Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL FROM *SATUREJA CALAMINTHA NEPETA* AGAINST PHYTOPATHOGENS FUNGI

RYMA LABIOD*, SAOUDI AOUADI, NABILA BOUHADDOUDA

Laboratory of Applied Biochemistry and Microbiology (LABM), Department of Biochemistry, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria
Email: labiod_ryma@hotmail.fr

Received: 27 Mar 2015 Revised and Accepted: 21 May 2015

ABSTRACT

Objective: The increasing incidence of resistance among pathogen towards synthetic fungicides is a cause of serious problem. For this, the fight against fungi is directed to the use of natural products. The main objectives of this study were to investigate the chemical composition and antifungal activity of the essential oil against two post harvest pathogenic fungi: *fusarium. sp* and *Aspergillus. sp* and then compare it with the effect of two fungicides (Vapcotop and Propineb) on the same stem.

Methods: Essential oil samples of *Satureja calamintha nepeta* were analyzed using a Gas Chromatography/Mass Spectrometry (GC-MS) technique. The *in vitro* antifungal activity of the essential oils was done by the poisoned food technique.

Results: The GC–MS analysis of the essential oil identified 35 components accounting for 99.40% of the total oil composition. The most abundant components were Menthone (26.46%), piperitone oxide (22.26%), and pulegone (14.04%). Essential oil was found effective against all the tested fungi, and was more potent then the two fungicides with minimum inhibitory concentration (MIC) value of 2 µl/ml.

Conclusion: The essential oil exhibited a significant reduction in mycelial growth with the two fungi species, and has a higher biological activity than those of the two fungicides. This study suggests that essential oil represents a good alternative to eliminate fungi that can be pathogens for plants.

Keywords: *Satureja calamintha nepeta,* Essential oils, Antifungal activity, *Aspergillus. sp*, *Fusarium. Sp*

INTRODUCTION

Plant pathogens that include fungi, nematodes, bacteria and viruses can cause diseases or damages in plants. They cause yield losses in numerous economically important crops [1].

Fungal species of *Aspergillus* and *Fusarium* genera are the most species producer's mycotoxins in food and in addition to diseases such as mildew, seeds corruption, stem rot, wilt and dwarf plants involved [2]. The application of synthetic fungicides has led to a number of environmental and health problems because they are themselves carcinogenic, teratogenic, and highly and acutely toxic with long degradation periods [3-4].

New awareness to reduce the usage of the chemical pesticides by developing alternative strategies or technologies in order to improve plant disease resistance and control of pathogens are being promoted. Therefore, there has been a growing interest in research concerning the alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils of aromatic plants [5-7].

Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals [8]. Previous *in vitro* and *in vivo* investigations suggested that the essential oils could be used as effective antifungal agents [9].

The genus *Satureja*, which belongs to the Lamiaceae, is represented by about 200 species of herbs and shrubs, often aromatic, widely distributed in the Mediterranean area, Asia and boreal America [10].

These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky regions. Many members of this genus are well known for their aromatic and medicinal character. They are used as culinary herbs and in folk medicine to treat various ailments, based on the different plant activities [11-12].

Several studies have been made on the genus *Satureja*. However, few works in Algeria have been devoted to the specie *Satureja calamintha nepeta* and limited to the study of the antimicrobial activity of plant extracts. This oil is highly volatile, and it can be used by spraying in warehouses of agricultural products. The present study was undertaken to investigate the phytochemical composition and the antifungal activity of the oil of the Algerian *Satureja calamintha nepeta* against *Fusarium. sp* and *Aspergillus. sp*, and then compare it with the effect of two fungicides using as standard (Vapcotop and Propineb) on the same stem.

MATERIALS AND METHODS

Plant material

Aerial parts of *Satureja calamintha nepeta* at theflowering stage (September 2012), were collected from Jijel in North-eastern of Algeria. The samples of the plant were identified and performed in a shady place at room temperature for 10 days

Isolation of the essential oil

Dried aerial parts (100 g) of the plant was cut and subjected to the hydro-distillation for 2 h, using Clevenger type apparatus (1.48% v/w). The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until tested.

