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Hyptis Suaveolens: A Potential Toxicant for the Management of Stored Grain Pests

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ABSTRACT
The plant Hyptis is a potent medicinal herb and a well known medicinal plant in herbal world. The efficacy of *Hyptis suaveolens* (L.) Poit oil as a preservative of the cereals, pulses, against fungal spoilage and has been widely used as a stimulant, carminative, lactagogue and to treat colic disorders. We found that eighteen fungal species were found to be associated with the stored cereals, pulses, Because of their wide distribution, high incidence as well as enormous potential to produce mycotoxins, *Aspergillus flavus* and *A. niger* were selected as test fungi for antifungal screening of *H. suaveolens* oil. *H. suaveolens* exhibited the strongest bioactivity. Minimum inhibitory concentrations (MIC) of the volatile antifungal oil fraction against *A. flavus and A. niger* were found to be 400 ppm to 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 40 days, at its exposure to 100ºC, and on autoclaving. Results indicated a potent antimicrobial activity of *Hyptis suaveolens.*

Keywords: *Hyptis suaveolens, A. flavus*, *A. niger,* Antimicrobial activity, Chemical composition.

1. INTRODUCTION

The plant, *H. suaveolens* commonly known as "Wilayati tulsi" belongs to the family Lamiaceae and is an ethno botanically important medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases (Asprey., 2004). It is approximately 2 meters high, having branches and long, white piliferous stems. Its flowers are purple or white, its leaves oval, wrinkled and pointed (Mohamed *et al.,* 2016).

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, Ali 2017). 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 196 higher plants have proved their usefulness in controlling biodeterioration and therefore are considered to have a bright future (Sharma, 1998). The efficacy of *Hyptis suaveolens* oil as a preservative of the cereals, pulses, nuts and spices against fungal spoilage and has been widely used as a stimulant, carminative, lactagogue and to treat colic disorders (Asprey G, F., 2004, Mandal *et al.,* 2007, (Feitosa-Alcantara *et al.,* 2017).

A wide range of chemical compounds including terpenoids, alkaloids, acidic polysaccharide and 33 constituents were identified in the oil of *Hyptis suaveolens* isolated from its leaves. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities (Asprey G, F., 2004; Ali, 2017). *Hyptis suaveolens* was targeted on the basis of folkloric uses which suggest its toxicity to microbes, coupled with its importance as food to humans (Shenoy *et al.,* 2009). Soluble solvent extracts of the plant were tested for phytochemicals which revealed the existence of alkaloids, flavonols, flavones, flavonones, terpenoids, tannins, aldehydes and ketones and the absence of steroids, saponins and anthraquinones (Verma 2011, Feitosa-Alcantara *et al.,* 2017). Wound Healing Activity of *Hyptis suaveolens* is a traditional pubescent annual herb found throughout India. A considerable number of studies have emphasized the research and development of herbal substances for controlling mosquitoes (Bielakovic et al. 2007, Fabricant and Farnsworth 2001, Okigbo, 2010). These botanical extracts could also be used along with other insecticides under integrated vector control. Studies carried out so far have shown that some photochemicals acts as general toxicant (insecticide/ Larvicide) both against adult as well as larval stage of mosquito while others interfere with reproduction (Okigbo, 2010, Ali 2017).

In this communication, the effect of a volatile obtained from *Hyptis suaveolens* on two different species of *Aspergillus spp.* was studied.

2. METHOD AND MATERIAL

Selection of Commodities

Samples of stored cereals (*Triticum vulgare* L; *Oryzae sativa* L; *Zea mays* L.), and Pulses (*Cajanus cajanus* L; *Lens esculentum* L., *Vigna mungo* L.), were collected from local market 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 27±2ºC and observed daily for 7 days.

Figure 1
Plant of *Hyptis suaveolens*

Extraction of essential oils

During the months of September to December the fresh plant parts of *Hyptis suaveolens* (L.) Poit. were collected from the left bank of 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 made anhydrous by treating it 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 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 *Hyptis suaveolens* ultimately resulted into stocks of two fractions an oil fraction and an aqueous fraction. The obtained essential oil was kept in sealed glass tube at 4°C until analysis.

GC/MS analysis

The GC/MS of essential oil was analysed on a Shimadzu QP-2000 instrument at 70 eV and 250°C. GC Column: ULBON HR-1 equivalent to OV-1, fused silica capillary –0.25mm×50M with film thickness-0.25 µ. The GC-Mass was operated under the following conditions- 60-5-5-250 means that the initial temperature was 60°C for 5 minutes and then heated at the rate of 5°C per minute to 250°C. Carrier gas (helium) flow was 2 ml per minute.

The identification of component 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*, and A. *niger* by poisoned food technique.

