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BIOACTIVITY OF ESSENTIAL OIL FROM *HYPTIS SUAVEOLENS* AGAINST STORAGE MYCOFLORA

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ABSTRACT

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. The volatile substances obtained from higher plants have proved their usefulness in controlling biodetioration as they form a vast cornucopia of defence chemicals, toxicant and antimicrobial agents. In this communication bioactivity of essential oil extracted from *Hyptis suaveolens* was tested against *Aspergillus flavu*s Link, *Aspergillus niger* Van Tieghem, and *Aspergillus ochraceous* Wilhelm. Minimum inhibitory concentration (MIC) of the volatile oil fraction against these biodeteriogens was found to be 500 ppm; higher concentrations (>1000 ppm) were fungicidal rather than fungistatic. The toxicity of the oil did not change even with high inoculum densities during storage periods of 250 days, after exposure to 100^0C or after autoclaving.

INTRODUCTION

The distinct propensity towards a 'trek back to nature' has become evident in the recent past, especially in the field of pesticides. This change is not sudden, but has resulted out of sheer necessity that has been realized due to the indiscriminate use of synthetic pesticides causing health problems, environmental pollution, pathogen resistance to chemicals and consequent pest resurgence. Utilization of regenerative resources such as plants and their products for newer strategies of pest pathogen management is the demand of this century. The millennia-old interaction and co-evolution between pathogens and plant hosts makes the latter ideal sources of new products for use in indigenous strategies in the management of pest control (Sharma, 1998). India being the home of such plants with medicinal and prophylactic properties can take advantage of these opportunities.

The apprehension that large-scale use of plant-based fungicides/insecticides may lead to resistance among pathogens has not substantiated. Unlike conventional pesticides based on a single active ingredient, the bioactive components in the natural

plant product will be made up by a complex array of novel chemicals that affect not only one physiological function but rather act in concert on several processes (Fawcett and Spencer, 1970)

Such products from higher plants are relatively bio-efficacious, economical and environmentally safe and can be an ideal candidates for use as agrochemicals (Macias et al., 1997, Cutler, 1999). Among these, essential oils (Ramezani *et al.,* 2002) from a number of plants have been reported to show bioactivity against a wide array of plant pathogenic and storage mycoflora (Rice, 1995; Sharma, 2001). However, as yet no work has been carried out to explore the fungicidal activity of the volatile oil *from Hyptis suaveolens* (L) Poit., an annual member of family Lamiaceae, growing abundantly as a weed in India.

Thus in this present communication, the effect of essential oil obtained from *Hyptis suaveolens* on fungi causing storage rot was studied.

MATERIALS AND METHODS

Fungi isolated from different surveyed sites:

For aeromycological investigation of the 10 different sites, Rotorod and Tilak air samplers were used to find out, both quantitatively and qualitatively, the presence of suspended fungal spores in the working environments. To obtain cultures of the fungal forms, the gravity plate method was also used (Mishra *et al.*, 2003).

Selection of Commodities:

Samples of stored cereals (*Triticum vulgare* L; *Oryzae sativa* L; *Zea mays* L.), pulses (*Cajanus cajanus* L; *Lens esculentum* L., *Vigna mungo* L.), kernels of ground nut (*Arachis hypogea* L.) cuddapah almond (*Buchanania lanzan*), cashewnut (*Anacardium occidentale*) and spices cumin (*Cumin cyminum*) and coriander (*Coriandrum sativum*) were collected from local markets in pre sterilized bags and were studied for their associated mycoflora by standard blotter and agar plate methods (Neergard and Saad, 1962). The plates were incubated at 25±2°C and observed daily for 7 days.

Extraction of essential oils

During the months of September to December the fresh plant parts of Hyptis suaveolens were collected from the left bank of the river Gomti, behind New Hyderabad, Lucknow, India, and used for extracting oil by hydro-distillation for 5h using Clevenger type apparatus (Guenther, 1948). In the Clevenger type apparatus 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 treated with 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 added to the oil collected earlier. The remaining aqueous fraction, free from smell was stored separately. Thus, the hydro-distilled volatile fraction from the leaves of *Hyptis suaveolens* was separated into two fractions, an oil fraction and an aqueous fraction. The essential oil obtained was kept in sealed glass tube at 4ºC until analysis.

