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Antifungal properties of essential oil of *Mentha spicata* L. var. MSS-5

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Abstract

Studies on various antifungal properties of essential oil of *Mentha spicata* L.var. MSS-5 showed cidal effect on mycelial growth of test fungi viz., 1100ppm against *Aspergillus ochraceus* Wilhelm, 1000ppm against *Penicillum digitatum* Sacc and *Pyricularia oryzae* Cavara and 700ppm against *Alternaria alternata* (fr.) Keissler. Oil was found to be thermo stable upto 80°C and remained active upto 24 months at room temperature, it possessed quick fungicidal action, activity on broad pH range, broad fungitoxic spectrum. Its volatile vapours was also found fungitoxic at 2200ppm concentration.

Key words: Fungitoxicity, Antifungal, *Mentha spicata.*

Introduction

Several plants have been reported to possess antifungal properties in their secondary metabolites. Essential oil of *M. Spicata* L.var.MSS-5 has already been found by the authors to exhibit strong fungitoxicity against some storage fungi (Yadav, 2002). The plant belongs to the family Lameaceae commonly called spearmint is an aromatic perennial herb grows successfully under the temperate and subtropical regions. The leaves have a characteristics aromatic odour and a slightly pungent taste. This is due to carvone content in the essential oil preserved in the glands of leaves present in the subcuticular region. In India it is commercially cultivated in Punjab, Himachal Pradesh, Uttranchal, Gangarate plains and Tarai regions of Uttar Pradesh. In the present investigation, various antifungal properties of the extracted oil of the *Mentha spicata* var. MSS-5 plant have been determined.

Materials and methods

The present investigation was carried out in 2002 at the Biological product laboratory, Deptt. of Botany, University of Allahabad, Allahabad. Folliage parts of *Mentha spicata* var. MSS-5 were subjected to hydro-distillation by using Clevenger apparatus (Clevenger, 1928). A clear yellowish brown oily layer was obtained at the top of aqueous distillate, which was separated from the latter and dehydrated over anhydrous sodium sulphate. The oil thus obtained was found acrid in taste and with typical odour. The oil fraction thus obtained was used for assay. *Alternaria alternata, Aspergillus ochraceus, Penicillium digitatum, Pyricularia oryzae* were used as test fungi. Fungitoxicity was estimated in terms of percentage of mycelial inhibition.

$$(MGI) = \frac{dc - dt}{dc} \quad 100$$

Where, dc & dt = average diameter of the fungal colony in control and in treatment respectively. Determination of the antifungal properties of the oil was done by "colony diameter measurement method" (Grover and Moore, 1962). The minimum killing time (MKT) of the oil against storage fungi was determined by the technique of Shahi *et al.* (1996) with slight modification (Shukla *et al*., 1997). Fungitoxic spectrum of the oil was determined at the conc. of 550ppm, 1100ppm and 2200ppm, i.e. sub-lethal, lethal and hyper-lethal conc. against twenty-five available fungi and nature of toxicity, i.e. static/cidal of the oil was also determined. Fungitoxicity of the volatile vapour of the oil was determined at 2200ppm and the experiment was repeated twice, each containing five replicates and the mean value was taken.

Result and discussion

As it is clear from table-1, the oil was fungitoxic against all the test fungi and its cidal conc. was found to be 1100ppm against *Aspergillus ochraceus,* 1000ppm against *Penicillium digitatum* and *Pyricularia oryzae* and 700ppm against *Alternaria alternata*. The oil retained activity upto 80°C. It remained completely active upto 24 months when stored at room temperature, killed the *A. ochraceues* within 30 minutes, *P. oryzae* within 15 minutes, *P. digitatum* and *A. alternata* within 10 minutes, possessed maximum fungitoxicity between pH levels 4.5 - 7.5. Volatile vapours emitted from the oil was also fungitoxic. (Table-1). It possessed a wide range of fungitoxicity inhibiting all the 25 fungi tested at 1100ppm and 2200ppm (Table-2).

Disease resistance in plants has been attributed to the various chemicals present in their tissues. It may be marked that some workers first screened the plants for their antifungal activity with their crude extracts and

Table 1. Antifungal properties of the oil

Properties studied		Mycelial inhibition of the test fungi (%)			
		Ao	Pd	Po	Aa
Cidal conc. (ppm)	1100	100	100	100	100
	1000	65	100	100	100
	700	-	60	-	100
Effect of temp. (°C)	40	100	100	100	100
	60	100	100	100	100
	80	100	100	100	100
Effect of storage (months)	03	100	100	100	100
	06	100	100	100	100
	09	100	100	100	100
	12	100	100	100	100
	15	100	100	100	100
	18	100	100	100	100
	21	100	100	100	100
	24	100	100	100	100
Effect of exposure duration for killing fungi (Minutes)	120	100	100	100	100
	60	100	100	100	100
	30	100	100	100	100
	15	70	100	100	100
	10	-	100	80	100
	5	-	65	60	73
Effect of pH	3.5	80.0	100	95	85
	4.5	100	100	100	100
	5.5	100	100	100	100
	6.5	100	100	100	100
	7.5	100	100	100	100
	8.5	70	75	72	81
Activity of volatile vapours (ppm)	2200	100	100	100	100

