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PRESERVATION OF DRIED FRUITS AND NUTS FROM BIODETERIORATION BY NATURAL PLANT VOLATILES

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ABSTRACT

Thirty fungal species were found to be associated with the stored kernels of cashewnut *(Anacardium occidentale* L.), chironji/cuddapah almond *(Buchanania lanzan* Spreng.), walnut (*Juglans regia* L.), dried fruits of cardamom *(Elettarra cardomomum* Matan), and raisin *(Vitis vinifera* L.). Because of their wide distribution, high incidence as well as enormous potential to produce mycotoxins, *Aspergillus flavus* Link: Fr. and *A. ochraceous* Wilhelm. were selected as test fungi for antifungal screening of leaf extracts of 126 species of higher plants belonging to 45 families of angiosperms. *Ocimum gratissimum* exhibited the strongest bioactivity. Minimum inhibitory concentrations (MIC) of the volatile antifungal oil fraction against *A. flavus and A. ochraceous* were found to be 400 ppm and 700 ppm, respectively; higher concentrations (>900 ppm) were fungicidal and not fungistatic. The toxicity of the oil did not change even with high inoculum density during storage periods of 270 days, at its exposure to 100ºC, and on autoclaving.

INTRODUCTION

In the tropical and subtropical parts of the world, the losses due to mould spoilage are of greater magnitude than in the temperate regions because of faulty storage methods and climatic conditions.

The protection of crops, stored food grains and pest control in the public health sector continues to place heavy reliance upon the use of chemicals. The history of pesticide development has been instructive to us in terms of benefits derived as well as the hazards, which accompany indiscriminate use of these poisons. Our lessons have been costly but educational. Therefore, in the future we must rely upon the development of strategies, which in addition to their efficiency must be safe and selective to the target pest or pathogen.

Plants are a very rich source of bioactive organic chemicals. Although only some 15,000 secondary plant metabolites have been chemically identified, their total number may exceed 4,000,000 (Saxena, 1993). They are a vast cornucopia of defense chemicals, comprising repellents, feeding deterrents, growth inhibitors, sterilants, toxicants and anti-microbial agents. In addition the volatile substances obtained from

higher plants have proved their usefulness in controlling biodeterioration and therefore are considered to have a bright future (Sharma, 1998). In this communication, the effect of a volatile obtained from *Ocmum gratissimum* on two different species of *Aspergillus* was studied.

MATERIALS AND METHODS

Samples of kernels of cashew nuts (*Anacardium accidentale*), cuddapah almond (*Buchanania lanzen*) and walnut (*Jugulans regia*), dried fruits of cardamon (*Elettaria cardamomum*), and raisin (*Vitis rinifera*) were collected from local markets in pre-sterilized bags and were studied for their associated mycoflora by standard blotter and Agar plate methods as recommended by Neergaard and Saad (1962). The plants were incubated at 28±2ºC and observed daily for 7 d. Since *Aspergillus flavus* and *Aspergillus niger* were found to predominate these species were selected as test organisms for fungitoxic investigations.

Screening of vapours of leaves of angiosperms for their toxicity against Aspergillus flavus and Aspergillus niger

Twenty grams of freshly collected leaves of plants belonging to different families were surface sterilized, chopped and macerated with 20 mL of sterile water (1:1 w/v) in a pestle and mortar. The extract was obtained by passing the pulp through double layered muslin cloth. The extract was assayed for its volatile antifungal activity against the test fungi by the inverted plate method of Bocher (1938).

Distribution of fungitoxicity in different parts of *Ocimum gratissi*mum

Root, stem, leaf and inflorescence of *Ocimum gratissimum* were collected and tested for their activity against mycelial growth of the test fungi by the usual inverted Petri plate method. The percent inhibition of mycelial growth was calculated from mean values of colony diameter of treated and control sets.

Extraction of volatile fungitoxic fraction from the leaves of *Ocimum gratissimum*

The volatile fraction from the leaves of *O. gratissimum* was extracted by hydro-distillation through Clevenger's apparatus. Five hundred g of fresh leaves were macerated and subjected to hydro-distillation. Two distinct fractions comprising an upper oily layer and a lower aqueous layer were obtained which were separated by carefully regulating the stopper of the apparatus. The upper oily layer was made anhydrous by treating it with 0.5 g anhydrous sodium sulphate in order to obtain the pure essential oil.