GC/MS analysis

The GC/MS of essential oil was analysed on a Shimadzu QP-2000 instrument at 70 eV and 250ºC. GC Column: ULBON HR-1 equivalent to OV-1, fused silica capillary –0.25mm_50M with film thickness-0.25 μ. The GC-MS was operated under the following conditions- the initial temperature was 60ºC for 5 minutes and then heated at the rate of 5ºC per minute to 250ºC. Carrier gas (helium) flow was 2 ml per minute.

The identification of components was based on comparison of their mass spectra with those of Mass Spectrometry Data Centre, the Royal Society of Chemistry. U.K. (Eight Peak Index of Mass Spectra, 3rd Ed. 1983)

Studies on the fungi-toxic properties of the essential oil

Antifungal activity assay: Antifungal activity was tested against *A. flavus, A. niger* and *A. ochraceous* by poisoned food technique and volatile activity assay.

Poisoned food technique: The fungi-toxicity of the oil and the aqueous fraction were evaluated against the test fungi separately by the poisoned food technique of Grover and Moore (1962). PDA (20ml) was poured into sterilized petri dishes and measured amounts of oil were added. The assay plates were rinsed carefully to ensure even distribution of the oil in the medium. For control sets, the medium was supplemented with the same amount of distilled water instead of oil. After the medium solidified, inocula of the test fungi were placed in the center of each assay plate, which were incubated at 28±2ºC. On the tenth day, the growth of the test fungi were recorded and percent inhibition was computed after comparison with the control.

Assay of Volatile Activity: Tests for volatile activity were carried out in 90mm petri plates containing 20 ml of solidified PDA. A 5 mm diameter disc of inoculum of the test species, cut from the periphery of an actively growing culture on PDA plates, was placed on the agar in each petri plate and then petri plates were kept in inverted position. In the lid of each petri plate, a sterilized cotton swab was placed on to which a different concentration of oil were poured. Petri plates were sealed by parafilm to check the release of volatile oil. For each corresponding control an equal

amount of water was poured on the sterilized cotton swab. The petri plates were kept at 28±1°C for 30 days.

Fungitoxicity was expressed in terms of percentage of mycelia growth inhibition and calculated as per formula of Pandey *et al.,* (1982).

$$\text{Percentage of mycelial growth inhibition} = \frac{dc-dt}{dc} \times 100$$

Where dc = Average diameter of fungal colony in control

dt = Average diameter of fungal colony in treatment

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: 100, 200, 500, 600, 700, 800, 900 and 1,000 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.

Effect on dry weight: To determine the effect of essential oil on the dry weight of the test fungi different concentrations of oil in PDB medium were prepared in an Erlenmeyer flask and inoculated with 5 mm discs of test fungi. In the corresponding control an equal amount of distilled water was added. After fifteen days the dry weight of the mycelium was determined

Nature of toxicity: The fungitoxicity (fungistatic/fungicidal) of the essential oil was tested by using the technique of Thomson (1989) and Carta and Arras (1987).

Effect of increased inoculum: The effect of increased inoculum density of the test fungi on the 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 mixing with 9.5 ml liquid Czapek-Dox medium to make the final concentration of 1,000 ppm. Assay discs 5 mm in diameter of *A. flavus, A. niger* and *A. ochraceous* in multiples of two i.e. 2, 4, 6, 8, 10, were inoculated separately in the sets containing 1,000 ppm 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 the toxicity of the oil was determined by storing a stock of the oil in an air tight glass vial at room temperature. The fungal

toxicity of the oil taken from the stock at a regular six months interval was tested at the MIC of the respective fungi by the poisoned food technique, volatile assay and observations on mycelial growth were recorded.

Effect of temperature: Experiments were performed to determine if the antifungal factor of the oil was thermostable or labile. Different glass vials each containing 3 ml oil were stored and subjected to different temperature treatments for three hours in incubators already adjusted to 40, 60, 80 and 100°C. Antifungal activity of oil was also tested after autoclaving it. The toxicity of the treated oil of each set was tested against the test fungi separately at their respective MIC by the usual poisoned food technique and volatile activity assay.

Efficacy of Hyptis suaveolens oil as a preservative of cereals, pulses, nuts and spices against fungal spoilage under storage: The cereals, pulses, nuts and spices were obtained from the market 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 *Hyptis suaveolens* oil as a preservative of the cereals, pulses, nuts and spices against fungal spoilage was determined as follows: 500g of the commodities was placed separately in pre-sterilized plastic containers of 1000 cc capacity. Different amounts of *Hyptis suaveolens* oil 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 commodities. 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 22-40°C and relative humidity between 57 to 87% for a period of six months. Thereafter, fungal infestation of the stored commodities of both the treatments and controls was determined by agar plate and serial dilution plate method.