Ao: *Aspergillus ochraceus*; Pd: *Penicillium degitatum*; Po: *Pyricularia oryzae*; Aa: *Alternaria alternata*

than isolated the essential oils from the active plants (Mishra, 1992; Caceres *et al.*, 1993). While, others directly assayed the commercial oils (Kishore and Dwivedi 1991; Shahi *et al*., 1996). Furthermore, most of the workers tested the oil for their fungicidal activity only and did not pay attention to their detailed fungicidal investigations, which were important in predicting the successfulness of an essential oil as fungitoxic. This difference is due to different chemical composition of the oil and different test pathogens used. According to Wellman (1967) a fungicide must not be affected by extremes of temperature. The effect of temperature on toxicity of oils has been studied by a few workers. Shukla *et al.* (1997) reported that oil of *Eucalyptus spp*. retains antifungal activity upto 100°C. However, in the present study the toxicity of *M. spicata* oil was found to be thermostable upto 80°C, the maximum temperature taken into consideration. A few efforts have been made to determine the expiry of toxicity of the natural principles in the past. The fungicidal activity of the *Adenocalymma allicea* oil was lost within 21 days of storage (Chaturvedi *et* al., 1987), while it is persisted for longer period in essential oils of *Caesulia oxillaries* (Pandey *et al*., 1982); *Cedrus deodara* (Dikshit, 1980), and *Cymbopogon flexuosus* (Shukla, 1998). Therefore, antifungal activity of the oil

Table 2. Fungitoxic spectrum of the oil

Fungi tested	MGI (%) of the oil at different conc.		
	Sub-Lethal 550ppm	lethal 1100ppm	Hyper-lethal 2200ppm
1. *Curvularia lunata* (Wakker) Boedijn[b]	70	100[s]	100[c]
2. *Helminthosporium oryzae* Breda de Haan[b]	80	100[s]	100[c]
3. *Botrytis cinerea* Pers[a]	100[s]	100[c]	100[c]
4. *Penicillium brevicompactum* Dierckx[a]	70	100[c]	100[c]
5. *Aspergillus niger* Van Tieghem[b]	85	100[s]	100[c]
6. *Penicillium funiculosum* Thom[a]	75	100[s]	100[c]
7. *Penicillum chrysogenum* Thom[a]	77	100[c]	100[c]
8. *Fusarium moniliforme* Sheld	86	100[c]	100[c]
9. *Colletotrium capsici* (Syd.) Butler & Bisby[b]	65	100[s]	100[c]
10. *Colletotricum falcatum* Went[b]	63	100[s]	100[c]
11. *Fusarium oxysporum* Schlecht ; fr[a]	82	100[s]	100[c]
12. *Helminthosporium maydis* Nesikado & Miyakel[b]	74	100[s]	100[c]
13. *Penicillium imlicatum* Bioarge[b]	70	100[s]	100[c]
14. *Penicillium italicum* Wwhmeg[b]	76	100[s]	100[c]
15. *Aspergillus parasiticus* Speare[b]	75	100[s]	100[c]
16. *Aspergillus versicolor* (Vull) Terboschi[b]	71	100[s]	100[c]
17. *Penicillium expansum* Link[b]	76	100[c]	100[c]
18. *Aspergillus candidus* Fres[b]	85	100[s]	100[c]
19. *Aspergillus fumigatus* Fres[b]	74	100[s]	100[c]
20. *Fusarium proliferatum* (Matsushima) Nirenberg[b]	87	100[s]	100[c]
21. *Penicillium citrinum* Thom[b]	73	100[s]	100[c]
22. *Aspergillus wentii* Wehmer[b]	80	100[s]	100[c]
23. *Aspergillus tamarii* Kita[b]	84	100[s]	100[c]
24. *Cladosporium cladosporioides* (Fres) devries[a]	82	100[s]	100[c]
25. *Fusarium graminaream* Schwabe	72	100[s]	100[c]

Where,
s: static activity; c: cidal activity; a: fungi obtained from IARI, New Delhi; b: fungi obtained from IMTECH, Chandigarh

have been found to be fungi dependent. In the present study, fungitoxicity of *M. spicata* oil persisted even after 24 month of storage at room temperature. Very little attention has been paid on the effect of pH amendment on fungicidal activity of the essential oils. Therefore, an attempt has been made in this direction to see if the activity of the essential oil can be increased by altering its original pH. Shukla (1998) and Shukla *et* al. (2000), have reported the remarkable studies with the essential oils of *Trachyspermum ammi* and *Cympogon flexuosus* against some storage spoilage fungi. However, in the present investigation the fungicidal activity of the oil of *Mentha spicata* at adjusted pH levels of 4.5 and 7.5 have been found most effective than the original pH against the test fungi *A. ochraceous, P. oryzae, P. digitatum* and *A. alternata*. On the contrary, at lower pH i.e. 3.5 and at higher pH i.e. 8.5, the toxicity got reduced (Table-1). In the present study the oils of *M. spicata* was found to have wide range of activity at its 1100ppm and 2200ppm conc., while at 550ppm it exhibited narrow range of toxicity (Table-2). Due to broad fungitoxic spectrum and quick killing action against storage fungi the oil of *Mentha spicata* var. MSS-5 indicates the possibility of its exploitation as an effective fungitoxicant in storage after undergoing successful *in vivo* trials. Moreover, the oil would constitute a cheap source of valuable fungitoxicant.

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