Gas chromatography-mass spectrometry (GC/MS) analysis

Analyses were realized in the university Ouled Aissa (Jijel), Algeria. The GC-MS used was a Shimudzu GCMS-QP 2010 Plus system (Shimudzu, Kyoto, Japan). The column was a 30 m × 0.25 mm DB-5 MS capillary with 0.25µm film thickness. The carrier gas was helium at a flow rate of 2.0 ml min-1. Samples were injected by placing the SPMe fiber in the GC inlet for 2 min. The starting temperature was 40 °C for 10 min, then raised to 200 °C at a rate of 5 °C min-1 and held at 220 °C for 5 min. The mass spectrometer was operated in the electron impact mode with ion source temperature of 250 °C, using an ionization voltage of 70 eV. The mass range was 40-450 amu. The components were identified by computerized bank ESO 2000 database: library Nist 05 and literature data [13].

Fungal strains

Two filamentous fungal strains *Aspergillus. sp* and *Fusarium. sp* causes damping off in wheat were obtained from the Laboratory of

Microbiology (BADJI Mokhtar University), Algeria. The fungal strain cultures were maintained on a Potato Dextrose Agar (PDA) slant at 4 °C.

Antifungal activity assay

Antifungal activity of the essential oil was tested against the two fungal strains reported above following the poisoned food technique [14].

Aliquots of the essential oil dissolved separately in 0.5 ml of 5% (v/v) Tween-20 were pipetted aseptically onto glass Petri dishes (9 cm×1.5 cm) containing 9.5 ml PDA medium at a temperature of 45–50 °C to produce concentrations of 1, 2, 3, 4, and 5 µl/ml. Control plates (without essential oil) were inoculated following the same procedure. A fungal disc (9 mm in diameter) of mycelium, cut from the periphery of a five-day-old culture using a cork borer, was inoculated aseptically into the center of each Petri dish. The plates were sealed with polyethylene film and incubated at a temperature of 28±2 °C. The efficacy of the treatment was evaluated daily for nine days by measuring the average of two perpendicular diameters of each colony. All tests were performed in triplicate. The percentage inhibition of the radial growth of the two tested fungi by the oils, compared with the control, was calculated at day 9, using the following formula [15]:

Percentage mycelial inhibition = $[(dc\text{-}dt)/dc] \times 100$

Where *dc* is the mean colony diameter for the control sets and *dt* is the mean colony diameter for the treatment sets. The lowest concentration that completely inhibited the growth of the fungus was considered the minimum inhibitory concentration (MIC).

In parallel we have tested activity of two fungicides: Propineb and Vapcotop on the same stem. Whose purpose is to compare their effect with that of the essential oil. Propineb was used at the concentration of (1.59 mg/Petri dishes), and Vapcotop at (2 mg/ml/Petri dishes).

Results and discussion

Chemical composition of essential oil

The aerial parts of *Satureja calamintha nepeta (C. nepeta)* yielded 1, 48% v/w essential oil by steam distillation. A total of thirty five different components of the essential oil, accounting for 99.40% of the total oil composition, were identified by GC–MS analyses. The identified chemical composition, retention time, retention indice and percentage composition are given in table 1. The most abundant components of the essential oil were Menthone (26.46%), piperitone oxide (22.26%), and pulegone (14.04%). The chemical composition of our essential oil appears different compared to those reported in the literature; this is may be due to many factors: the climate, geographical area, seasons, soil conditions, crop period and extraction technique [16-17].

Previous studies on the essential oil of many samples of *C. nepeta* grown from seeds in the Botanical Garden of the State University of Gent (Belgium), have shown that it contains Pulegone (11.5 to 33.2%), Piperitenone oxide (5.9 to 37.8%) and Menthone (4.9 to 8.9%) as major components [18]. That from Molina di Quosa (Italy) on rocky, dry and calcareous soil showed that pulegone (46%), piperitenone oxide (2.53%), piperetone oxide (2.29%), and piperitenone (2.00%) were the dominant components [19]. The study realized in Morocco found that: ρ-cymene (20.9%), γ-terpinene (18.7%), and thymol (34.94%) were the most abundant constituents [20].