To isolate the oil from the aqueous portion, the oil was extracted with solvent ether in a separating funnel. The ether was removed at reduced pressure, which resulted in an oily residue that was stocked with the oil collected earlier. The remaining aqueous fraction, free from smell was stocked separately. Thus, the hydro-distilled volatile fraction from the leaves of *O. gratissimum* ultimately resulted into stocks of two fractions - an oil fraction and an aqueous fraction.

The fungi-toxicities of the oil and the aqueous fraction were evaluated against the test fungi separately by the poisoned food technique of Grover and Moore (1962).

Studies on the fungi-toxic properties of the essential oil

Minimum inhibitory concentration (MIC): To find the minimum concentration of the oil needed to obtain absolute inhibition of mycelial growth of the test fungi, experiments were carried out following the poisoned food technique. Treatment sets comprising the following concentrations of the oil: 600, 700, 800, 900, 1,000, 1,100 and 1,200 ppm, were prepared by dissolving the requisite amounts in 0.5 mL acetone and mixing with 9.5 mL Czapek-Dox agar medium. In controls, requisite amounts of sterile water were added to the medium.

Nature of toxicity: To ascertain the fungistatic/fungicidal nature of the oil, tests were performed according to the method adopted by Garber and Houston (1959). Requisite quantities of the oil were dissolved in 0.5 mL acetone and finally mixed with Czapek-Dox agar medium to get final concentrations of 800, 900, 1,000, 1,500, 2,000, 2,500, and 3,000 ppm. Control sets were prepared using requisite quantities of sterile water in place of the oil. Mean values of colony diameters of treatment and control sets were recorded.

Effect of increased inoculum - The effect of increased inoculum density of the test fungi on fungi-toxicity of the oil was studied by the poisoned food technique using Czapek-Dox liquid medium as recommended by Misra (1975). The poisoned liquid medium was prepared in different conical flasks by supplementing requisite quantities of the oil dissolved in 0.5 mL acetone and then mixed with 9.5 mL liquid Czapek-Dox medium to make the final concentration of 900 and 1,000 ppm. Assay discs 5 mm in diameter of *A. flavus,* in multiples of two i.e. 2, 4, 6, 8, 10 were inoculated separately in the sets containing 1,000 ppm oil. Similarly the assay discs of *A. niger* were also inoculated separately in the sets containing 900 ppm of oil. For controls, the oil was replaced by sterile water, which was dissolved in acetone and mixed with Czapek-Dox liquid medium.

Effect of storage: The effect of storage on fungi-toxicity of the oil was determined by storing a stock of the oil in an air-tight glass vial at room temperature. The fungi-toxicity of the oil taken from the stock at a regular 30-day interval was tested at the MIC of the respective fungi by the poisoned food technique, and observations on mycelial growth were recorded.

Effect of temperature: Experiments were performed to determine if the antifungal factor of the oil was thermo stable or labile. Different glass vials each containing 3 mL oil were stoppered and subjected to different temperature treatments for three hours in incubators already adjusted to 40, 60, 80, and 100ºC. Fungitoxicity of the

treated oil of each set was tested against both the test fungi separately at their respective MIC by the usual poisoned food technique

Fungitoxic spectrum: The fungitoxic spectrum of the oil was determined at 500 ppm, 1,000 ppm and 2,000 ppm (the hypotoxic, toxic and hypertoxic concentrations with respect to the test fungi) against the fungi isolated excluding the test fungi by the usual poisoned food technique.

Isolation and identification of antifungal principle from the oil of Ocimum gratissimum: Since the oil exhibited the presence of phenols it was thought desirable to separate phenolic and non-phenolic components of the oil by the technique adopted by Singh *et al.* (1983) and to test their fungi-toxic activity. The plates were incubated at 28±2ºC for 6 d. On the 7th day, the treatment and control sets were observed for the growth of fungi.

Thin-layer chromatography (solvent benzene:ethyl acetate - 98:2) of the NaOH-insoluble fraction exhibited the presence of three spots with Rf 0.89, 0.74, 0.41, when the chromatogram was developed in an iodine chamber. The three components were separated by preparative layer chromatography by the method of Sobti *et al.* (1982).