Comparison of the Hyptis suaveolens oil with selected fungicides: The efficacy of essential oil was compared with that of prevalent fungicides namely: Bavistin, Bayleton, Blitox, Calixin, Captaf, Emisan, Indofil, Karathane, Prochloraz, Ridomil, Thiabendazole and Vitavex. The relative effectiveness was determined in the form of their minimum concentration required to inhibit the test fungi by poisoned food technique.

RESULTS

Ninety-seven fungal species were isolated from the 10 different surveyed sites. Out of ten surveyed sites 48 fungal species were found in a grain shop/storage godown. Three major species groups; *Aspergillus, Penicillium* and *Cladosporium* were identified during the survey. (Table1).

From the different stored commodities; cereals pulses, nuts and spices, nineteen fungi were isolated out of which *A. flavus*, *A. niger* and *A. ochraceous* were the most

common species (Table 2 and Fig. 1). These fungi were selected for further experiments.

The oil was extracted from different plant parts of *Hyptis suaveolens* and the maximum percentage of oil was recovered at the flowering stage from the small leaves and inflorescence (Table 3). Fungal toxicity of the essential oil and the aqueous fraction of *H. suaveolens* against mycelial growth of *A. flavus, A. niger* and *A. ochraceous* were compared (Table 4). The oil obtained from leaves caused 100% inhibition of mycelial growth of all the test fungi (Table 5).

It was found that increasing oil concentrations of *H. suaveolens* significantly inhibited mycelial growth and dry weight of the fungi examined. At 100 ppm concentration of oil the growth was reduced to more than half than that of controls in all the test fungi. At 1000 ppm concentration of oil, growth of all the three fungi was completely inhibited (Table 6)

Volatile activity assay method was found to be more effective than poisoned food technique in checking the growth of test fungi (Plate 1). Vapour toxicity of oil on inhibition of radial growth of the test fungi were assayed and found that this showed complete inhibition at 100 ppm of oil after 10 days but after 30 days the level of inhibition was somewhat reduced (Table 7).

The percent spore germination of test fungi was also assayed against different concentrations of oil and it was observed that at 50 ppm more than 60% inhibition of spore germination was recorded while at 1000 ppm no spores germinated (Table 8).

The minimum inhibitory concentration (MIC) of the oil required to stop mycelial growth of test fungi was 500 ppm for all species (Table 9). At 1000 ppm the oil became fungitoxic (Table 10). Temperature treatment (40-100ºC), autoclaving and storage (up to 24 months) had no adverse effect on the bioactivity and toxicity of the oil (Table 11 and 12). Various physico-chemical properties of oil are shown in Table 13.

The chemical composition of essential oil of the *H. suaveolens* was elucidated employing GC with MS. *H. suaveolens* oil consisted of 24 components with 1,8-Cineole accounting for 44.4% of the total constituents. Out of 24 components 21 were identified with a typical library search match exceeding 90%. Besides 1-8 cineole the other major constituents are β-caryophyllene, β-pinene and camphene (Table 14 and Fig. 2). A typical GC-MS chromatogram of essential oil was shown in Fig. 2, while the results of the quantitative analysis are presented in Table 14. From the observed antifungal activity of the oil it is hypothesised that the major chemical principle of the oil 1,8- cineole plays an important role.

When the fungal population from untreated commodities and treated commodities at 1000 ppm and 2000 ppm of oil respectively were compared (CFU/g) after six months of storage, in treated commodities a drastic decrease in CFU was recorded (Table 15).

When different fungicides were compared with essential oil at 500 ppm on test fungi, the oil showed a greater inhibitory effect than many. Emisan and Prochloraz and Hyptis oil showed 100% inhibition against all the test fungi. In A. niger and A. ochraceous Calixin, Indofil, Ridomil and Thiabendazole also showed 100% inhibition whereas in A. flavus Bavistin and Blitox did so (Fig. 3 and Plate 2).