Table 1: Chemical composition of the essential oil of *calamintha nepeta*

S. No.	Compounds[a]	RT[b]	IR[c]	% composition
1	α-Thujene	4.46	924	0.09
2	1R-α-Pinene	4.60	932	0.7
3	L-β-Pinene	5.54	980	1.33
4	3-Octanol	5.61	988	0.96
5	α-Terpinene	6.16	1014	0.27
6	(+)-4-Carene	6.87	1015	0.31
7	D-Limonene	7.19	1024	1.94
8	B-Phellandrene	7.27	1025	0.26
9	β-cis-Ocimene	7.66	1035	0.76
10	γ-Terpinene	8.29	1054	0.23
11	Cis-Sabinene hydrate	9.38	1065	0.14
12	Cyclohexanone,2-(1 methylethylidene)-	11.42	1099	9.37
13	3-Octanol-acetate	11.84	1120	0.35
14	Menthone	12.04	1148	26.46
15	Cis-Carveol	12.95	1226	0.16
16	Pulegone	14.82	1233	14.04
17	Piperitone oxide	16.42	1253	22.26
18	Thymol	17.42	1289	0.26
19	Diosphenol	17.57	1309	0.43
20	Piperitenone	17.89	1340	0.2
21	Jasmatone	18.74	1378	0.25
22	β-Cubebene	19.84	1387	5.51
23	β-Elemen	19.98	1389	0.27
24	Caryophyllene	21.10	1419	4.33
25	Z-β-Farnesene	22.22	1440	0.48
26	α-Caryophellene	22.51	1455	0.73
27	Elixene	22.69	1456	1.93
28	*Allo*-aromadendrene	22.79	1458	0.6
29	γ-Muurolene	22.90	1478	0.16
30	α-Muurolene	23.19	1500	0.93
31	γ-cadinene	24.47	1513	0.77
32	(-)-α-Panasinsen	24.63	1530	0.23
33	α-Cadinene	25.36	1537	1.84
34	3-Oxo-α-ionone	25.98	1675	0.25
35	Phytol	39.92	1942	0.56
TOTAL				99.40

[a]: Compounds listed in order of their elution., [b]: Retention time (as min)., [c]: Retention indices.

Antifungal activity assay

Phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment [21].

Propineb and Vapcotop are an active substance of plant protection products (phytopharmaceutical products or pesticide) which have a fungicidal effect.

Propineb is a dithiocarbamate fungicide of German origin (Bayer), (1962), the molecule is quite persistent, insignificant toxicity (LD50 for rats by ingestion of 8500 mg • kg^{-1}), the propineb is used alone or in combination, to the fight against late blight of potato, tobacco, scab, botrytis garlic and onion. The first brand name is marketed Antracol [22].

Vapcotop fungicide is belonging to the Thiophanate-methyl (TM) group. TM is used on a variety of trees, vine, and root crops, as well as on canola and wheat. It may be applied as a dip treatment for cut flowers, rose bud wood, or nursery stock; and as a seed treatment for peanuts and potato pieces. TM was first registered as a pesticide in the United States in 1973 for use as a fungicide. EPA issued a Registration Standard for TM in March, 1996.

TM generally has been shown to have low acute oral/dermal/inhalation toxicity (toxicity categories III/IV). TM is not an irritant to the skin and only a slight occular irritant (toxicity category IV) and is a skin sensitizer. TM is classified as "likely to be carcinogenic to humans based on dose-dependent increases in liver tumors in male and female mice [23].

The activity of propineb and Vapcotop on our steam is giving in table 2. These two fungicides were used in our work that aims to compare their effects to that of the essential oil.

As already mentioned above Vapcotop and propineb were used at concentrations (2 mg/ml/Petri dish) and (1.59 mg/Petri dish), respectively. (Concentrations were selected based on the concentrations mentioned on bags of both products).