Studies on the active principle - citral

Efficacy of citral as a preservative of cashew nut and walnut against fungal spoilage under storage. Freshly harvested kernels of Anacardium occidentale (cashew nut) and Jugulans regia (walnut) were obtained from the market in the month of June and brought to the laboratory. Moisture contents of the seeds were determined by the method of Lawrence and found to be 8.7%. The efficacy of citral as a preservative of the nut kernels against fungal spoilage was determined as follows: Twenty g of the kernels were placed separately in pre-sterilized plastic containers of 200 cc capacity. Different amounts of citral were soaked separately in sterilized cotton swabs so as to obtain final concentrations of 1,000 ppm and 2,000 ppm with respect to the volume of the containers. One swab of each concentration was placed in a sterilized perforated polythene bag, which was introduced into each plastic container containing the kernels. In this way treatment sets comprising 5 containers for each concentration were prepared. A control set was run parallel to each treatment set using un-soaked sterile cotton swabs. All the sets were stored at room temperature ranging between 22 to 40ºC and relative humidity between 57 to 87% for a period of six months. Thereafter, fungal infestation of the stored kernels of both the treatments and controls was determined by agar plate and dilution plate method.

TABLE 1
Fungi isolated from different dried fruits and nuts

Fungi isolated	Anachardium occidentale (kernel)	*Buchanania lanzan* (kernel)	Elettaria cardamomum (dried fruit)	*Juglans regia* (kernel)	*Vitis vinifera* (dried fruit)
1	2	3	*4*	5	6
Absidia spinosa	-	-	-	-	-
Alternaria alternata	+	-	-	+	-
A. tenuissina	-	-	+	-	-
A. fumigatus	+	-	-	+	+
A. fischeri	-	-	+	-	-
A. flavipes	-	-	-	-	-
A. flavus	+	+	+	+	+
A. luchuensis	-	-	-	-	-
A. niger	+	+	+	+	+
A. nidulans	+	+	-	-	-
A. ochraceus	+	+	-	-	-
A. ruber	-	-	-	-	-
A. sydowi	-	-	-	-	-
A. versicolor	-	-	-	-	-
Cladosporium cladosporioides	+	-	-	-	-
C. herbarum	-	-	+	-	-
Emericella quadrilineata	-	-	-	-	+
Fusarium oxysporum	-	-	-	-	-
Mucor hiemalis	-	-	-	-	-
Penicillium citrinum	-	-	-	+	-
P. expansum	+	-	-	-	-
P. funiculosum	+	-	+	-	-
P. oxalicum	-	+	-	-	-
Rhizopus arrhizus	+	+	+	-	+
R. nigricans	-	-	-	+	-
Syncephalastrum racemosum	-	-	-	-	+
White sterile mycelium	-	-	-	-	-
Yeast-like fungi	-	-	-	-	+

Effect of citral on the appearance and the taste and flavour of stored kernels: The treated and stored kernels of cashew nuts were compared with those of the control samples after 10 months. Organoleptic tests were carried out on individuals of four different groups. The samples were distributed among 100 individuals representing 25 adult males, 25 adult females, 25 boys and 25 girls.

The evaluation of treated and untreated stored kernels of cashew nuts was carried out using the following ratings: (a) poor quality with bitter taste; (b) poor quality flavourless; (c) good quality.

Comparison of the citral with some preservatives: The efficacy of citral was compared with that of prevalent preservatives namely: acetic acid, calcium propionate and ammonia. The effectiveness was determined in the form of their minimum concentration required to inhibit the test fungi by poisoned food technique.

RESULTS

Twenty eight fungal species were found associated with all the commodities out of which 9 were isolated from *A. accidentale*, 9 from *B. lanzen*, 7 from *E. cardamomum*, 5 each from *J. regia* and *V. vinifera*. As evident from Table 1, *A. flavus* and *A. niger* were the most common species associated with all the dried fruits.

TABLE 2

Percent recovery of the oil from various parts of the plant on fresh weight basis

Plant parts	Percent recovery of the oil
Roots	0
Stem	0.06
Leaf	0.37
Inflorescence	0.18

Most of the species of angiosperms revealed either poor or moderate activity. *Ocimum sanctum* showed absolute toxicity against *A. flavus* but was moderately active against *A. niger*. However, *O. gratissimum* was found to exhibit absolute toxicity against both the test fungi and was, therefore, selected for further studies (Table 2). The leaves *O. gratissimum* showed complete inhibition of mycelial growth of both the test fungi (Table 3). The essential oil extracted from the leaves demonstrated strong fungitoxicity against the test fungi when compared with the aqueous fraction (Table 4). The minimum inhibitory concentration at which the oil checked the mycelial growth completely was 900 ppm for A. *flavus* and 800 for *A. niger* (Table 5). The test fungi revived their growth after being re-inoculated on fresh medium indicating thereby the fungistatic nature of the toxicity of the oil. However at higher concentrations the fraction was fungicidal (Tables 6 and 7).