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 amount of oil was 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 27±2°C. On the tenth day, the growth of the test fungi were recorded and percent inhibition was computed after comparison with the control.

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

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

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 was prepared in Erlenmeyer flask and inoculated with 5mm disc of test fungi. In the corresponding control equal amount of distilled water was added. After fifteen days dry weight of 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 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 1,000 ppm. Assay discs 5 mm in diameter of *A. flavus,* and *A. niger* 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 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 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 thermo stable 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, 100°C. Antifungal activity of oil was also tested after autoclaving it. The fungi toxicity 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 and volatile activity assay.

Efficacy of Hyptis suaveolens oil as a preservative of cereals, pulses, against fungal spoilage under storage: The cereals and pulses 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 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.

3. RESULTS

From the different stored commodities; cereals, pulses, eighteen fungi were isolated out of which *A. flavus, A. niger* were the most common species associated with all the stored commodities (Table 1). So these fungi were selected for further experiments. The oil was extracted from different plant parts of *Hyptis suaveolens* and maximum percentage of oil was recovered at flowering stage from the small leaves and inflorescence (Table 2). Fungi-toxicity of essential oil and the aqueous fraction of *H. suaveolens* against mycelial growth of *A. flavus,* and *A. niger* were compared and it was found absolute toxicity occurred by the essential oil. The oil obtained from leaves showed 100% inhibition of mycelial growth of all the test fungus (Table 3).

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 control in all the test fungus. The concentration of 1000 ppm of oil was found to completely inhibit growth of all the three fungi (Table 4). Volatile activity assay method was found to be more effective than poisoned food technique in checking the growth of test fungi. 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 percent inhibition was found somewhat reduced (Table 5). The percent spore germination of test fungi was also assayed against different concentration of oil and it was observed that at 50 ppm more than 60% inhibition of spore germination was recorded while at 1000 ppm no spore germination was found (Table 6). The minimum inhibitory concentration (MIC) of the oil required to inhibit the mycelial growth of test fungi was 500 ppm for all the test fungus (Table 7). Nature of toxicity of the oil was studied and it was observed that at 500 ppm the oil was fungistatic and at 1000 ppm oil showed fungitoxic nature (Table 8). Temperature treatment (40-100ºC), autoclaving and storage (up to 24 months) had no adverse effect on the bioactivity and toxicity of the oil (Table 9 and 10).

Table 1 Fungi isolated from different stored commodities

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

Table 2 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
	Large	0.14	0.24
Leaf	Small	0.23	0.30
Small leaves+ inflorescence		-	0.46

Table 3 Fungi-toxicity of the essential oil and the aqueous fraction of *Hyptis suaveolens* against mycelial growth of A. flavus, and A. niger

Plant parts	Percent inhibition by the oil	
	A. flavus	***A. niger***
Essential Oil	100	100
Aqueous fraction	7.4	5.1

Table 4 Effect of different concentrations of *Hyptis suaveolens* oil in inhibition of Radial growth, Dry weight and Sporulation of fungus; *A. niger* and, *A. flavus* after 10 days of incubation at 27±1ºC

Conc of oil (ppm)	*% Inhibition*		*Dry Weight (mg)*		Sporulation (*Log_{10} spore cm^{-2}*)	
	A. flavus	***A. niger***	***A. flavus***	***A. niger***	***A. flavus***	***A. niger***
Control	-	-	730.0	710	7.7	6.2
25	2.20	0.0	675.0	670.0	7.2	6.2
50	7.24	6.32	640.0	635.0	7.4	6.2
100	54.39	52.29	610.0	600.0	7.5	6.4
200	60.87	58.54	453.0	400.0	7.4	6.5
500	85.42	80.21	195.0	180.0	7.4	6.5
1000	100	100	0.0	0.0	-	-
2000	100	100	0.0	0.0	-	-

Table 5 Vapour toxicity of *Hyptis suaveolens* oil on inhibition of radial growth of *A. niger* and *A. flavus* after 10 days and 30 days of incubation at 27±2ºC

Concentration of oil (ppm)	***Percent Inhibition of radial growth (mm)***			
	A. flavus		**A. niger**	
	10 days	**30 days**	**10 days**	**30 days**
25	82.0	54.0	79.0	09.0
50	93.0	64.0	86.0	36.0
100	100.0	71.3	100.0	38.3
200	100.0	78.0	100.0	45.0
500	100.0	91.14	100.0	85.7
1000	100.0	100.0	100.0	100.0

Table 6 Effect of different concentrations of *Hyptis suaveolens* oil on inhibition of spore germination of fungus; *A. flavus*, and *A. niger*, after 10 days of incubation at 27±2ºC