TABLE 1
Fungi isolated from different surveyed sites

	Fungi	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1.	*Alternaria alternata* (Fr.) Keissler	+	+	+	+	-	+	-	+	+	-
2.	*A. citri EII & Pierce*	-	-	-	-	-	-	-	+	-	-
3.	*A. solani* Sorauer	-	-	+	+	-	-	-	+	-	-
4.	*A. tenuis* Nees	-	-	-	-	+	-	-	-	-	-
5.	Alternaria sp.	-	-	-	-	-	+	-	+	-	-
6.	*Aspergillus* candidus Link	+	+	-	-	+	+	-	+	+	-
7.	A. carbonarius (Bainer) Thom	-	+	-	-	-	-	-	-	+	-
8.	*A. carneus* (Van tieghem) Bloch.	-	+	-	-	-	-	-	-	+	-
9.	*A. clavatus* Desmazieres	-	-	-	-	-	+	-	+	-	-
10.	*A. flavus Link Fr.*	+	+	+	+	+	+	+	+	+	-
11.	*A. fumigatus Fres.*	+	+	-	+	-	+	+	+	+	-
12.	*A. glaucus Link*	-	-	+	+	-	+	-	+	-	-
13.	*A. japonicus Saito*	+	+	-	-	+	-	-	-	+	-
14.	*A. melleus* Yukawa*	-	+	-	-	+	-	-	-	+	-
15.	*A. nidulans* ukawa*	+	+	-	-	+	+	-	-	+	-
16.	*A. niger* van Tiegh.*	+	+	+	+	+	+	-	+	+	-
17.	*A. niveus Bloch.*	-	+	-	-	-	-	-	-	+	-
18.	*A. ochraceous Wilhelm*	+	-	-	-	-	+	-	-	-	-
19.	*A. oryzae* (Ahlb.) Cohn	-	-	+	-	-	+	-	-	-	-
20.	*A. parasiticus Speare*	-	-	+	+	-	+	-	-	-	+
21.	*A. restrictus G. Smith*	-	-	-	-	-	+	-	+	-	-
22.	*A. sydowi (Bainier& Sartory) Thom and Church*	+	+	-	-	-	+	-	-	+	-
23.	*A. tammarii Kita*	-	+	-	-	+	+	-	-	+	-
24.	*A. terreus Thom*	-	+	+	-	+	+	-	+	+	-
25.	*A. ustus (Bain) Thom & Chrch*	-	+	-	-	-	-	-	-	+	-
26.	*A. versicolor (Vuil) Tiraboschi*	+	+	-	-	+	+	-	-	+	-
27.	*A. wentii Wehmer.*	+	-	-	-	-	-	-	+	-	-
28.	*Candida albicans (Robin)*	-	-	-	-	-	+	-	-	-	-
29.	*Chaetomium globosum Kunze fr.*	-	-	-	-	-	+	-	-	-	-
30.	*Cladosporium cladosporiodes (fresen.)*	+	-	-	-	+	+	-	-	+	-
31.	*C. epiphyllum Persoon*	-	+	-	-	-	-	-	-	+	-
32.	*C. herbarum* (Pers.) Link ex. S.F.Gray*	-	+	-	+	-	-	-	+	+	-
33.	*C. lignicolum Corda*	-	+	-	-	-	-	-	-	+	-
34.	*C. macrocladium*	+	-	-	-	-	-	-	-	-	-