TABLE 3
Fungi-toxicity in different parts of *Ocimum gratissimum* against mycelial growth of *Aspergillus flavus* and *A. niger*

Parts tested	Percent inhibition of mycelial growth	
	A. flavus	*A. niger*
Roots	1.2	2.5
Stem	21.5	22.2
Leaf	100	100
Inflorescence	80.8	85.2

TABLE 4
Fungi-toxicity of the essential oil and the aqueous fraction of *Ocimum gratissimum* against mycelial growth of *Aspergillus flavus* and *Aspergillus niger*

Fractions at 5000	Percent inhibition of mycelial growth	
	A. flavus	*A. niger*
Essential oil	100	100
Aqueous fraction	6.2	7.1

TABLE 5
Minimum inhibiting concentrations (MIC) of *Ocimum gratissimum* oil against mycelial growth of *Aspergillus flavus* and *Aspergillus niger*

MIC (in ppm)	Percent inhibition of mycelial growth	
	A. flavus	*A. niger*
1500	100	100
1400	100	100
1300	100	100
1200	100	100
1100	100	100
1000	100	100
900	100	100
800	85	100
700	70	81
600	65	75
500	62	69

TABLE 6
Nature of toxicity of *Ocimum gratissimum* oil against *Aspergillus flavus*

Concentration (ppm)	Percent inhibition of mycelial growth	
	Treated set	Re-inoculated
800	100	47
900	100	86
1,000	100	100
1,500	100	100
2,000	100	100

TABLE 7
Nature of toxicity of *Ocimum gratissimum* oil against *Aspergillus niger*

Concentration (ppm)	Percent inhibition of mycelial growth	
	Treatment set	Re-inoculated
800	100	68
900	100	100
1,000	100	100
1,500	100	100
2,000	100	100

The toxicity of the oil was thermostable and it remained unaltered against both the test fungi for the duration of 12 months, the maximum period taken into consideration (Tables 8 - 9).

TABLE 8
Fungitoxicity of *Ocimum gratissimum* oil treated to different temperature

Temperature (ºC)	Percent inhibition of mycelial growth	
	A. flavus (1,000 ppm)	*A. niger* (900 ppm)
40	100	100
60	100	100
80	100	100
100	100	100

TABLE 9
Fungi-toxicity of *Ocimum gratissimum* oil stored for different periods

Storage period (d)	Percent inhibition of mycelial growth	
	*A. flavus (*1,000 ppm)	*A. niger* (900 ppm)
30	100	100
60	100	100
90	100	100
120	100	100
150	100	100
180	100	100
210	100	100
240	100	100
270	100	100
300	100	100
330	100	100
336	100	100

As evident from Table 10 the oil demonstrated a broad range of bioactivity inhibiting 7, 20 and 29 fungi out of the 30 organisms tested at 500, 1,000 and 2,000 ppm respectively. However, *F. moniliformae* and *P. chryosogenum* were the most resistant fungi, which could not be checked even at the hypertoxic concentrations.

On the basis of its physicochemical properties (Table 11) the oil was identified as citral. Citral showed strong activity as a preservative against fungi because the treated kernels were free from any fungal infestation while untreated stored kernels showed heavy infestation (Table 12). Minimum inhibitory concentration of citral was 200 and 300 ppm for *A. flavus* and *A. niger* respectively. Comparison of citral with other preservatives such as acetic acid and ammonia demonstrated that citral was the most effective at 200 and 300 MIC (Table 13).

There was no visual change in the appearance of treated and stored kernels of cashew nut, rather they appeared more glossy and healthy as compared to those of controls, which were mouldy and damaged. As evident from Table 14, the untreated stored samples were rated poor with either bitter taste or as off flavour by all the members of the taste panel. However, the treated stored samples were rated as good quality with no deviation from the normal taste of healthy kernels.