The results show that propineb had a less effective on *Fusarium. sp* than Vapcotop. However, it gave a percentage mycelial inhibition greater than 50% on Aspergillus. Unlike Vapcotop gave a percentage inhibition greater than 50% over the two fungal strains.

Recently, the scientific interest in biological properties of essential oil (EO) has been increased. New researches about biological active secondary compounds present in EO of plants have been seen as a potential way to control fungal contamination. Our study had assessed potential antifungal activity of essential oil *C. nepeta*.

Table 2: Percentage of mycelial inhibition of the two fongicides

fungicides	Percentage mycelial inhibition %[a]	
	Fusarium. sp	Aspergillus. sp
Propineb	11.11±0.00	56.11±3.85
Vapcotop	55.55±0.00	67.22±7.69

[a]: Values expressed are means±SD of three parallel measurements.

The growth of the two fungal species over the nine days is shown in (fig.1), and Percentage of mycelial inhibition of essential oil is shown in table 3. The results showed that growth increased with incubation time but mycelial growth was considerably reduced with increasing concentration of essential oil. Growth was delayed by five days for *Fusarium sp*, and six days for *Aspergillus sp* at 1 µl/ml concentration. A MIC of 2 µl/ml was obtained after nine days of incubation.

The percentage inhibition of mycelia growth was determined at day 9. The oil produced a significant reduction in mycelial growth with the two fungi species at 1 µl/ml concentration with percentage reduction of 66,29±1,69 for *Fusarium. sp* and 62,72±0,64 for *Aspergillus. sp.* The growth of fungi was completely inhibited at concentration of 2, 3, 4 and 5 µl/ml which indicates the excellent antifungal property of the oil.

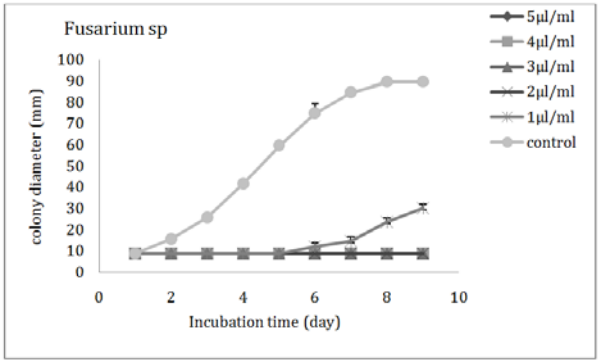

Fig. 1: Effects of the different concentrations of EO on colony diameter (mm) growth of *Fusarium sp*. Values are means (n=3)±standard deviations. Sample size: 9 mm

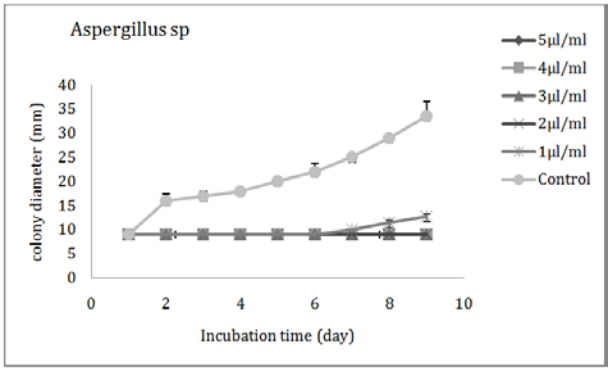

Fig. 1: Effects of the different concentrations of EO on colony diameter (mm) growth of *Aspergillus sp*. Values are means (n=3)±standard deviations. Sample size: 9 mm

Table 3: **Percentage of mycelial inhibition of essential oil of *Satureja calamintha nepeta***

Concentrations(µl/ml)	Percentage mycelial inhibition %[a]	
	Fusarium. sp	Aspergillus. sp
1	66,29±1,69	62,18±1,72
2	100±0,00	100±0,00
3	100±0,00	100±0,00
4	100±0,00	100±0,00
5	100±0,00	100±0,00

[a]: Values expressed are means±SD of three parallel measurements

It would seem possible that the antifungal mode of action of essential oils might be attributed to the presence of menthone, pulegone and pipertone oxide. These three compounds have been shown to possess strong antimicrobial properties: Carvone, Menthol and Menthone are structurally related fungicidal bioactive compounds having low MIC values and negligible cytotoxicity.