	Fungi	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
35.	*C.psoraleae M.B.Ellis*	-	-	-	-	-	-	-	+	-	-
36.	*Circinella simplex van Tiegh.*	-	-	-	-	-	+	-	-	-	-
37.	*C. umbellate van Tiegh.&Le Mom.*	-	-	-	-	-	+	-	-	-	-
38.	*Cunninghamella echinulata Thaxt.*	-	-	-	-	-	+	-	-	-	-
39.	*C. lunata (Wakker)Boedijn*	-	-	-	+	+	-	-	+	-	-
40.	*C. lunata Lima & Vas.) Ellis*	-	+	-	-	-	-	-	-	+	-
41.	*C. pallescens Boedinj*	+	-	-	-	-	+	-	+	-	-
42.	*C. tetramera (Mc Kinney)*	-	+	-	-	-	-	-	-	+	-
43.	*C. brachyspora Boedijn*	-	-	-	-	-	+	-	-	-	-
44.	*Drechslera hawaiiensis (Bugnicourt) Subram. &Jain ex Ellis*	-	+	-	-	-	+	-	-	+	-
45.	*Emercella nidulans var. lata (Thom &Raper)Subram.*	-	+	-	-	-	-	-	-	-	-
46.	*Fusarium annifosporem*	-	-	-	-	-	-	-	+	-	-
47.	*F. equiseti (Corda)Sacc.*	-	-	-	-	-	-	-	+	-	-
48.	*F.moniliformis Sheldon*	+	-	-	-	-	-	-	+	-	-
49.	*F. oxysporum Schlech.ExFr.*	-	+	-	-	-	+	-	+	+	-
50.	*F. roseum Link*	-	+	-	-	-	-	-	-	+	-
51.	*F. trichothecoides Wollenweber*	-	+	-	-	-	-	-	-	+	-
52.	*Geotrichum candidum*	+	-	-	-	-	+	-	+	-	-
53.	*Helminthosporium harwaiiense Bugnicout*	-	+	-	-	-	-	-	-	-	-
54.	*H. oryzae Breda de Haam*	-	-	+	-	-	-	-	-	-	-
55.	*H. sativum Pannel, King& Bakke*	-	-	+	+	-	-	-	-	-	-
56.	*Humicola grisea traen*	-	-	-	-	-	+	-	-	-	-
57.	*Monilia sitophila (Montagne)Sacc.*	-	+	-	-	-	+	-	-	-	-
58.	*Monodictys levis (Wilsire) Hughes*	-	-	-	-	-	+	-	-	-	-
59.	*Mucor hiemalis Wilshire)*	-	+	-	-	-	-	-	-	+	-
60.	*M. varians Povah*	+	-	-	-	-	-	-	-	-	-
61.	*Neurospora sitophila*	+	-	-	-	-	-	-	-	-	-
62.	*Nigrospora sphaerica (Sacc.) Mason*	-	+	-	-	-	+	-	-	+	-
63.	*Nigrospora sp.*	+	-	-	-	+	+	-	+	-	-
64.	*Oidiodendron, sp.*	+	-	-	-	-	-	-	-	-	-
65.	*Oidium sp.*	+	-	-	-	-	-	-	-	-	-
66.	*Paecilomyces fusisporus Sakena*	-	-	-	-	-	-	-	-	+	-
67.	*P. varioti Bain*	+	+	-	-	-	+	-	+	+	-
68.	*Penicillium chrysogenum Thom*	+	-	-	-	-	-	-	-	-	-
69.	*P. citrinum Thom*	+	+	-	-	-	+	-	-	+	-
70.	*P. cyclopium Westilin*	+	-	-	-	-	-	-	-	-	-
71.	*P. expansum (Link) Thom*	+	-	-	-	-	+	-	-	-	-
72.	*P. fellutanum Biourge*	-	-	-	-	-	+	-	-	-	-
73.	*P. frequentans Westling*	-	-	-	-	-	+	-	-	-	-
74.	*P. funiculosum Thom.*	-	-	-	-	-	+	-	-	+	-
75.	*P. lanosum Westling*	-	-	-	-	-	-	-	+	-	-
76.	*P. lapidosum Raper&Fennell*	-	-	-	-	-	-	-	+	-	-
77.	*P. miczynskii Zaleski*	-	-	-	+	-	-	-	-	-	-
78.	*P. oxalicum Currie&thom*	+	-	-	-	-	+	-	-	-	-

	Fungi	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
79.	*P. porulum*	-	-	-	-	-	-	-	+	-	-
80.	*P. puberulum Bainier*	-	-	-	-	-	-	-	+	-	-
81.	*P. purpurogenum stoll.*	-	-	-	-	-	+	-	-	-	-
82.	*P. thomii Maire*	-	-	-	-	-	+	-	-	-	-
83.	*P. vinaceum Gilman&abbott*	-	-	-	-	-	+	-	-	-	-
84.	*Pestalotia pezizoides de Not*	-	-	-	-	-	-	-	-	+	-
85.	*P. herbarum Westd.*	-	-	-	-	-	-	-	-	+	-
86.	*Phytophthora infestans (Mont.). de Bary*	-	-	-	-	-	-	-	+	-	-
87.	*Rhizopus nigricans Ehrenb.*	-	-	+	+	-	-	-	-	-	-
88.	*R. oryzae Went&Gerlings*	-	-	+	-	-	-	-	-	-	-
89.	*R. stolonifer (Ehrenberg:Fr.) Vuillemin*	+	-	-	-	-	+	-	-	-	-
90.	*Scolecabasidium constrichum Abbott*	-	-	-	-	-	+	-	-	-	-
91.	*Stemphylium sp.*	-	+	-	-	-	-	-	-	+	-
92.	*Sterile mycelium*	-	-	-	-	-	-	-	-	+	-
93.	*Syncephalastrum racemosum (Cohn) Schroeter*	-	+	-	-	-	+	-	-	+	-
94.	*Torula ellisi Yadav &Lal*	-	-	-	-	-	-	-	+	+	-
95.	*Trichoderma harzianum*	-	-	-	-	-	+	-	-	-	-
96.	*T. viride Pers.*	+	-	-	-	-	+	-	-	-	-
97.	*Trichothecium roseum (Person)Link ex S.F. Gray*	+	+	-	-	-	-	-	-	+	-

