, the fungitoxic efficacy of each tested oil remained almost the same against both toxigenic strains of *A. flavus*. Essential oils of *B. serrata, C. zeylanicum* and *S. indicum* exhibited more than 50% fungitoxicity (Table 1). Only the essential oil of *P. graveolens* showed complete inhibition of the inocula of both toxigenic strains of *A. flavus*. Therefore, the oil from leaves of *P. graveolens* was selected for further study. It is evident from Table 2 that the essential oil of *P. graveolens* exhibited absolute toxicity against both toxigenic strains of *A. flavus* at 0.75 g L^{-1} (MIC). The oil exhibited a fungistatic nature up to 1.0 g L^{-1} as the completely inhibited fungal discs on reinoculation to fresh medium (without oil) resumed their growth. Hence it may be concluded that the mode of fungitoxic action of the oil against both strains was same. The oil also exhibited a broad fungitoxic spectrum against all the fungi tested as it absolutely inhibited the growth of *A. fumigatus, A. terreus, A. alternata, F. oxysporum, H. oryzae* and *T. viride* (Table 3). As a fungitoxicant, *P. graveolens* essential oil was recorded to be better than most of the synthetic fungicides compared. The MICs of the synthetic fungicides viz. benzimidazole, diphenylamine, zinc dimethyl dithiocarbamate and phenylmercuric acetate, against toxigenic strains of the fungus (*A. flavus*) were found to be >1.0 g L^{-1} (Table 4). Hence the oil of *P. graveolens* (MIC 0.75 g L^{-1}) was found to be

Table 1. Screening of some essential oils against toxigenic strains of *Aspergillus flavus*

		Percentage inhibition of growth of *A. flavus* at 1.0 g L^{-1}	
Essential oil	Family	Navjot 4NSt	Saktiman 3NSt
Ageratum haustonianum Mill. (leaf)	Asteraceae	36.0 ± 0.57	47.3 ± 1.20
Artemisia pallens Wall. (leaf)	Asteraceae	23.6 ± 0.33	32.3 ± 0.88
A. vulgaris Linn. (leaf)	Asteraceae	13.4 ± 0.71	11.0 ± 0.35
Boswellia serrata Roxb. ex Colebr. (bark)	Burseraceae	80.7 ± 0.21	91.6 ± 0.31
Caesulia axillaris Roxb. (leaf)	Asteraceae	9.28 ± 0.19	7.92 ± 0.51
Chrysanthelum indicum Dc. (leaf)	Asteraceae	25.5 ± 0.30	17.9 ± 0.57
Cinnamomum zeylanicum L. (leaf)	Lauraceae	61.5 ± 0.31	57.6 ± 0.32
Elettaria cardamomum Maton. (leaf)	Zingiberaceae	8.85 ± 0.45	10.8 ± 0.11
Pelargonium graveolens Linn. ex Ait (leaf)	Geraniaceae	100.00 ± 0.00	100.0 ± 0.00
Salvia plebeia R. Br. (leaf)	Lamiaceae	30.2 ± 0.82	18.6 ± 0.17
Seseli indicum Wight and Arn. (leaf)	Apiaceae	56.8 ± 0.42	52.3 ± 0.19

Values are mean (n = 3) ± standard error.

Table 2. Minimum inhibitory concentration (MIC) and nature of toxicity of *Pelargonium graveolens* oil against two toxigenic strains of *Aspergillus flavus*

	Navjot 4NSt		Saktiman 3NSt	
Concentration of oil (g L^{-1})	MIC	Nature of toxicity	MIC	Nature of toxicity
0.15	16.1 ± 0.38	–	21.8 ± 0.17	–
0.25	32.2 ± 0.35	–	25.9 ± 0.51	–
0.50	42.2 ± 0.39	Static	45.5 ± 1.36	Static
0.75	100.00 ± 0.00	Static	100.00 ± 0.00	Static
1.00	100.00 ± 0.00	Static	100.00 ± 0.00	Static

Values are mean (n = 3) ± standard error.

comparatively more efficacious than these synthetic fungicides. In addition, the oil completely inhibited aflatoxin B_1 production (Table 5) at a concentration (0.50 g L^{-1}) lower than its fungitoxic concentration (MIC 0.75 g L^{-1}). At 0.50 g L^{-1} mycelial growth was recorded in *Pelargonium*-treated sets, but aflatoxin B_1 production was completely inhibited. Thus, at 0.50 g L^{-1} the oil of *Pelargonium* completely inhibited aflatoxin B_1 synthesis, but at 0.75 g L^{-1} it completely stopped mycelial growth. The mode of fungal mycelial growth inhibition and aflatoxin B_1 suppression appear to be different as the oil expressed these properties at different concentrations. *P. graveolens* oil in the present investigation showed specific virtues, viz. checking growth of fungi responsible for deterioration of food commodities as well as inhibitory effects on aflatoxin synthesis against toxigenic strains of *A. flavus*. Hence the oil may be recommended in control of qualitative as well as quantitative losses of food commodities due to fungal infestation. Although there are reports on the mycelial growth inhibition by essential oils, the literature is mostly silent on their efficacy in inhibition of aflatoxin. A product inhibitory to fungal growth and aflatoxin synthesis would be definitely economical in control of postharvest fungal contamination of food commodities. The efficacy of *P. graveolens* oil as aflatoxin B_1 suppressor is reported for the first time in the present investigation.