Mint essential oil and its three lead compounds (Carvone, Menthol and Menthone) not only reduce the transition of Candida albicans from yeast to the invasive and more pathogenic hyphal form at sub-inhibitory concentrations but has also a significant effect on the production of the hydrolytic enzymes (Proteinase and phospholipase) secreted by the fungal cell during an interaction with a substance to be possessed antifungal activity [24]. The pulegone has pronounced activity against fungi and bacteria [25].

When we compare between the effects of *C. nepeta* essential oil with that of the two fungicides, we find that the essential oil was more effective against Fusarium than both fungicides, it was more

powerful than propineb against Aspergillus, and its effect was very similar to that of Vapcotop on the same stock.

CONCLUSION

The results of *in vitro* study are a clear demonstration of the excellent antifungal property of *C. nepeta* essential oil against *Fusarium sp* and *Aspergillus sp*, and have a higher biological activity than those of the two fungicides. The antifungal activity of the oil can be attributed by its high content of menthone, piperetone oxide or pulegone.

This study indicated that *C. nepeta* essential oil can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread.

These results are also of interest for the use of this oil in the food industry in order to increase the life of many food products, in the control of the food borne pathogens, as well as in the prevention of lipid oxidation. However, the presence of pulegone should be considered, according to the regulations described in the application guide of EFFA [26] this molecule is counted among the undesirable substances as such. For this purpose it is necessary to improve the quality of our essential oil by the method of fractional distillation in vacuo. It is also interesting to identify the products of hydrolyse resulting to the interaction Essential oil/fungi, because some of the microorganisms are able to neutralize the inhibitory effect of essential oil. This can be exploited in the field of fine chemistry for the bioconversion of molecules [27-28].

ACKNOWLEDGEMENT

Authors are thankful to Pr. Ladjama Ali, the director of Applied Microbiology and Biochemistry laboratory (Department of Biochemistry, Annaba, Algeria). Our sincere thanks also go to Dr. Sebti Mohammed laboratory of Phytochemistry and Pharmacology (Department of Animal Biology and vegetable, Jijel, Algeria) for providing necessary facilities in the analysis.

CONFLICT OF INTERESTS

The authors have no conflict of interest in publication of this article.