+ = Present, - = Absent

1.	Bakery	6.	Grain shops/storage godown
2.	Flour mill	7.	Oil mill
3.	Food storage places	8.	Poultry farm
4.	Fruit shop/market	9.	Saw mill
5.	Ginnery	10.	Scientific laboratory

TABLE 2

Fungi isolated from different stored commodities.

Fungi isolated	Cereals	Pulses	Nuts	Spices
1. *Absidia spinosa*	-	-	-	+
2. *Aspergillus tenuissina*	+	-	-	+
3. *A. fumigatus*	-	-	+	-
4. *A. fischeri*	-	-	-	+
5. *A. flavus*	+	+	+	+
6. *A. niger*	+	+	+	+
7. *A. ochraceous*	+	+	+	+
8. *A. nidulans*	+	+	-	-
9. *Cladosporium cladosporiodes*	+	-	-	+
10. *C. herbarum*	-	+	-	-
11. *Emericella quadrilineata*	+	+	+	+
12. *Penicillium expasusm*	-	-	+	+
13. *P. citrinum*	-	-	+	+
14. *P. oxalicum*	-	-	+	+
15. *P. funiculosum*	-	-	+	-
16. *Rhizopus arrhizus*	+	+	+	-
17. *R. nigricans*	-	+	+	+
18. *Syncephalastrum racemosum*	+	+	+	-
19. *Yeast-like fungi*	-	-	+	-

TABLE 3

Percent recovery of the oil from various parts of the *Hyptis suaveolens* on fresh weight basis at different stages

Plant parts		% Oil before flowering	% Oil at flowering
Roots		0.004	0.005
Stem		0.008	0.009
Leaf	Large	0.14	0.24
	Small	0.23	0.30
Small leaves+ inflorescence		-	0.46

TABLE 4

Fungi-toxicity of the essential oil and the aqueous fraction of *Hyptis suaveolens* against mycelial growth of *A. flavus, A. niger* and *A. ochraceous*

Plant parts	Percent inhibition by the oil		
	A. flavus	*A. niger*	*A. ochraceous*
Essential Oil	100	100	100
Aqueous fraction	7.6	5.2	4.5

TABLE 5

Fungi-toxicity of *Hyptis suaveolens* oil in different parts against mycelial growth of *A. flavus*, *A. niger* and *A. ochraceous*

Plant parts	Percent inhibition of the mycelial growth		
	A. flavus	*A. niger*	*A. ochraceous*
Roots	3.2	5.5	6.5
Stem	25.5	29.2	20.0
Leaf	100.0	100	100
Inflorescence	91.8	90.2	89.5

TABLE 6

Effect of different concentrations of *Hyptis suaveolens* oil in inhibition of Radial growth, Dry weight and Sporulation of fungus; *A. niger, A. flavus and A. ochraceous* after 10 days of incubation at 28±1ºC

Conc of oil (ppm)	% Inhibition			Dry Weight (mg)			Sporulation (Log_{10} spore cm^{-2})		
	A. flavus	*A. niger*	*A. ochraceous*	*A. flavus*	*A. niger*	*A. ochraceous*	*A. flavus*	*A. niger*	*A. ochraceou*
Control	-	-	-	729.0	720	626.0	7.8	6.4	6.9
25	2.19	0.0	0.0	685.0	680.0	600.0	7.8	6.4	6.9
50	8.24	6.04	4.04	650.0	645.0	596.0	7.6	6.4	6.9
100	55.49	53.29	51.00	620.0	615.0	500.0	7.6	6.5	7.1
200	70.87	68.68	65.38	500.0	495.0	495.0	7.6	6.6	7.2
500	82.41	80.21	78.23	200.0	195.0	385.0	7.6	6.6	7.2
1000	100	100	100	0.0	0.0	0.0	-	-	-
2000	100	100	100	0.0	0.0	0.0	-	-	-

TABLE 7

Vapour toxicity of *Hyptis suaveolens* oil on inhibition of radial growth of *A. niger, A. flavus and A. ochraceous* after 10 days and 30 days of incubation at 28±1ºC.