Concentration of Oil (ppm)	Spore germination (%)	
	A. flavus	***A. niger***
Control	100.00	100.00
25	70.00	70.18
50	30.00	36.15
100	10.00	08.00
200	3.00	4.18
500	2.00	2.50

1000	0.00	0.00
2000	0.00	0.00

Table 7 Effect of MIC of *Hyptis suaveolens* against mycelial growth of *A. flavus* and *A. niger*

Concentration (ppm)	Percent inhibition of radial growth	
	A. flavus	***A. niger***
1000	100	100
900	100	100
800	100	100
700	100	100
600	100	100
500	100	100
200	86	81
100	81	68

Table 8 Nature of toxicity of oil against *A. flavus*, and *A. niger*

Conc. (ppm)	Percent inhibition of the fungus			
	A. flavus		***A. niger***	
	Treated set	**Re-inoculated**	**Treated set**	**Re-inoculated**
500	100	58	100	74
600	100	72	100	78
700	100	78	100	84
800	100	85	100	87
900	100	91	100	95
1000	100	100	100	100
1100	100	100	100	100
1200	100	100	100	100
1300	100	100	100	100
1400	100	100	100	100
1500	100	100	100	100
2000	100	100	100	100

Table 9 Fungitoxicity of oil treated at different temperature

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

Table 10 Fungi-toxicity of oil stored for different periods

Storage period (months)	Percent inhibition of mycelial growth	
	A. flavus	***A. niger***
10	*100*	*100*
20	*100*	*100*
30	*100*	*100*
40	*100*	*100*

Table 11 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 12 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 13 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	2.3×10^{6}	1.0×10^{2}	1.54×101
Pulses	7.0×10^{6}	1.0×10^{2}	1.1×10^{1}

Various physico-chemical properties of oil are shown in (Table 11).The chemical composition of essential oil of the *H. suaveolens* was elucidated employing GC with MS. *H. suaveolens* oil consisted of 24 compounds with 1-8 cineole accounting for 44.4% of the total constituents. Out of 24 constituents 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 12). From the observed antifungal activity of oil it emerge that the major chemical principle of the oil 1-8 cineole play an important role in its antifungal activity. Fungal population from untreated commodities and treated commodities at 1000 ppm and 2000 ppm of oil respectively were recorded (CFU/g) after six months of storage. As compared to untreated in all the commodities, in treated commodities drastic decrease in CFU was recorded (Table 13).

4. DISCUSSION

The essential oil from the leaves of *H. suaveolens* has been reported earlier by Saxena *et al.* (1978). Earlier the antifungal and antimicrobial activities of H. suaveolens oil have been reported by Iwu *et al.*, (1990) and Pandey *et al.*, (1982). Oil was more effective as vapour phase and there was no report of its use as a fungitoxic fumigant. Plants parts (leaves) of *H. suaveolens* exhibited absolute toxicity against the test fungi and therefore, was selected for further detailed investigation. Since the fungitoxic fraction obtained from the leaves of *H. suaveolens* exhibited thermostability at higher temperature without altering the toxicity, therefore 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 was higher from the plants growing in Lucknow when compared with those from Bangalore (31.51%) and Hyderabad (35.30%) by Mallavarapu *et al.* (1993) and Australia (32%) by Peerzada (1997). This may be due to geographical differences.

So our investigation that 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 they were found to be the most common bio-deteriogens.

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, oil of a plant investigated by different workers has shown variation in MIC (Singh and Handique, 1997 and Pandey *et al.*, 1982, Ali 2017). 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 *H. suaveolens* was 500 ppm against *A. flavus* and *A. niger* respectively.

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 possess 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. 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. Comparison of oil with different fungicides against the test fungi was showed that oil was equally or more effective as fungicides. The risk of fungal resistance against fungicides can be eliminated 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 bio management of diseases of stored commodities. In pilot experiments it can be concluded that this oil can serve as natural fungicides or at least template for the synthesis of noble 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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REFERENCE