REFERENCES

1. Fletcher J, Bender C, Budowle B, Cobb WT, Gold SE, Ishimaru CA, *et al*. Plant pathogen forensics: capabilities, needs, and recommendations. Microbiol Mole Biol 2006;70:450-71.
2. Fandohan P, Hell K, Marasas WFO, Wingfield MJ. Infection of maize by Fusarium species and contamination on with fumonisis in Africa. Afr J Biotech 2003;2(12):570-9.
3. Lingk W. Health risk evaluation of pesticide contamination in drinking water. Gesunde Pflanz 1991;43:21–5.
4. Tian J, Ban X, Zeng H, He J, Huang B, Wang Y. Chemical composition and antifungal activity of essential oil from Cicuta virosa L. var. latisecta Celak. Int J Food Microbiol 2011;145:464–70.
5. Pradhanang PM, Momol MT, Olson SM, Jones JB. Effects of plant essential oils on Ralstonia solanacearum population density and bacterial wilt incidence in tomato. Plant Dis 2003;87:423–7.
6. Kotan R, Cakir A, Dadasoglu F, Aydin T, Cakmakci R, Ozer H, *et al*. Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. J Sci Food Agric 2010;90:145–60.
7. Kotan R, Dadaso˘gl F, Karagoz K, Cakir A, Ozer H, Kordalia S, *et al*. Antibacterial activity of the essential oil and extracts of Satureja hortensis against plant pathogenic bacteria and their potential use as seed disinfectants. Sci Hortic 2013;153:34–41.
8. Sokmen A, Jones BM, Erturk M. The *in vitro* antibacterial activity of Turkish medicinal plants. J Ethnopharmacol 1999;67:79-86.
9. Adam K, Sivropoulu A, Kokkini S, Lanaras T, Arsenakis M. Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia and Salvia fruticosa essential oils against human pathogenic fungi. J Agric Food Chem 1998;46:1739–45.
10. Skocibusic M, Bezic N, Dunkic V. Phytochemical composition and antimicrobial activities of essential oils from Satureja subspicata Vis. Growing in Croatia. Food Chem 2006;96:20–8.
11. Azaz D, Demirci F, Satil F, Kurkcuoglu F, Baser KHC. Antimicrobial activity of some Satureja essential oils. Z Naturforsch 2002;57:817–21.
12. Azaz AD, Kurkcuoglu M, Satil F, Baser KHC, Tumen G. *In vitro* antimicrobial activity and chemical composition of some Satureja essential oils. Flavour Fragrance J 2005;20:587–91.
13. Adams RP. Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy. 4th ed. Allured Publishing Corporation, Carol stream, IL; 2001.
14. Singh P, Srivastava B, Kumar A, Kumar R, Dubey NK, Gupta R. Assessment of Pelargonium graveolens oil as plant-based antimicrobial and aflatoxin suppressor in food preservation. J Sci Food Agric 2008;88:2421–5.
15. Albuquerque CC, Camara TR, Marian RDR, Willadino L, Marcelino C, Ulisses C. Antimicrobial action of the essential oil of Lippia gracilis Schauer. Braz Arch Biol Technol 2006;49:527–35.
16. Bertini LM, Pereira AF, Oliveira CLL, Menezes EA, Morais SM, Cunha FA, *et al*. Perfi de sensibilidade de bactérias frente a óleos essenciais de algumas plantas do nordeste do Brasil. Infarma 2005;17:80-3.
17. Gende LB, Mendiara S, Fernández NJ, Van Baren C, Di Leo Lira A, Bandoni A, *et al.* Essentials oils of some Mentha spp. and their relation with antimicrobial activity against Paenibacillus larvae, the causative agent of American foulbrood in honey bees, by using the bioautography technique. B Insectol 2014;67:13-20.
18. De Pooter HL, Goetghebeur P, Scamp N. Variability in the composition of the essential oil of Calmaintha nepta. Phytochemistry 1987;26:3355-6.
19. Panizzi L, Flamini G, Cioni PL, Morelli I. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. J Ethnopharamacol 1993;39:167-70.
20. Satrani B, Farah A, Fechtal M, Talbi M, Blaghen M, Chaouch A. Composition chimique et activité antimicrobienne des huiles essentielles de Satureja calamintha et Satureja alpina du Maroc. Ann Falsif Expert Chim 2001;94:241-50.
21. Harris CA, Renfrew MJ, Woolridge MW. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Addit Contam 2001;18:1124-9.
22. http://fr.wikipedia.org/wiki/Propinèbe
23. Anonymous. US EPA Archives Document, R. E. D. FACTS, Thiophanate-Methyl. United States; 2004.
24. Samber N, Varma A, Manzoor N. Evaluation of Mentha piperita essential oil and its major constituents for antifungal activity in candida spp. Int J Innov Res Sci Eng Tech 2014;3(2):9404-11.
25. Javidnia K, Tabatabaiee M, Shafiee A. Composition and antimicrobial activity of essential oil of Ziziphora teniur, population Iran. DARU 1996;6:56-9.
26. Guide d'application de l'EFFA sue le règlement européen relatif aux aromes ; 2009.
27. Shimoni E, Ravid U, Shoham Y. Isolation of a Bacillus sp. Capable of transforming isoeugenol to vanillin. J Biotechnol 1999;78:1-9.
28. Furukawa H, Zenno S, Jwasawa Y, Morita H, Yoshida T, Naagasawa T. Ferulic Acid Production from clove oil by Pseudomonas fluorescens E118. J Biosci Bioeng 2003;96:404-5.