Concentration of oil (ppm)	Percent Inhibition of radial growth (mm)					
	A. flavus		*A. niger*		*A. ochraceous*	
	10 days	30 days	10 days	30 days	10 days	30 days
25	84.0	56.0	81.0	11.0	78.0	15.0
50	95.0	66.0	89.0	38.0	85.0	40.0
100	100.0	73.3	100.0	38.3	100.0	50.3
200	100.0	80.0	100.0	50.0	100.0	56.5
500	100.0	94.44	100.0	87.7	100.0	80.7
1000	100.0	100.0	100.0	100.0	100.0	100.0

TABLE 8

Effect of different concentrations of *Hyptis suaveolens* oil on inhibition of spore germination of fungus; *A. flavus, A. niger, and A. ochraceous* after 10 days of incubation at 28±1ºC

S. No.	Concentration of Oil (ppm)	Spore germination (%)		
		A. flavus	*A. niger*	*A. ochraceous*
1.	Control	100.00	100.00	100.00
2.	25	72.00	72.28	72.00
3.	50	32.00	38.19	32.00
4.	100	12.00	10.00	12.00
5.	200	5.00	5.20	5.00
6.	500	4.00	4.50	4.00
7.	1000	0.00	0.00	0.00
8.	2000	0.00	0.00	0.00

TABLE 9

Effect of MIC of *Hyptis suaveolens* against mycelial growth of *A. flavus, A. niger and A. ochraceous*

Concentration (ppm)	Percent inhibition of radial growth		
	A. flavus	*A. niger*	*A. ochraceous*
1000	100	100	100
900	100	100	100
800	100	100	100
700	100	100	100
600	100	100	100
500	100	100	100
200	90	85	89
100	85	79	83

TABLE 10

Nature of toxicity of oil against *A. flavus*, *A. niger* and *A. ochraceous*

Conc. (ppm)	Percent inhibition of the fungus					
	A. flavus		*A. niger*		*A. ochraceous*	
	Treated set	Re-inoculated	Treated set	Re-inoculated	Treated set	Re-inoculated
500	100	60	100	76	100	78
600	100	74	100	80	100	80
700	100	80	100	86	100	85
800	100	87	100	89	100	90
900	100	95	100	96	100	94
1000	100	100	100	100	100	100
1100	100	100	100	100	100	100
1200	100	100	100	100	100	100
1300	100	100	100	100	100	100
1400	100	100	100	100	100	100
1500	100	100	100	100	100	100
2000	100	100	100	100	100	100

TABLE 11

Fungitoxicity of oil treated at different temperature

Temperature (°C)	Percent inhibition of mycelial growth		
	A. flavus	*A. niger*	*A. ochraceous*
40	100	100	100
60	100	100	100
80	100	100	100
100	100	100	100
121	100	100	100

TABLE 12

Fungi-toxicity of oil stored for different periods

Storage period (months)	Percent inhibition of mycelial growth		
	A. flavus	*A. niger*	*A. ochraceous*
6	100	100	100
12	100	100	100
18	100	100	100
24	100	100	100

TABLE 13

Physico-chemical properties of the oil of *Hyptis suaveolens*

Parameter	Values
Colour	Light yellow
pH	2.8
Solubility	Soluble in acetone, solvent ether, ethanol, methanol and carbon tetra chloride
Ester value	48.1132
Saponification value	48.1132
Carbonyl percentage	13.370
Acid value	0.2
Phenolic content	Nil

TABLE 14
GC/MS of *Hyptis suaveolens* oil

Peak no.	R. Time	Area	Height	Retention area	Components	% Total
1.	9.83	45119	6952	1.9	α-Thujene	0.8
2.	12.63	60296	15027	2.5	3-Carene	1.1
3.	12.93	13539	44117	5.7	α-Pinene	2.5
4.	14.50	641502	96884	26.4	β-Pinene	11.7
5.	14.66	292316	79311	12.0	β-Myrcene	5.3
6.	16.83	2430888	246778	100.0	**1,8-Cineole**	**44.4**
7.	17.53	94954	36129	3.9	Linalool	1.7
8.	18.30	61066	23834	2.5	Camphor	1.1
9.	18.73	311860	63197	12.8	Camphene	5.7
10.	21.73	119121	36083	4.9	Terpinen-4-ol	2.2
11.	27.46	14317	6448	0.6	α-Cubebene	0.3
12.	28.40	86052	30737	3.5	β-Gurjunene	1.6
13.	28.70	64525	19713	2.7	β-Elemene	1.2
14.	29.86	547191	96524	22.5	β-Caryophyllene	10.0
15.	30.00	151019	48331	6.2	α-Farnesene	2.8
16.	30.66	50841	22222	2.1	β-Selinene	0.9
17.	31.33	24167	10456	1.0	Longifolene	0.4
18.	31.53	26916	11984	1.1	γ-Humulene	0.5
19.	31.76	30521	10517	1.3	α-Copaene	0.6
20.	32.26	16828	7597	0.7	γ-Codinene	0.3
21.	36.66	93149	22293	3.8	α-Bergamotene	1.7
22.	44.20	87136	28212	3.6	Unidentified	1.6
23.	44.86	42600	14659	1.8	Unidentified	0.8
24.	45.80	39732	11930	1.6	Unidentified	0.7