TABLE 10
Fungitoxic spectrum of the oil of *Ocimum gratissimum* at hypotoxic, toxic and hypertoxic concentrations

Fungi	Percent inhibition of mycelial growth		
	500 ppm	1,000 ppm	2,000 ppm
Alternaria alternata	100	100	100
A. tenuissima	100	100	100
A. awamori	86	100	100
A. candidus	65	100	100
A. fumigatus	100	100	100
A. nidulans	88	92	100
A. ochraceous	42	100	100
A. oryzae	77	84	100
A. parasiticus	60	100	100
A. repens	71	86	100
A. ruber	60	85	100
A. sulphureus	63	78	100
A. tamarii	63	100	100
A. terreus	79	100	100
Cladosporium cladosporioides	69	100	100
C. herbarum	100	100	100
Curvularia lunata	100	100	100
Epicoccum nigrum	50	100	100
Fusarium acuminatum	100	100	100
F. moniliformae	40	63	91
Mucor hiemalis	95	100	100
Penicillium citrinum	100	100	100
P. chrysogenum	63	82	100
P. expansum	86	100	100
P. funiculosum	75	100	100
P. italicum	90	100	100
Penicillium sp.	62	89	100
Rhizopus arrhizus	50	90	100
R. nigricans	100	100	100
Syncephalastrum racemosum	74	91	100

DISCUSSION

A perusal of Table 1 shows that various saprophytic as well as parasitic fungi were found associated with the stored products examined. As this study was designed to find out the possibility of utilizing volatile constituents of higher plants as preservatives of food commodities against fungal deterioration, *A. flavus* and *A. niger* were selected as the test organisms since these fungi were found to be the most common storage fungi during the present mycofloral analysis.

TABLE 11
Physico-chemical properties of the oil of *Ocimum gratissimum*

Parameters	Values
Specific gravity	0.9335
Optical rotation	± 0
Refractive index	1.5190
Acid value	11.19
Saponification value	30.09
Ester value	18.90
Solubility inorganic solvents	Soluble in acetone (1:1), ether (1:1); insoluble in Benzene, Hexane and Petroleum ether
Phenolic test	Present

In this study, out of 114 plant species belonging to 45 families screened for their antifungal activity only the leaves of *O. gratissimum* exhibited absolute toxicity against both the test fungi and were, therefore, selected for further detailed investigation. Extraction by hydro-distillation using Clevenger's apparatus offers several advantages namely, compactness, complete distillation and separation of the essential oil and an accurate determination of yield of the essential oil content using only small quantities of plant material. Moreover, in the case of *O. gratissimum,* since the fungitoxic fraction, was thermostable at high temperature, there was no risk of losing the toxicity during hydro-distillation.

TABLE 12
Fungi isolated from treated (1,000 ppm and 2,000 ppm citral) and untreated kernels of cashew nut after six months of storage

Fungi	Appearance of fungi		
	Untreated	Treated stored kernels	
		T_1 (1,000 ppm)	T_2 (2,000 ppm)
Alternaria alternata	+	-	-
Aspergillus flavus	+	-	-
A. fumigatus	+	-	-
A. nidulans	+	-	-
A. niger	+	-	-
Aspergillus ochraceus	+	-	-
A. ruber	+	-	-
Cladosporium oxysporum	+	-	-
Curvularia lunata	+	-	-
Penicillium oxalicum	+	-	-
Rhizopus arrhizus	+	-	-
R. oryzae	+	-	-

\+ : Presence
\- : Absence

TABLE 13
Comparative efficacy of citral with some prevalent preservatives against *Aspergillus flavus* and *Aspergillus niger*

Preservatives and active principle	Minimum inhibitory conc. (ppm)	
	A. flavus	*A. niger*
Acetic acid	9000	5000
Ammonia	9000	5000
Calcium propionate	11000	9000
Citral	200	300

In many other studies, the oils have been tested only for their antifungal activity with no attention paid to investigate detailed fungitoxic properties. It may be mentioned that simply recording fungitoxicity in an oil, on the basis of its antifungal activity may not indicate its successfulness during *in vivo* trials.