1. Ali H.K., Ahmed, M. J. and Ahmed, S, H, 2017. Studying Efficiency Inhibition of Some Medicinal Plant Extracts against Some Fungal Int.J.Curr.Microbiol.App.Sci 6(1): 108-116
2. Asprey G.F., 2004. MedIcinal Plants of Jamaica, *West Indian Medical Journal*, 2(4)
3. Bielakovic G., Nikolova D., Gluud L.L. And Simonetti. R.G., Gluud C., 2007. Mortality in randomised trials of antioxidant supplements for primary and secondary prevention: systematic review and meta analysis, *JAMA* , 297(8), 842-857
4. Carta, C., and Arras, G. (1987) Azione inhibitice in vitro di olii essenziali net confronti di aleuni pathogeni di plante ornamentali. *La Difesa Delle Plante* 10, 195-202.
5. Fabricant D.S. and Farnsworth N.R., 2001. The value of plants used in traditional medicine for drug discovery, *Environ. Health Perspect*., 109(1), 69–75
6. Feitosa-Alcantara R., Bacci L , Blank A F., Alves Péricles B. , Silva I. M. d. A., Soares C. A. , Sampaio T S., Nogueira and Arrigoni-Blankd F, 2017. Essential Oils of *Hyptis pectinata* Chemotypes: Isolation, Binary Mixtures and Acute Toxicity on Leaf-Cutting Ants. Molecules, 22, 621; doi: 10.3390/molecules22040621
7. Grover, R.K. and Moore, J.D. 1962. Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology* 52, 876-880.
8. Guenther, E. 1948. The Essential Oils Vol.1 pp 774, D. Van Nostrand, Princeton York.
9. Iwu, M.M., Ezeugwa, C.O., Okunji, C.O., Sanson, D.R. and Tempesta, M.M. 1990 Antimicrobial activity and terpenoids of essential oil of *H. suaveolens*. *Journal of Crude Drug Resistance* 28, 73-76.

10. Mallavarapu, G.R., Ramesh, S, Kaul, P.N., Bhattacharya, A.K and Rao, R.R. 1993. The essential oil of *Hyptis suaveolens* (L.) Poit. *Journal of Essential Oil Research* 5, 321- 323.
11. Mandal S.M., Mondal K.C., Dey S. and Patil B.R., 2007. Antimicrobial activity of the leaf extracts of Hyptis suaveolens (L.) poit, *Indian Journal of Pharmaceutical Sciences,* 69(4), 568-569
12. Misra, S.B. 1975. Antifungal properties of leaf extract of *Ranunculus scleratus* L. *Experimentia* 34, 1442.
13. Mohamed D , Seydou T , Ouedraogo S. Leonard , Brahima C , Antoine BBI B , Hilaire K. T., and c Daouda K. 2016. Assessment of the efficiency of essential oils of *Ocimum gratissimum* L. (Lamiaceae) and *Hyptis suaveolens* (L.) Poit. (Lamiaceae) in the biological control against sclerotinia of *Abelmoschus esculentus* (L.) Moench (Malvaceae) or okra (cultivars Volta and Hiré). *Int. J. Agri. Agri. R*. 8, 3, p. 38-51
14. Neergard, P. and Saad, A. 1962. Seed health testing of rice. *Indian Phytopathology*, 15, 85-87.
15. Okigbo R.N., Okeke J.J. and Madu N.C., 2010. Larvicidal effects of Azadirachta indica, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae, *Journal of Agricultural Technology,* 6(4), 703-719
16. Pandey, D.K., Tripathi, N.N., Tripathi, R.D., and Dixit, S.N. 1982. Fungitoxic and phytotoxic properties of the essential oil of *H. suaveolens*. Zeitschrift fur Pflanzenkrankheit und. Pflanzenschutz 89, 344-349.
17. Peerzada, N.1997. Chemical composition of the essential oil of *Hyptis suaveolens*. *Molecules* 2,165-168.
18. Saxena, H. O., Dutta, D.K. and Brahman, M. 1978. A survey of Vetiver, Lemongrass and *Hyptis suaveolens* in Orissa for their essential oils. *Indian Perfumer* 22, 298-299.
19. Sharma, N. 1998. Control of post harvest diseases with natural plant products. In: Post Harvest Diseases of Horticultural Perishables. (Edited by: Sharma N. and Mashkoor Alam M.). IBDC, New Delhi, pp. 254-262.
20. Sharma, N. 2001. Preservation of dried fruits and nuts from biodeterioration by natural plant volatiles In: Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, (Edited by: Donahaye, E.J., Navarro, S. and Leesch, J.) Fresno, California. Executive Printing Services, Clovis, CA USA, pp. 195-208.
21. Shenoy Chitra, Patil M.B. and Kumar Ravi, 2009. Wound Healing Activity of Hyptis suaveolens (L.) Poit (Lamiaceae), *International Journal of Pharm Tech Research*, 1(3), 737-744
22. Singh, H.B., AND Handique, A.K. 1997. Antifungal activity of the essential oil of *Hyptis suaveolens* and its efficacy in biocontrol measures in combination with *Trichodermaharzianum*. *Journal of Essential Oil Research* 9. 683-687.
23. Thomson, D.P. 1989. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia,* 81, 151-153.
24. Verma A., Mukerjeea, A, Vijayakumarc M. and Mishraa S. B., 2011. Anti-hyperglycemic activity of leaves extract of *Hyptis suaveolens* L. Poit in streptozotocin induced diabetic rats, *Asian Pacific Journal of Tropical Medicine*, 4(9), 689–693