TABLE 15

Fungi isolated from treated (1000 ppm and 2000 ppm oil of *Hyptis suaveolens*) and untreated grains after six months of storage

Commodities	Appearance of fungi (log$_{10}$ CFU/g)		
	Untreated	Treated stored seed grains	
		T$_1$ (1,000 ppm)	T$_2$ (2,000 ppm)
Cereals	2.5_10^6	1.0_10^2	1.5_101
Pulses	8.0_10^6	1.0_10^2	1.2_10^1
Nuts	3.0_10^5	1.0_10^2	Not detected
Spices	2.0_10^4	1.0_10^1	Not detected

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, A. niger* and *A. ochraceous* were selected as the test organisms since they were found to be the most common bio-deteriogens during the survey of 10 selected sites.

The essential oil from the leaves of *H. suaveolens* has been reported earlier by Saxena et al. (1978). Earlier the antifungal and antimicrobial activity of H. suaveolens oil was reported by Iwu et al. (1990) and Pandey et al. (1982). Oil was more effective in the vapour phase but there was no report of its use as a fungitoxic fumigant. Plants parts (leaves) of *H. suaveolens* exhibited the highest toxicity against the test fungi and therefore these were selected for further detailed investigation. Since the fungitoxic fraction obtained from the leaves of *H. suaveolens* exhibited thermostability at higher temperatures without altering the toxicity, extraction by hydro-distillation using Clevenger type apparatus was employed (Sharma, 2001). The fungicidal activity is attributed to 1,8- cineole found in the concentration 44.4% of oils tested. The GC-MS analysis of essential oil obtained from leaves of H. suaveolens had 44.4% of 1, 8-cineole. It appears that the fungicidal / fungitoxic nature of the oil is due to this compound. Interestingly it was also observed that the percentage of this compound in the essential oil was higher from the plants growing in Lucknow when compared with those from Bangalore (31.51%) and Hyderabad (35.30%) (Mallavarapu *et al*., 1993), or from Australia (32%) (Peerzada, 1997). This is probably due to local geographical differences.

The determination of the MIC of oil is necessary for prescribing its appropriate dose. Clearly, unnecessarily high doses of oil 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. It is noteworthy that in some instances, the oil of a plant investigated by different workers has shown variation in the MIC (Singh and Handique, 1997 and Pandey *et al.,* 1982). Such variations may be due to the use of different test fungi or different techniques adopted. However, in the present work the MIC of the oil of H. suaveolens was 500 ppm against A. flavus and A. niger and A. ochraceous.

A fungitoxicant may act as a fungistat or a fungicide inhibiting the growth of fungus temporarily or permanently respectively. In this study the oil of H. suaveolens exhibited a fungistatic nature at its MIC against 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, *H. suaveolens* 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 *H. suaveolens* was found to retain its fungitoxicity for up to two years, which was the maximum period for which it was tested, thus showing that this 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 up to 100°C and even after autoclaving. Comparison of oil with different fungicides against the test fungi was showed that oil was at least as effective as several fungicides. The risk of fungal resistance against fungicides may be reduced by the use of oil.

Based upon the present study it could be concluded that volatile oil from *H. suaveolens* and its major constituents 1,8-cineole possess fungitoxic activity worth exploiting for the management of spoilage of stored commodities. In pilot experiments it can be concluded that this oil can serve as a natural fungicide or possibly as a template for the synthesis of novel fungicides.

The findings suggest that the *Hyptis suaveolens* oil can be exploited as a potent and ecofriendly fungitoxic fumigant against storage mycoflora because of its high yield, strong and durable fungitoxicity and thermostability.

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