Gas chromatographic–mass spectrometric analysis of the leaf essential oil of *P. graveolens* has been done by Ganapaty and Beknal[26] and revealed the presence of citronellol (178.5 g kg^{-1}), geranyl acetate (274.3 g kg^{-1}), geraniol (90.5 g kg^{-1}), citronellyl formate (66.4 g kg^{-1}) and linalool-l (54.9 g kg^{-1}). The minor components consisted of α- and β-pinene (25.8 and 4.20 g kg^{-1}), α-phellandrene (17.2 g kg^{-1}), *o*-cymene (4.1 g kg^{-1}), *cis*-rose oxide (12.2 g kg^{-1}) and geranyl tiglate (7.0 g kg^{-1}).

Table 3. Fungitoxic spectrum of *Pelargonium graveolens* oil at 0.75 g L^{-1}

Fungi	Percentage inhibition
Aspergillus fumigatus Fres.	100.00 ± 0.00
Aspergillus niger Van Teigh.	78.87 ± 0.11
Aspergillus terreus Thom.	100.00 ± 0.00
Alternaria alternata Fr. Keissl.	100.00 ± 0.00
Cladosporium herbarum Pers.	84.99 ± 0.57
Curvularia lunata Wakk. Boed.	95.29 ± 0.64
Fusarium oxysporum Schlecht ex Fr.	100.00 ± 0.00
Helminthosporium oryzae Breda de Hann	100.00 ± 0.00
Trichoderma viride Pers.	100.00 ± 0.00

Values are mean ($n = 3$) ± standard error.

The present investigation further shows some interesting findings regarding the essential oil of *P. graveolens*. The oil was found to be highly efficacious, showing fungitoxicity against *A. flavus* at concentrations lower than the earlier reported oils of *Cymbopogon martini*,[27] *Chrysactinia mexicana*[28] and *Cymbopogon citratus*.[29] Because of lower MIC compared with synthetic fungicides and a broad fungitoxic spectrum, the oil of *P. graveolens* would possess a high market value as a botanical fungitoxicant.

P. graveolens has been used for a long time in the Indian system of medicine. The plant is commonly used for skin care and as a food flavouring.[30] Hence its essential oil may be recommended as a safe antimicrobial agent against fungi causing contamination of food commodities. The findings of the present study are relevant in enhancing shelf-life of commodities by controlling microorganisms and minimizing health hazards by inhibiting aflatoxin B_1 elaboration in food by use of the essential oil of *P. graveolens*. The oil may be recommended as an easily available and renewal source of antifungal and anti-aflatoxigenic agent over the synthetic antimicrobials

Table 4. Toxicity of *Pelargonium graveolens* oil and synthetic fungicides against the two toxigenic strains of *A. flavus*

	Minimum inhibitory concentration (g L^{-1})	
Fungicides/oil	Navjot 4NSt	Saktiman3NSt
Benzimidazole (benomyl)	>5.0	>5.0
Diphenylamine (DPA)	2.0[a]	3.0[a]
Phenylmercuric acetate (Ceresan)	1.0[a]	1.0[a]
Zinc dimethyl dithiocarbamate (ziram)	>5.0	>5.0
Pelargonium graveolens oil	0.75	0.75

[a] Cidal nature of toxicity.

Table 5. Efficacy of *Pelargonium graveolens* oil on biomass of mycelium and aflatoxin B_1 by a toxigenic strain (Saktiman 3NSt) of *A. flavus*

	Concentration of *Pelargonium graveolens* oil (g L^{-1})				
Parameters	Control	0.25	0.50	0.75	1.00
Mycelial biomass (g)	0.779 ± 0.017	0.569 ± 0.005	0.228 ± 0.004	0.00 ± 0.00	0.00 ± 0.00
Aflatoxin B_1 (μg kg^{-1})	521.7 ± 0.53	188.2 ± 0.64	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Each data point represents the mean of three replicates ± standard error.

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used in food processing, most of which are reported to possess residual toxicities.

In conclusion, *P. graveolens* oil, owing to its antifungal, anti-aflatoxigenic properties and broad fungitoxic spectrum, may be recommended for its practical application as a botanical fungitoxicant for enhancing the shelf-life of food commodities by controlling fungal infestation.

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