TABLE 14
Consolidated data about responses of individuals on quality of kernels of cashew nut with respect to taste and flavour

Group of individuals	Responses					
	Untreated			Treated		
	PQBT	PQOF	GQ	PQBT	PQOF	GQ
MA	17	11	0	0	0	20
MC	16	10	0	0	0	20
FA	19	16	0	0	0	20
FC	18	7	0	0	0	20

PQBT	Poor quality with bitter taste	PQOF	Poor quality with off-flavour
GQ	Good quality	MA	Male adult
MC	Male child	FA	Female adult
FC	Female child		

The determination of the MIC of a fungicide is necessary for prescribing its appropriate dose. Clearly, unnecessarily high doses of a fungicide increase wastage and may cause considerable harm to the quality of the commodity treated. A perusal of the MIC's of most of the oils shows a range between 1,000 to 5,000 ppm. However, some of the oils are effective even at very low concentrations. It is noteworthy that in some instances, oil of a plant investigated by different workers has shown variation in MIC. Such variations may be due to the use of different test fungi or different techniques adopted, as was shown where the MIC of the oil of *O. gratissimum* was 900 ppm and 800 ppm against *A. flavus* and *A. niger* respectively.

A fungitoxicant may act as a fungistat or a fungicide inhibiting the growth of a fungus temporarily or permanently respectively. In this study the oil of *O. gratissimum* exhibited a fungistatic nature at its MIC against both the test fungi, but, at higher concentrations it became fungicidal. Its fungistatic properties do not indicate an ineffectiveness to control fungal deterioration, and it is noteworthy that fungistats have been found to be most successful in preventing fungal development on stored products. The efficacy of antibiotics depends upon the number of organisms they have to combat. In this context, *O. gratissimum* oil exhibited a capability to be fungitoxic even at high doses of inoculum, thereby, indicating the possibility of its exploitation as an ideal fungitoxicant.

A fungicide should be able to retain its activity over a long period of shelf-life. The essential oil of *O. gratissimum* was found to retain its fungitoxicity for up to 356 days, which was the maximum period for which it was tested, thus showing that the oil possesses another attribute of an ideal fungicide. A fungicide must also retain its fungitoxicity at temperature extremes. In this case the fungitoxicity of the oil was found to be thermostable at up to 100ºC. A chemical may exhibit a broad antifungal spectrum inhibiting many fungi, or may be effective against a few specific fungi. If it possesses a narrow range of fungitoxicity it cannot be successfully employed in controlling diseases incited by a complex of pathogens. Concentration also plays an important role during the study of fungitoxic spectrum. Also, an essential oil may show a broad range of activity at higher concentrations. Therefore, the fungitoxic spectrum should be studied at its hypotoxic, toxic, and hypertoxic concentrations. Here, the oil of *O. gratissimum* was found to exhibit broad a fungitoxic spectrum, thereby adding to its success e for the control of fungal deterioration of food-stuffs during storage. It is noteworthy that the oil has also been reported to possess antibacterial activity as well.

The active principle - citral - isolated from *O. gratissimum* oil was found more potent than the oil itself. It appears therefore that other compounds present in the oil mask or decrease the antifungal activity of citral. Furthermore, it is noteworthy that even the fungitoxic nature of an oil and its active principle isolated from the same plant species may vary from sample to sample.

This study revealed that, citral was more fungistatic than some prevalent chemical fungistats namely, acetic acid, ammonia and calcium propionate, thereby, further indicating the possibility of its exploitation as an ideal antifungal agent for the protection of food-stuffs during storage. It also showed that the appearance of the treated and stored samples of cashew nuts remained unchanged and looked better than the controls, which became grey and looked mouldy with wrinkled and damaged seed coats. Moreover, the treated kernels imparted no off-flavour and were palatable in contrast to the controls, which imparted some off-flavour and tasted bitter.

For commercial application, an ideal post harvest fungi-toxicant should not adversely affect the appearance and the quality of the treated commodity. Many synthetic chemicals used for the control of fungal deterioration have been found to have phytotoxic effects and to alter the palatability of the treated commodities. In

contrast, fungi-toxicants of plant origin, chiefly the essential oils, have been found to be non-injurious to the treated commodities. Citral, the active principle of the oil derived from *O. gratissimum* has both an ideal mild lemon-flavour and is a natural preservative, superior to some synthetic ones against fungal spoilage of dried fruits and nuts, and so, this study indicates a newer application beyond that used so far in the pharmaceutical, confectionery and cosmetic industries for flavour only.

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